

Summer 2017

FOUNDATIONS FOR A GEOBIOCHEMICAL CHARACTERIZATION OF MUDPOTS IN YELLOWSTONE NATIONAL PARK

Georgia Dahlquist
Montana Tech

Follow this and additional works at: http://digitalcommons.mtech.edu/grad_rsch

 Part of the [Geochemistry Commons](#)

Recommended Citation

Dahlquist, Georgia, "FOUNDATIONS FOR A GEOBIOCHEMICAL CHARACTERIZATION OF MUDPOTS IN YELLOWSTONE NATIONAL PARK" (2017). *Graduate Theses & Non-Theses*. 132.
http://digitalcommons.mtech.edu/grad_rsch/132

This Thesis is brought to you for free and open access by the Student Scholarship at Digital Commons @ Montana Tech. It has been accepted for inclusion in Graduate Theses & Non-Theses by an authorized administrator of Digital Commons @ Montana Tech. For more information, please contact ccote@mtech.edu.

FOUNDATIONS FOR A GEOBIOCHEMICAL CHARACTERIZATION OF MUDPOTS IN YELLOWSTONE NATIONAL PARK

by
Georgia Dahlquist

A thesis submitted in partial fulfillment of the
requirements for the degree of

Master of Science in Geoscience:
Geochemistry Option

Montana Tech
2017



Abstract

Mudpots are acidic, turbid thermal features formed by the argillic or sericitic alteration of rock with enough fluid to create a viscous feature. Prior to this research, the combination of interdisciplinary sampling for geochemistry, mineralogy, and microbiology of rhyolite hosted mudpots, particularly in chemically distinct subregions of an area, remained largely unavailable. This work discusses mudpots and nearby hot springs sampled in Yellowstone National Park (YNP) in July 2016 and the measured *in situ* pH, temperature, and conductivity values, and dissolved oxygen concentrations. Water, filtered via gravity pre-filtration and 1.2 μm and 0.8/0.2 μm syringe filtration, yielded δD and $\delta^{18}\text{O}$ values and major anions, cations, and trace element concentrations. Sediment collected for biological and mineralogical analysis revealed that mudpots, with approximate viscosities ranging from 5 to 100 centipose ($\text{mPa}\cdot\text{s}$), had trace element geochemical “fingerprints” that can be linked with underlying rock units. Turbid hot springs of similar pH and temperature lacked consistent chemical similarity to mudpots, however mudpots shared chemical similarity with respect to major anions and cations to hot springs from similar thermal source waters. Chloride concentrations and δD determined similarities between source waters. Despite having similar concentrations of major anions and cations with nearby acid sulfate hot springs, mudpots differ from these hot springs in their trace element relative abundance “fingerprints” that make connections between surficial geochemistry and variations in chemical composition of underlying rock units. Such comparisons are not possible with hot springs. 16S and 18S rRNA gene microbial diversity analyses show that mudpots host organisms with a variety of known metabolisms, such as methanogenesis, nitrification, and ammonia oxidation. There are microbes common to thermal areas in mudpots, however no genus common to all mudpots. Mudpots, formed by alteration of volcanic rock by acidic steam, serve as both indicators of underlying geology and distinct microbial ecosystems offering insight into potentially ancient analogs.

Keywords: mudpot, geochemistry, Yellowstone, montmorillonite, kaolinite, methanogenesis, Central Plateau Member rhyolite

Dedication

To E. T. Allen and Arthur L. Day, the first of few to find sampling mudpots “disagreeable and tedious, not [an] impossible task” and to my parents for support, encouragement, and help building a wealth of custom made equipment to make mudpot sampling a little less disagreeable and tedious.

Acknowledgements

This work would not be possible without the time and dedication of my advisor, Alysia Cox, members of LEGEND (Laboratory Exploring Geochemical Engineering in Natural Dynamics), Renée Schmidt, Shanna Law, Mallory Nelson, James Foltz, McKenzie Dillard, Cynthia Cree and Jordan Foster; the GEOPIG (Group Exploring Organic Processes in Geochemistry) 2016 field team, particularly Everett Shock and Vince Debes; sample analysis from Jackie Timmer, Ashley Huft, and Gary Wyss; and my graduate thesis committee, Chris Gammons, Katie Hailer, and Bill Drury, for their collective wisdom. In addition to all of the help, encouragement, and advice, this undertaking was made possible by the generous funding from the Montana Tech Faculty Development Initiative Grant to Alysia Cox and Georgia Dahlquist, the Montana Tech Faculty Seed Grant to Alysia Cox, the Montana Water Center Grant to Alysia Cox, the Montana Research and Economic Development Initiative (MREDI) Grant to Jerry Downey, Ed Rosenberg, Hsin-Hsiung Huang, and Alysia Cox, and the NASA Exobiology Award number NNX-16AJ61G to Everett Shock, Alysia Cox, Jason Raymond, Jordan Okie, Kris Fecteau, and Grayson Boyer. Permission to collect and analyze samples was granted by National Park Service permit number YELL-2016-SCI-7008 to Alysia Cox.

Table of Contents

ABSTRACT	II
DEDICATION	III
ACKNOWLEDGEMENTS	IV
LIST OF TABLES	IX
LIST OF EQUATIONS	XII
LIST OF FIGURES.....	XIII
 1. INTRODUCTION	 1
1.1. Mudpot Formation.....	1
1.2. Summation of Mudpot Discoveries	3
1.3. Yellowstone Geology.....	4
1.4. Thesis Objectives/Statement of Purpose.....	8
2. METHODS	10
2.1. Cleaning Protocols.....	10
2.1.1. Trace Metal Cleaning Procedure	10
2.1.2. Other Bottle Cleaning Procedures.....	11
2.1.2.1. δD and $\delta^{18}O$	11
2.1.2.2. Dissolved Organic Carbon	11
2.1.3. Sterilization Procedures	11
2.1.3.1. Field Ethanol Sterilization.....	11
2.1.3.2. Microwave Sterilization	11
2.2. Field Methods.....	12
2.2.1. Filtration	12
2.2.1.1. Water	12

2.2.1.2.	Mud	12
2.2.1.	<i>In situ</i> Meters	16
2.2.2.	Spectrophotometry	16
2.2.3.	Biological Matter Collection	18
2.2.3.1.	Suspended Sediment	18
2.3.	<i>Lab Methods</i>	18
2.3.1.	Biological Analysis	18
2.3.1.1.	DNA Extraction	18
2.3.1.2.	Polymerase Chain Reaction (PCR)	21
2.3.1.3.	Gel Electrophoresis	21
2.3.1.4.	16S and 18S rRNA Gene Diversity Analysis	22
2.3.2.	Chemical Analysis	25
2.3.2.1.	δD and $\delta^{18}O$	25
2.3.2.2.	Inductively Coupled Plasma – Optical Emission Spectroscopy (ICP-OES)	25
2.3.2.3.	Ion Chromatography	26
2.3.2.4.	Carbon Analysis (Dissolved Organic and Inorganic Carbon)	26
2.3.2.5.	Inductively Coupled Plasma – Mass Spectrometry (ICP-MS)	27
2.3.3.	Solid Preparation and Analysis	27
2.3.3.1.	Desiccation	27
2.3.3.2.	TerraSpec Halo Short Wave Infrared Portable Spectrometer	27
2.3.3.3.	Scanning Electron Microscopy	28
3.	RESULTS	29
3.1.	<i>Geochemical</i>	29
3.1.1.	Background Context for Geochemical Observations	29
3.1.2.	Water Isotopes	33
3.1.3.	Major Cation and Anion Concentrations	35
3.1.4.	Trace Element Concentrations	40
3.2.	<i>Geologic</i>	49

3.2.1.	Clay Compositions	49
3.2.2.	Mineral Solubility Predictions via EQ3 and CHNOSZ	53
3.2.3.	Clay Stability Plots	53
3.3.	<i>Microbial Diversity</i>	54
4.	DISCUSSION.....	60
4.1.	<i>Thermal Source Waters</i>	60
4.2.	<i>Rock-Pore Water Chemistries</i>	65
4.3.	<i>Mudpots are Dynamic (Resurgent Domes/Seasonal Variations)</i>	67
4.4.	<i>Preliminary Summary of Mudpot Characteristics</i>	69
4.5.	<i>Comparison of Mudpots and Adjacent Hot Springs by Thermal Area</i>	72
4.6.	<i>Comparisons with Known International Mudpot Geochemistry</i>	73
4.7.	<i>Microbial Communities in YNP Mudpots</i>	74
4.8.	<i>Future Work</i>	76
5.	CONCLUSION	79
6.	REFERENCES CITED.....	82
7.	APPENDIX A: BIOLOGICAL DATA	89
8.	APPENDIX B: GEOCHEMICAL DATA.....	95
9.	APPENDIX C: SAMPLING BLANKS AND DETECTION LIMITS	123
10.	APPENDIX D: FIELD GUIDE TO SAMPLING LOCATIONS	128
10.1.	<i>Eastern Sites</i>	129
10.1.1.	Geology	129
10.1.2.	Eastern Thermal Areas	131
10.2.	<i>Western Sites</i>	138
10.2.1.	Geology	138
10.2.2.	Thermal Areas	138
11.	APPENDIX E: CHNOSZ INPUT FILE	144

<i>CHNOSZ input file</i>	144
--------------------------------	-----

List of Tables

Table I: Surficial geology for thermal areas and nearby older outcrops.....	7
Table II: Methods and reagents for dissolved silica.	17
Table III: Methods and reagents for ferrous iron.....	17
Table IV: Methods and reagents for dissolved sulfide.	18
Table V: DNA concentration and A260/280.	21
Table VI: 16S and 18S rRNA gene primer sequences and melting temperatures.	24
Table VII: TerraSpec Halo portable Near Infrared Spectrometer and mudpot solids calculations.	50
Table VIII: Mineral supersaturation in mudpots (modelled predictions).	53
Table IX: Archaeal and bacterial genera.	56
Table X: Explanation of lines from Truesdell plot.	62
Table XI: Relative abundance of bacterial classes.	89
Table XII: Total counts of bacterial classes.....	89
Table XIII: Relative abundance bacterial genera.....	90
Table XIV: Total counts of bacterial genera.....	91
Table XV: Relative abundance of archaeal classes.	91
Table XVI: Total counts of archaeal classes.....	92
Table XVII: Relative abundance of archaeal genera.	92
Table XVIII: Total counts of archaeal classes.	93
Table XIX: <i>In situ</i> dissolved oxygen measurments.	95
Table XX: <i>In situ</i> tempearture and pH mesurments.	98
Table XXI: Major cation concentrations.	102

Table XXII: Major anion concentrations.	103
Table XXIII: Lithium, boron, aluminum, iron, and titanium concentrations.	104
Table XXIV: Beryllium, vanadium, chromium, and manganese concentrations.	106
Table XXV: Arsenic, rubidium, molybdenum, and cesium concentrations.	108
Table XXVI: Cobalt, nickel, copper, and zinc concentrations.	110
Table XXVII: Gallium, selenium, strontium, and zirconium concentrations.	112
Table XXVIII: Antimony, barium, lanthanum, and cerium concentrations.	113
Table XXIX: Praseodymium, neodymium, tungsten, thallium, and uranium concentrations.	116
Table XXX: Palladium, silver, cadmium, lead, and thorium concentrations.	118
Table XXXI: Relative abundances of trace elements in sampled mudpots.	119
Table XXXII: $\delta^{18}\text{O}$ and δD vs VSMOW values for mudpots and hot springs.	121
Table XXXIII: Lithium, boron, aluminum, iron, and titanium concentrations for blanks.	123
Table XXXIV: Lithium, boron, aluminum, iron, and titanium detection limits.	123
Table XXXV: Manganese, arsenic, rubidium, molybdenum, and cesium concentrations for blanks.	124
Table XXXVI: Beryllium, vanadium, chromium, manganese, arsenic, rubidium, molybdenum, cesium, cobalt, nickel, and copper detection limits.	124
Table XXXVII: Zinc, selenium, strontium, barium, and tungsten concentrations for blanks.	125
Table XXXVIII: Sodium and calcium concentrations for blanks.	125

Table XL: Zinc, gallium, selenium, strontium, zirconium, niobium, palladium, silver, cadmium, tin, antimony, barium, tungsten, lanthanum, cerium, praseodymium, neodymium, thallium, lead, thorium, and uranium detection limits.	126
Table XLI: Lithium, sodium, potassium, magnesium, and calcium detection limits.	126
Table XLII: Fluoride, sulfate, and nitrate concentrations for blanks.	127
Table XLIII: Fluoride, chloride, bromide, sulfate, phosphate, nitrite, and nitrate detection limits.	127
Table XLIV: Same as Table I.	130

List of Equations

Alteration of K-feldspar to sericite. (Equation 1)	2
Alteration of plagioclase to sericite. (Equation 2)	2
Alteration of sericite to kaolinite. (Equation 3)	2
Alteration of plagioclase to kaolinite. (Equation 4)	2
Alteration of K-feldspar to alunite. (Equation 5)	2

List of Figures

Figure 1: Qualitative mineral formation experiments.....	3
Figure 2: Yellowstone National Park Map with sampling sites.	5
Figure 3: YNP surficial geology map showing sampling areas.....	6
Figure 4: Generic stratigraphy for Quaternary rock units in Yellowstone National Park. ..	8
Figure 5: Mud gravity filtration apparatus.....	13
Figure 6: Chloride, aluminum, zinc, and iron concentrations for blanks.	15
Figure 7: Elements analyzed by the Montana Bureau of Mines and Geology (MBMG). ..	25
Figure 8: pH vs temperature.	29
Figure 9: Difference in specific conductivity of clay suspensions vs clay concentration.	30
Figure 10: Conductivity vs temperature..	31
Figure 11: Dissolved oxygen concentration vs temperature.....	32
Figure 12: δD vs $\delta^{18}O$	33
Figure 13: Changes in water isotope values based on environmental conditions.....	34
Figure 14: δD vs total dissolved chloride..	35
Figure 15: Major cation and anion concentrations for Sylvan Springs.	36
Figure 16: Major cation and anion concentrations for GOPA.....	36
Figure 17: Major cation and anion concentrations for Forest Springs.....	37
Figure 18: Major cation and anion concentrations for Rabbit Creek North.	37
Figure 19: Major cation and anion concentrations for Pocket Basin.....	38
Figure 20: Major cation and anion concentrations for Boulder Spring.	38
Figure 21: Dissolved sulfate vs pH.....	39

Figure 22: Chloride concentrations vs pH.	40
Figure 23: Trace elements vs pH for hot springs with concentrations higher than mudpots.	42
Figure 24: Trace elements vs pH for hot springs with concentrations lower than mudpots.	43
Figure 25: Trace elements vs pH for mudpots and hot springs with pH trends.	44
Figure 26: Arsenic vs chloride concentrations.	45
Figure 27: Boron concentration vs pH in hot springs and mudpots.	46
Figure 28: Boron speciation with pH.	47
Figure 29: Trace element relative abundances in YNP mudpots.	48
Figure 30: Trace element relative abundance grouped by iron or aluminum dominance.	49
Figure 31: SEM images from mudpots in the Crater Hills Area.	52
Figure 32: SEM images of mudpots, Princess Peach and Muddy Delight.	52
Figure 33: Eh-pH diagram for alunite and kaolinite at 92.5°C.	54
Figure 34: Venn diagrams of archaeal and bacterial genera extracted from four mudpots.	55
Figure 35: Archaeal classes identified with 16S rRNA gene diversity.	58
Figure 36: Archaeal genera identified with 16S rRNA gene diversity.	58
Figure 37: Bacterial classes identified with 16S rRNA diversity.	59
Figure 38: Bacterial genera identified with 16S rRNA gene diversity.	59
Figure 39: δD vs chloride concentrations from Fig. 14 with Truesdell lines.	62
Figure 40: Gel electrophoresis image of PCR product from GOPA mudpot	94
Figure 41: Same as Figure 2.	128
Figure 42: Same as Figure 3.	129
Figure 43: Same as Figure 4.	131
Figure 44: Forest Springs thermal area mudpot unofficially named, The Good Stuff.	133

Figure 45: Forest Springs thermal area mudpot unofficially named, Choco Mocha.....	133
Figure 46: Crater Hills thermal area mudpot cluster.	134
Figure 47: GOPA mudpot unofficially named, The Gomper.	135
Figure 48: GOPA mudpot unofficially named, Muddy Delight.	136
Figure 49: GOPA mudpot unofficially named, Phantom Pants.....	136
Figure 50: GOPA mudpot unofficially named, Pedro’s Tar Pit.	137
Figure 51: Sylvan Spring thermal area mudpot unofficially named, Ol’ Chunky.....	139
Figure 52: Pocket Basin thermal area mudpot unofficially named, Consolation Prize...140	
Figure 53: Pocket Basin thermal area mudpot unofficially named, Urinal Cake.	140
Figure 54: Boulder Spring thermal area mudpot unofficially named, Ugluk’s Door.	141
Figure 55: Rabbit Creek North thermal area mudpot unofficially named, Princess Peach.142	
Figure 56: Shoshone Geyser Basin mudpot unofficially named, Mudder’s Milk..	143

.

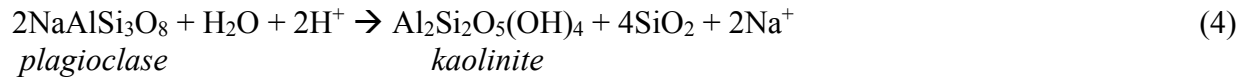
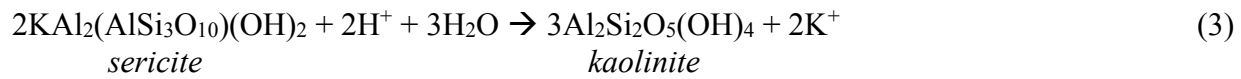
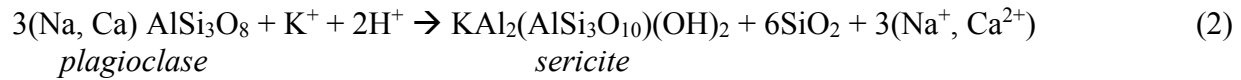
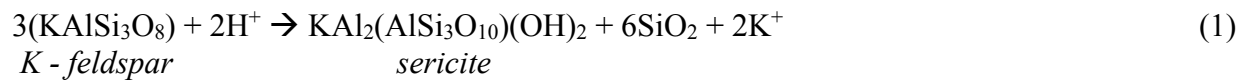
1. Introduction

1.1. Mudpot Formation

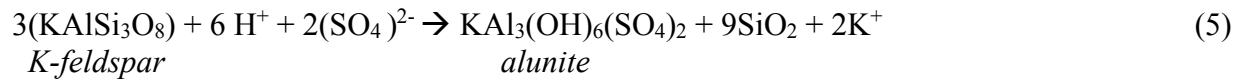
Thermal features, including hot springs, fumaroles, geysers, and mudpots, have been historically separated into acid sulfate and alkaline chloride, based on deep thermal fluid behavior. At depth, hydrothermal fluids separate into a volatile rich steam fraction and a briny liquid fraction. The formation of acidic thermal features is attributed to the rising volatile rich steam that mixes with aerated shallow groundwater, thereby oxidizing hydrogen sulfide to form sulfuric acid (Xu *et al.*, 1998). Acidic hot springs, mudpots, and fumaroles are all thought to be acid sulfate features. Likewise, the formation of alkaline thermal features is attributed to the briny liquid fraction rising from depth. Alkaline hot springs and many geysers are thought to be alkaline chloride features (Allen and Day, 1935). While the characteristics of the hydrothermal fluid reservoirs are less well known, surficial chemical measurements of acidic and alkaline features support this hypothesis.

A more current theoretical categorization of thermal features in Yellowstone National Park considers three major categories; neutral-chloride, acid sulfate, and calcium-bicarbonate sulfate (Fournier, 1989). The calcium-bicarbonate sulfate features are primarily in the northern reaches of YNP, including the hot springs and carbonate terraces at Mammoth Hot Springs. This work does not consider this category of thermal feature, because sampled mudpots do not fit into this major anion and cation characterization. The more current categorization builds upon the work of Allen and Day and shares the definitions and conceptualizations of formation for alkaline chloride (also called neutral chloride) and acid sulfate features.

Mudpots are acid sulfate thermal features that alter surficial rock units to form suspensions of clay minerals. There are many possible hydrothermal alteration pathways for kaolinite formation from plagioclase or K-feldspar (Yuan *et al.*, 2014). Sericite is often formed as an intermediate (Eqns 1, 2) which can react to form kaolinite (Eqn 3), however kaolinite can also be formed directly from sodium rich plagioclase (Eqn 4) (Yuan *et al.*, 2014). It is possible for rhyolitic flows and tuffs to undergo argillic and sericitic alteration to form kaolinite in the presence of acid and water, in a hot, acidic environ such as a mudpot (Yuan *et al.*, 2014).



Acidic fluids in hydrothermal systems can alter rock to form amorphous silica and alunite (Mayer *et al.*, 2016). In mudpots, the oxidation of hydrogen sulfide forms sulfuric acid and K-feldspar is found in rhyolite, which may provide an explanation for finding K-alunite in nearly all sampled mudpots (Eqn 5). All mudpots hosted in rhyolite would be expected to contain K-alunite as a detectable constituent.



The iron montmorillonite formed on the eastern half of YNP is somewhat less straightforward. Experiments synthesizing montmorillonite-group minerals at 300°C from combinations of kaolinite, illite, feldspar, and dolomite showed that felsic hydrothermal

environments can produce montmorillonite at depth (Levinson and Day, 1968). These experiments provide a theoretical basis for montmorillonite found in the eastern mudpots at Greater Obsidian Pool Area (GOPA) and Crater Hills. The experimental results are for both sodium and calcium montmorillonite, which are stable in more alkaline hot springs (Raymahashay, 1968). Substitution of predominately ferric iron into the montmorillonite structure may lend stability in more acidic conditions (Johnston and Cardile, 1987; Wilson *et al.*, 2006).

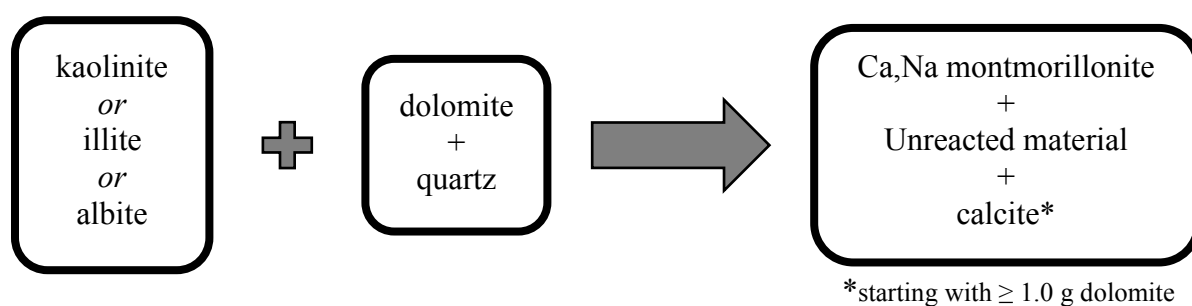


Figure 1: Qualitative mineral formation experiments.

1.2. Summation of Mudpot Discoveries

The body of scientific literature concerning mudpots can be divided into five major categories: broad survey of features in a given area (Allen and Day, 1935; Kanellopoulos, 2016), geochemical study focusing on a certain chemical parameter (Shevenell *et al.*, 1987; Trainer *et al.*, 2000; Szykiewicz *et al.*, 2012), mineralogical assessment of a subregion (Raymahashay, 1968; Bortnikova *et al.*, 2009; Marcucci *et al.*, 2013), organic geochemical analysis of an individual mudpot (Bradley *et al.*, 2005), or microbiological studies of a volcanic region containing one or more mudpots (Prokofeva *et al.*, 2006; Pol *et al.*, 2014). The discovery of microbes using methyldehydrogenases with rare earth element cofactors in an Italian mudpot (Pol *et al.*, 2014), the mineralogical assessment of mudpots in Yellowstone, Nicaragua, and Kamchatka (Raymahashay, 1968; Bortnikova *et al.*, 2009; Marcucci *et al.*, 2013), and the

original survey and rudimentary chemical assessment of mudpots in thermal areas in Yellowstone and Valles Caldera, New Mexico (Allen and Day, 1935; Shevenell *et al.*, 1987) represent the most groundbreaking works on mudpots. From these milestone publications, it may be asserted that mudpots are acidic thermal features, comprised of clays with the potential for methanotrophic microbial life.

1.3. Yellowstone Geology

Sites sampled in July 2016 can be divided into eastern and western thermal areas in YNP (Fig. 2). As further discussed in the results section, dividing the samples into eastern and western sites is partly based on differing trace element relative abundances. These chemical variations in filtered mudpot samples may be due to changes in magma composition which causes the bulk composition of different rhyolite flows to vary with regard to trace elements.

The eastern sampling sites are hosted in younger rock, whereas the western sampling sites may be in more eroded areas, with older outcrops nearby (Table I). The three eastern sampling sites are underlain by the Hayden Valley Flow. The Hayden Valley and Solfatara Flows of the Pleistocene Central Plateau Member have variation in trace element bulk compositions in relation to the other flows in the Central Plateau Member (Girard *et al.*, 2010). The western sites may be divided into three groups based on underlying geology: Sylvan Springs, the Midway Geyser Basin (Pocket Basin, Boulder Spring, and Rabbit Creek), and Shoshone Geyser Basin. Sylvan is underlain by the Gibbon River Flow, Shoshone by the Spring Creek Flow and Shoshone Lake Tuff member, and the Midway Geyser Basin by the Upper Basin Member. The far western edge of the park has columnar basalt outcrops and many older, more mafic volcanics (Figs. 3 and 4).

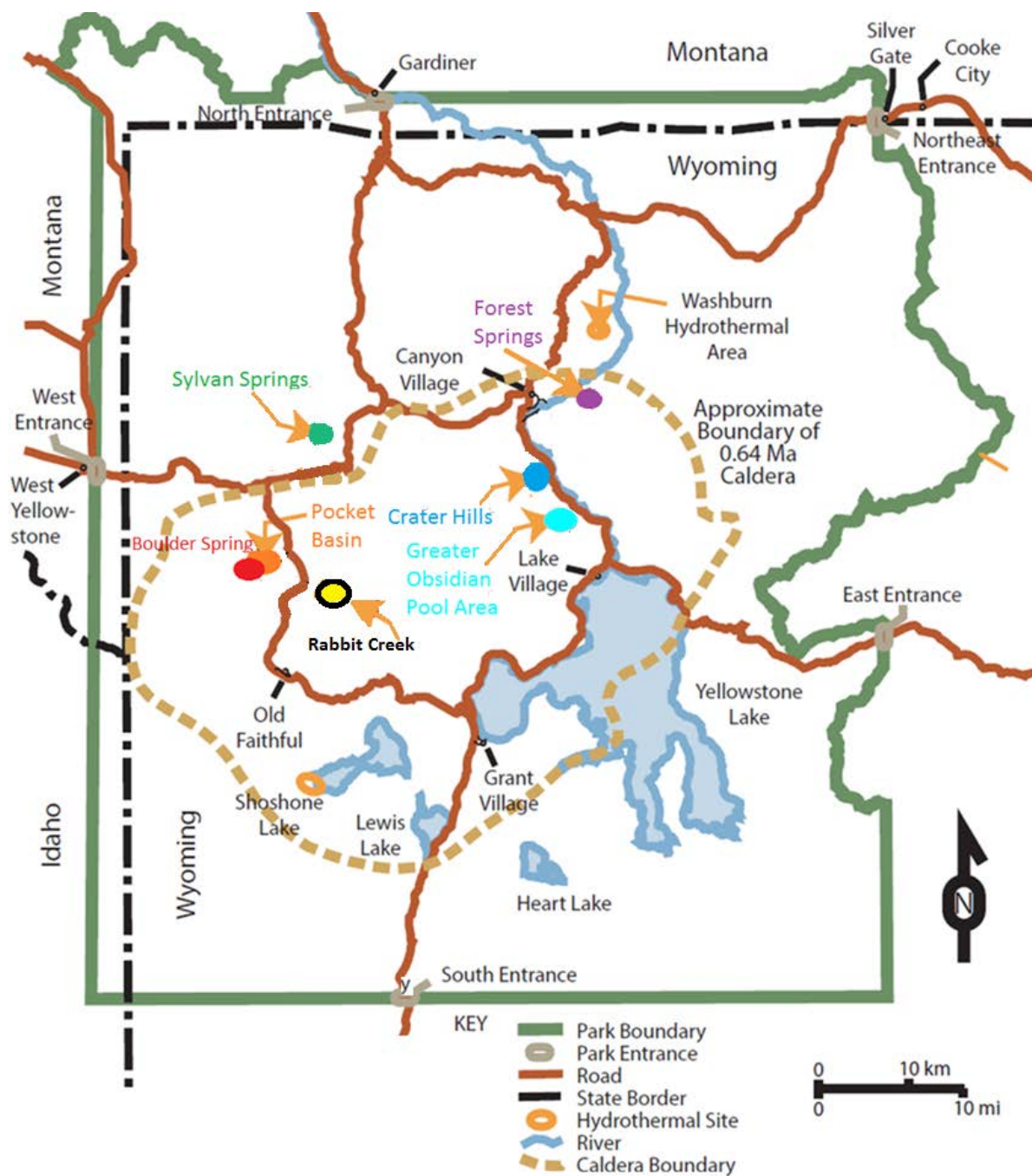


Figure 2: Yellowstone National Park Map with sampling sites. Modified from Havig *et al.*, 2011.



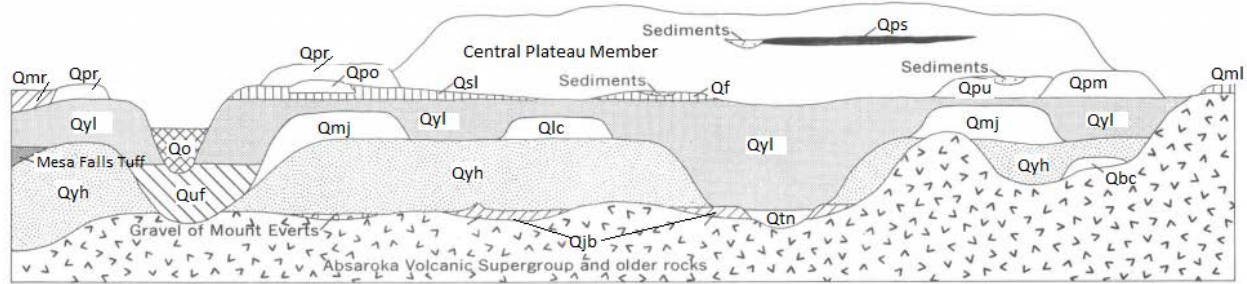
Figure 3: YNP surficial geology map showing sampling areas. Modified from Richmond, 1972.

Table I: Surficial geology for thermal areas and nearby older outcrops.

<u>Thermal Area</u>	<u>Surficial Geology*</u>	<u>Probable Next Rock Unit**</u>
Forest Springs	Qpu, Upper Basin Member (260 ka) Qs, detrital deposits (Holocene)	Qpu, Upper Basin Member (260 ka) Qpch, Hayden Valley Flow (102 ka)
Crater Hills	Qs, detrital deposits (Holocene) Qci, cemented ice contact deposits localized by hot springs (Holocene)	Qpch, Hayden Valley Flow (102 ka) Qpcf, Solfatara Plateau Flow (102.8 ka)
GOPA	Qs, detrital deposits (Holocene) Qci, cemented ice contact deposits localized by hot springs (Holocene)	Qpch, Hayden Valley Flow (102 ka) Qpce, Elephant Back Flow (150 ka) Qpcw, West Thumb Flow (144 ka)
Sylvan Springs	Qyl, Lava Creek Tuff (620 ka)	Qpo, Obsidian Creek Member (500 ka) Qpci, Gibbon River Flow (80 ka)
Pocket Basin	Qh, hot spring deposits (Holocene) Qhe, hydrothermal explosion deposits (Holocene)	Qpu, Upper Basin Member (260 ka) Qpcy, West Yellowstone Flow (114 ka) Qpce, Elephant Back Flow (150 ka)
Rabbit Creek	Qh, hot spring deposits (Holocene)	Qpu, Upper Basin Member (260 ka) Qpm, Mallard Lake Member (164 ka)
Shoshone	Qh, hot spring deposits (Holocene) Qci, cemented ice contact deposits localized by hot springs (Holocene)	Qps, Shoshone Lake Tuff Member (180 ka) Qpcc, Spring Creek Flow (160 ka)

Numbers parentheses indicate age in ka (kilo-annum) (Richmond, 1972; Christiansen and Blank, 1972; Beus, 1986; Girard, 2010). Standard abbreviations for rock units precede the rock unit name and age. *Read from USGS map (Richmond, 1972). **Inferred from nearby outcrops from USGS map (Richmond, 1972).

The general stratigraphic diagram of Yellowstone National Park shows volcanic rock units layering in a psudeo-sedimentary fashion, however the older eruptive cycles have caused subregional rock units (Fig. 4). These subregional rock units, such as the Shoshone Lake Tuff Member, outcrop in a relatively small area of the park and may be dissimilar in composition from larger and more widespread flows and tuffs, such as most flows in the Central Plateau Member Rhyolite or the Lava Creek Tuff. The localized rock units may have a dramatic effect on the chemistry of surficial thermal features.



Rock Unit	Rock Unit Abbreviation	Relative Age
Roaring Mountain Member*	Qpr	Third Volcanic Cycle
Shoshone Lake Tuff Member*	Qps	
Obsidian Creek Member*	Qpo	
Upper Basin Member*	Qpu	
Mallard Lake Member*	Qpm	
Osprey Basalt	Qo	
Madison River Basalt	Qmr	
Swan Lake Flat Basalt	Qsl	
Falls River Basalt	Qf	
Basalt of Mariposa Lake	Qml	
Lava Creek Tuff	Qyl	
Undine Falls Basalt	Quf	
Mount Jackson Rhyolite	Qmj	
Mesa Falls Tuff		Second Volcanic Cycle
Sediments and Basalts of The Narrows	Qtn	First Volcanic Cycle
Lewis Canyon Rhyolite	Qlc	
Huckleberry Ridge Tuff	Qyh	
Rhyolite of Broad Creek	Qbc	
Junction Butte Basalt	Qjb	

Figure 4: Generic stratigraphy for Quaternary rock units in Yellowstone National Park. Figure modified from Christiansen and Blank, 1972. Rock units with an asterisk are part of the Plateau Rhyolite that underlies many sampled thermal areas.

1.4. Thesis Objectives/Statement of Purpose

This thesis builds upon previous mudpot discoveries with field data to identify characteristics and trends for fifteen mudpots in Yellowstone National Park. This work begins a long-term sampling scheme to integrate mudpot investigation into a wider study of thermal features in YNP. These preliminary mudpot characterizations and the establishment of a

backcountry field filtration method provide a basis for sampling international mudpots which may be hosted in rock of intermediate or mafic compositions. The interdisciplinarity of this work allows connections to be made between surface fluid geochemistry, the underlying rock units, and the microbial communities hosted in mudpots. If this work considered any single facet of mudpots in isolation, broad conclusions such as characteristics of the Yellowstone subsurface and its thermal aquifers would be impossible. Microbial communities alter the surficial geochemistry, which is largely controlled by underlying geologic rock units. An interdisciplinary skill set and theoretical approach is crucial in extending predictive capability beyond the current modeling and traditional single discipline field methods. Potential far reaching applications of this work include but are not limited to: selection of microbes suitable for bioremediation processes, “fingerprinting” chemically unique rock units from filtered mudpot fluids, or deciphering past geochemical processes on Mars by comparing the formation and stability of iron-montmorillonites.

2. Methods

2.1. Cleaning Protocols

2.1.1. Trace Metal Cleaning Procedure

New Nalgene 30 mL high density polyethylene (HDPE) bottles were rinsed 3 times with Millipore Q 18.2 MΩ/cm ultrapure water (hereafter referred to as “MilliQ water”), soaked in citranox for 2 days, rinsed 7 times with MilliQ water, soaked in 10% HCl (v/v) (made with MilliQ water and J.T. Baker trace metal grade HCl) for 6 days, rinsed 7 times with MilliQ water, soaked in pH 2 HCl for 6 days, and rinsed 7 times with MilliQ water. The cleaned bottles were then stored in new plastic bags to avoid dust contamination. Two thirds of the cleaned 30 mL bottles were filled with MilliQ water until collection of filtered sample for major cations and anions analyses. The remaining third of cleaned 30 mL bottles were acidified with 300 µL of concentrated nitric acid (Fisher TraceMetal Grade A505-P212) and stored until collection of filtered sample for trace element analysis.

Steel support rings and paper tags were removed from the 100 µM and 50 µM polypropylene felt bag filters, then the filters were rinsed 3 times with MilliQ water, soaked in bins filled with citranox for 2 days, rinsed 12 to 14 times with MilliQ water, soaked in approximately 10% HCl (v/v) for 6 days, rinsed 12 to 14 times with MilliQ water, soaked in dilute pH 2 HCl for 6 days, and rinsed 12 to 14 times with MilliQ water. Cleaned filters were hung in the laminar flow HEPA hood overnight to dry. The clean filters were then stored in new plastic bags to avoid dust contamination.

2.1.2. Other Bottle Cleaning Procedures

2.1.2.1. δD and $\delta^{18}O$

New 10 mL and 2 mL glass vials were rinsed 3 times with MilliQ water, wrapped in aluminum foil, and dried in an oven. The new lids were rinsed 3 times with MilliQ water and soaked in pH 2 HCl until the vials were out of the oven and cool. Lids were rinsed 7 times with MilliQ water and set in a laminar flow HEPA hood to dry before capping the vials.

2.1.2.2. Dissolved Organic Carbon

New 40 mL glass vials were rinsed 3 times with MilliQ water, wrapped in aluminum foil packets, and muffled at 450°C for 4 hours. The new lids were rinsed 3 times with MilliQ water, soaked in pH 2 HCl until the vials were out of the oven and cool, approximately 24 hours, and rinsed 7 times with MilliQ water. Lids were set on muffled aluminum foil in a laminar flow HEPA hood to dry before capping the vials. Half of the muffled vials were acidified with 100 μ L of 85% phosphoric acid and stored until collection of filtered sample for dissolved organic carbon analysis. The other half of the muffled vials were capped and stored until collection of filtered sample for dissolved inorganic carbon analysis.

2.1.3. Sterilization Procedures

2.1.3.1. Field Ethanol Sterilization

Trace metal cleaned plastic spatulas, scoops, and tweezers used in the collection of sediment for later biological analysis were sterilized directly before use with 70% ethanol.

2.1.3.2. Microwave Sterilization

MilliQ water used for lab biomolecule extractions was sterilized by microwaving 300 mL of water in a 500 mL acid washed polycarbonate bottle for a total of 10 minutes. An autoclave

was not used for sterilization of ultrapure water due to potential contamination of DNA fragments.

2.2. Field Methods

2.2.1. Filtration

2.2.1.1. Water

Hot spring sample, collected in trace metal cleaned and triple sample-rinsed one liter Nalgene bottles, was syringe filtered with 1.2 μm and 0.8 μm /0.2 μm Pall Laboratory Acrodisc[®] sterile syringe filters with a hydrophilic polyethersulfone (PES) Supor[®] membrane into appropriately cleaned vials for later analysis. Vials of filtered sample for major anions and cations analysis, and dissolved inorganic and organic carbon were stored on ice. Vials for δD and $\delta^{18}\text{O}$ isotopes, and trace elements were stored in shaded areas at ambient temperatures. A blank of MilliQ water was filtered first with the same method at each field site to quantify contamination from field sampling (Fig. 6 and Tables XXXIII and XLI).

2.2.1.2. Mud

Gravity filtration via a PVC field ringstand (Fig. 5) was used to collect a prefiltrate from a mudpot sample which was further filtered for preservation and analysis. The mudpot samples were filtered using a polypropylene bag pre-filter (Duda Diesel, Size #1 POG Filter Bag) and in-line filtration using syringe tip filters (100 μm / 1.2 μm / 0.8 μm / 0.2 μm) (see above) to fill a suite of appropriately cleaned vials for later analysis of water isotopes, major anions, major cations, dissolved organic carbon, and trace elements.



Figure 5: Mud gravity filtration apparatus.

Mudpot viscosities were initially measured with a hand crank rheometer (Ofite 132-00) for viscosities up to 100 cP. Given previous experience testing a wide range of viscous solutions from 5 to 1000 cP with a Brookfield digital viscometer, visual assessment was effectively used to determine which mudpots to sample. Clay composition samples were collected in trace metal clean HDPE bottles to assess bulk density, which can be used as a relative measure of clay concentration, a relatively reliable analog to viscosity in mudpot systems.

Mudpots with low viscosities likely have particulate much less than 100 μm , but much greater than 1.2 μm , making gravity prefiltration ineffective and syringe filtration tedious and resource intensive. Mudpots with extremely high viscosities clog 100 μm bag filters quickly and

lack enough fluid per sampling aliquot to syringe filter a full suite of vials for geochemical sampling. These constraints on gravity prefiltration determined mudpot suitability for sampling.

A field blank using MilliQ water was performed for the Supor 1.2 μm / 0.8 μm / 0.2 μm filtering apparatus each sampling day. In addition, MilliQ water was run through the 100 μm or 50 μm bag filter and then the 1.2 μm / 0.8 μm / 0.2 μm filtering apparatus as a “filter blank”.

These blanks serve as an indication of contamination from field sampling (Fig. 6). Chloride, aluminum, and zinc concentrations in field and filter blanks are above the detection limit. The elevated and variable chloride concentrations in filter blanks, but well below concentrations found in samples, may indicate a need for additional rinses or a final soak in MilliQ water for the polypropylene felt bag filters to remove any dilute HCl from the washing process. Elevated aluminum and iron concentrations in field and filter blanks may result from dust input during field sampling. Elevated zinc concentrations are likely due to contact of blank water with the black rubber in the sampling syringe. (Tables XXXIII and XLI).

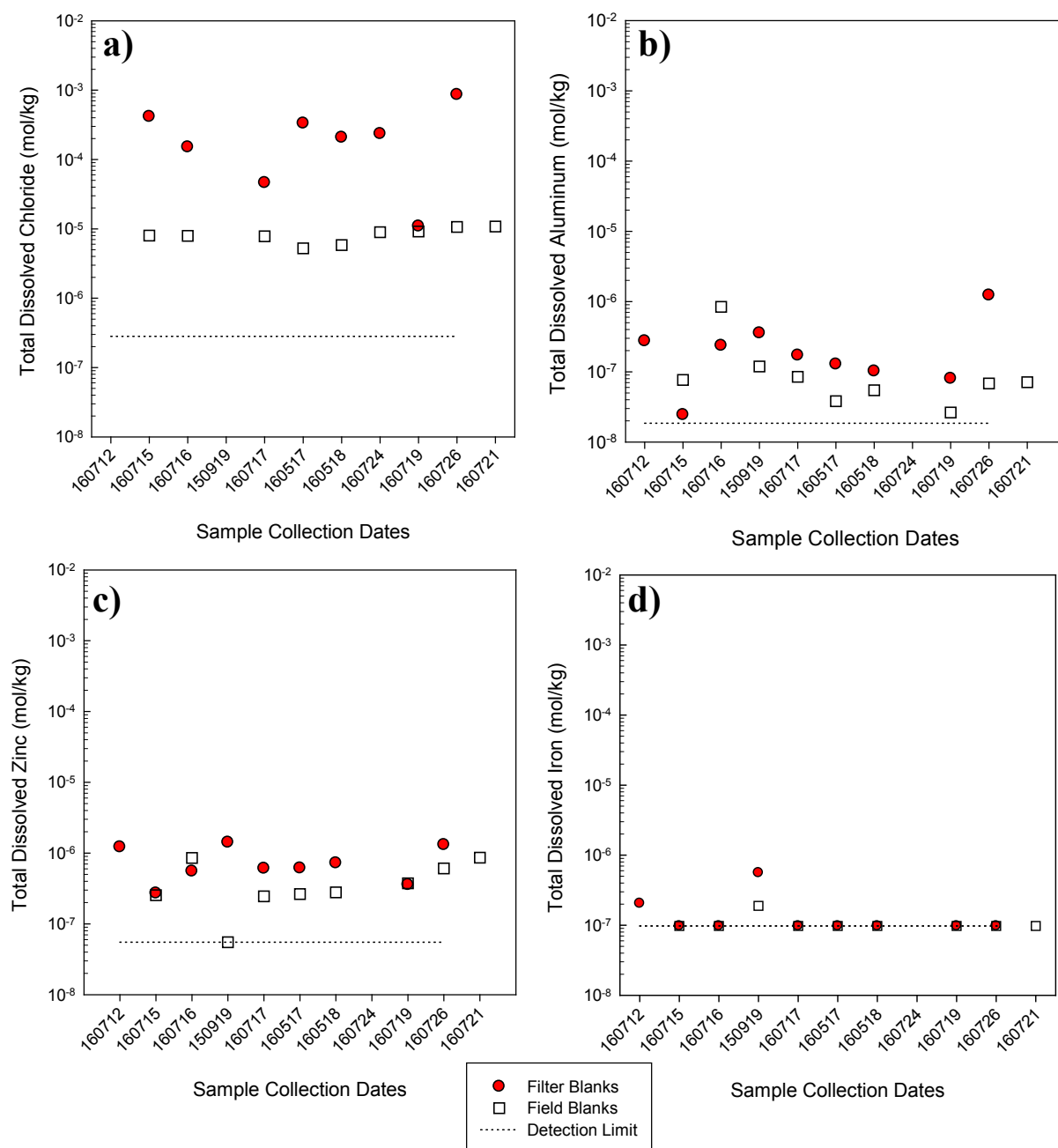


Figure 6: Chloride (a), aluminum (b), zinc (c), and iron (d) concentrations for blanks. Error bars, representing instrument error, were omitted because they were smaller than the symbols (Tables XXXIII and XLI). Sample collection dates are in the format of YYMMDD.

2.2.1. *In situ* Meters

Conductivity, dissolved oxygen, pH, and temperature measurements were made at each sampling site using hand-held meters. Conductivity values (temperature corrected and raw) and corresponding temperatures were collected using a YSI 30 conductivity meter calibrated with a ~3000 $\mu\text{S}/\text{cm}$ KCl calibration solution. Dissolved oxygen concentrations and corresponding temperatures were collected using a factory precalibrated Presens Fibox 4 meter and a fiber optic oxygen dipping probe. The detection limit for the oxygen probe is 15 ppb dissolved oxygen (0.47 μM). Temperatures and corresponding pH values were collected using a WTW 3110 ProfiLine pH meter with a Sentix 41-3 probe. For each day of sampling, the pH meter and probe were calibrated with three pH buffers that reflected the expected range of environmental conditions. For predominately acid sulfate thermal areas, the pH meter and probes were calibrated with pH 2, 4, and 7 buffers. For thermal areas with alkaline chloride hot springs as well as mudpots, the pH meter and probes were calibrated with pH 2, 7, and 10 buffers.

2.2.2. Spectrophotometry

Dissolved silica, ferrous iron, and sulfide concentrations were analyzed via field spectrophotometry (Hach DR/2010 spectrophotometer) for hot spring water samples. Methods were summarized from Schmidt, 2017. Dissolved silica was analyzed using the Silicomolybdate EPA Method 8185 with testing limits between 17 – 1664 μM . Samples were read by absorbance at 452 nm and converted to concentration. Water was filtered to 0.2 μm for both the blank and sample. Limitations to this test included interferences from high concentrations of iron and sulfide (Hach, 2014). Table II provides the reagents and methods used in the silica test.

Table II: Methods and reagents for dissolved silica.

Sample type	Matrix	Reagents used	Method
Blank	0.2 μ m filtered sample water	None	Added 10 mL filtered sample water to cuvette and zeroed instrument prior to reading sample.
Sample	0.2 μ m filtered sample water	HACH Molybdate Reagent Powder Pillows for HR Silica, 10 mL (sodium molybdate) HACH Acid Reagent Powder Pillows for HR Silica, 10 mL (sulfamic acid, sodium chloride) HACH Citric Acid Reagent Powder Pillows for Silica, 10 mL (citric acid)	Added molybdate reagent to 10 mL cuvette. Immediately added acid reagent. Developed reagents for 10 min. Added citric acid reagent and developed for 2 min.

Ferrous iron was analyzed using the 1,10-phenanthroline method (Tamura *et al.*, 1974; St. Clair, 2017). Samples were read by absorbance at 510 nm and converted to concentration. Testing limits were between 0.3 – 35 μ M Fe(II). Collected water was filtered to 0.2 μ m for the sample. The methods and reagents were added following the instructions in Table III.

Table III: Methods and reagents for ferrous iron.

Sample type	Matrix	Reagents used	Method
Blank	0.2 μ m filtered sample water	None	Added 10 mL filtered sample water to cuvette and zeroed instrument prior to reading sample.
Sample	0.2 μ m filtered sample water	100 μ L of 0.178M 1,10-phenanthroline solution	Added 10 mL of sample to a 10 mL cuvette. Added 100 μ L of 1,10-phenanthroline. Developed 1 min.

Dissolved sulfide was analyzed using the Methylene Blue EPA Method 8131 with testing limits between 0.2 – 9.4 μ M sulfide. Samples were read by absorbance at 665 nm and converted to concentration. Water was unfiltered to prevent increased volatilization. Limitations to this test include interferences from turbidity, volatilization, and rapid oxidation of sulfide to sulfate in more alkaline samples (Cline, 1969). Table IV provides the reagents and methods used in the sulfide test.

Table IV: Methods and reagents for dissolved sulfide.

Sample type	Matrix	Reagents used	Method
Blank	Q water	HACH Sulfide 1 Reagent (sulfuric acid) HACH Sulfide 2 Reagent (methylene blue)	Added 25 mL Q water to cuvette. Added 1 mL of sulfide reagent 1. Added 1 mL of sulfide reagent 2. Developed 5 min before zeroing instrument.
Sample	Unfiltered sample water	HACH Sulfide 1 Reagent (sulfuric acid) HACH Sulfide 2 Reagent (methylene blue)	Added 25 mL unfiltered sample water to cuvette. Added 1 mL of sulfide reagent 1. Added 1 mL of sulfide reagent 2. Developed 5 min.

2.2.3. Biological Matter Collection

2.2.3.1. Suspended Sediment

In a sterile Dynarex specimen container, sediment was collected from mudpots and hot springs. For each sample, mud was homogenized and transferred with field sterilized materials from the specimen container to labeled sterile 2 mL centrifuge tubes and immediately frozen on dry ice for later DNA extraction. Collected samples were stored at -80°C at Montana Tech until DNA extraction.

2.3. Lab Methods

2.3.1. Biological Analysis

2.3.1.1. DNA Extraction

A modified DNA extraction procedure after Huber, 2002 and Brazelton, 2010; was used because the mudpot samples did not extract well using the FastPrep kit (MP Biomedical). DNA extraction buffer was prepared by combining equal volumes of 0.1 M Tris(hydroxymethyl)aminomethane hydrochloride (Tris HCl) (Sigma T1503-500G), 0.1 M Na-EDTA (Mallinckrodt Chemicals 4931-02), 0.1 M NaH₂PO₄ (Acros Organics 206512500), 1.5 M NaCl (OmniPur 7710), and 1% cetyltrimethylammonium bromide (CTAB) (Amresco 0833-

500G), all adjusted to pH 8. Each salt solution was made with microwave sterilized MilliQ water. Two mL centrifuge tubes of collected suspended sediment from field mudpot samples were thawed and redistributed into sterile 2 mL centrifuge tubes, with masses of sediment not exceeding 0.7 grams. For each 0.5 grams of sediment in a sterile tube, 500 μ L of DNA extraction buffer was added. After adding extraction buffer and inverting several times by hand, samples underwent three freeze-thaw cycles of 12 minutes at -80°C and 65°C , respectively. After the third freeze-thaw cycle, 36 μ L of 50 mg/mL lysozyme was added for every 2mL of approximate tube volume. Tubes were incubated for 30 minutes at 37°C and 200 rpm in a digital shaking dry bath (Thermo Scientific 88880027). After the incubation, 45 μ L of 20 mg/mL proteinase K for every 2 mL of approximate tube volume and 90 μ L of 20 wt.% SDS for every 2 mL of approximate tube volume were added and the tubes incubated for 60 minutes at 65°C and 200 rpm. After the incubation, tubes were inverted for 10 minutes, then centrifuged at $4000 \times g$ for 1 minute to pellet the sediment. The fluid was then transferred to sterile 2 mL centrifuge tubes and equal volumes of phenol/chloroform/isoamyl alcohol (VWR Amresco 0883-400) were added. Tubes were centrifuged at $14000 \times g$ for 1 minute and then the aqueous phase was pipetted to sterile 2 mL centrifuge tubes. The new tubes were centrifuged at $14000 \times g$ for 1 minute and then equal volumes of chloroform/isoamyl alcohol was added. The tubes were centrifuged at $14000 \times g$ for 1 minute. After centrifuging the tubes, the aqueous phase was pipetted into sterile 2 mL centrifuge tubes, with no aliquot exceeding 550 μ L. To the new tubes, twice the pipetted volume of isopropyl alcohol was added and one-tenth the pipetted volume of 3M sodium acetate (OmniPur 7610) as added. The tubes were stored at -4°C for 24 to 168 hours to precipitate.

After freezer precipitation, the tubes for each sample site were combined in 15 mL Falcon tubes and for every 4 mL of solution, 1 mL of 5M NaCl (OmniPur 7710) was added. To

each tube, 1 mL of shaken binding matrix (MP Bio 6540-408) was added and tubes were inverted by hand for 5 minutes. After the 5 minute inversion, the tubes were centrifuged at 3260 x g for 1 minute. Most of the supernatant was then decanted from the tubes, leaving a pellet of solids and roughly 500 μ L of supernatant in the Falcon tube. The solids were resuspended in the remaining supernatant and transferred to spin filter tubes (MP Bio 6560-210). The tubes were centrifuged at 14000 x g for 1 minute. After centrifuging, the flow through was discarded and 500 μ L of SEWS (MP Bio 6540-405) was added to the solids in the filter. Tubes were agitated to resuspend solids and centrifuged at 14000 x g for 1 minute. After centrifuging, the flow through was again discarded, the tubes were centrifuged at 14000 x g for 2 minutes. After discarding the flow through liquids, the tubes were left uncapped in the HEPA hood (Enviroco Corp, filter model 69600S-00HPLXX) and allowed to air dry for 5 minutes. To the solids, 100 μ L of sterile water was added and the tubes were incubated for 5 minutes at 55°C in the digital shaking dry bath (Thermo Scientific, 88880027). After the incubation, the tubes were centrifuged at 14000 x g for 1 minute. The filters were discarded and the tubes closed. The elute in the tubes contained the DNA extract. Nanodrop was used to determine approximate concentration and relative purity (A260/A280) of extract of 1 μ L aliquots of DNA extract (Table V).

Table V: DNA concentration and A260/280.

Sample code	Sample Name	Extraction Date	Nanodrop (ng/ μ L)	Nanodrop A260/280*	Yield (ng DNA/g wet sediment)	Approx Total DNA (ng)
160724K	Phantom Pants	160918	67.9	1.56	3889	6790
160720	Pedro's Tarpit	160918	91.0	1.50	3772	9100
160715T	Urinal Cake	161016	136.2	1.39	10234	13620
160715V	Consolation Prize	161023	125.6	1.48	7086	12560
160715X	Vitamin C source	161030	177.5	1.39	13908	17750

*The A260/280 ratio represents the ratio of sample measured at 260 nm and 280 nm to assess purity of sample. Pure DNA is expected to have a theoretical A260/280 ratio of ≥ 1.8 . The presence of proteins lowers this ratio (Held, 2001).

2.3.1.2. Polymerase Chain Reaction (PCR)

Water was sterilized by microwaving approximately 300 mL of MilliQ water dispensed directly into a 500 mL polycarbonate bottle for 10 total minutes, in 3.3 minutes intervals with pauses of 1.5 minutes. An autoclave was not used for sterilization of ultrapure water due to potential contamination of DNA fragments. Master mix was prepared by combining 80 μ L of 50mM MgCl₂ (Invitrogen), 80 μ L of 5000nM BSA (Roche 10735078001), 400 μ L of 10x PCR buffer (Invitrogen), 24 μ L of Taq polymerase (Invitrogen 10342-020), 4 μ L of each dNTP (Promega U1220), and 1400 μ L of microwave sterilized water. In 200 μ L certified DNase/RNase free tubes, 12.5 μ L master mix, 11.7 μ L microwave sterilized water, 0.4 μ L forward primer (IDT), 0.4 μ L reverse primer (IDT), and 100 ng of template DNA were added. Bacterial, archaeal, eukaryotic, and universal primers were used for each sample (Table VI). PCR conditions were set after Boyd, et. al. 2010.

2.3.1.3. Gel Electrophoresis

A 1% agarose gel was prepared by microwaving 1.20 grams of agarose (OmniPur) and 120 mL 1X TBE buffer in a 250 mL Erlenmeyer flask for 30 second intervals (total time of 2

minutes) until clear and boiling. Once the agarose solution was cool enough to handle, 1.2 μ L of 10 mg/mL ethidium bromide (OmniPur) was added. The agarose solution was poured into a gel box with combs for sample wells and left to set for 30 minutes. After the gel was set, it was repositioned, the combs were removed and the gel box was filled with 1X TBE buffer. In each well, 1 μ L blue/orange 6X loading dye (Promega) and 5 μ L PCR product were added. In the first and last well of each line of wells, 1 μ L of loading dye and 5 μ L of 1kb DNA ladder (Promega) were added. Gel electrophoresis was run at 100 V for 1 hour. Gels were imaged with UV. DNA extracts with corresponding bands for Bacteria or Archaea in gel electrophoresis were sent for PCR amplification and sequencing to Molecular Research (Mr. DNA, Shallowater, TX) (see below).

2.3.1.4. 16S and 18S rRNA Gene Diversity Analysis

The 16S ribosomal RNA (rRNA) is part of the 30S subunit of bacterial and archaeal ribosomes and the 16S rRNA gene is the gene that codes for the part of the subunit in the ribosome. The 16S rRNA gene exhibits a slow evolution rate, therefore it has been used to reconstruct phylogenies and identify microorganisms (Woese and Fox, 1977). Similarly, the 18S rRNA is part of the 40S subunit of eukaryotic ribosomes and the 18S rRNA gene codes for the part of the subunit in eukaryotic ribosomes. Due to the importance of the 16S and 18S rRNA subunits to the function of the ribosomes, they are conserved and used to track evolutionary changes.

The gene sequences are cataloged in a database, such as SILVA (from the Latin *silva* for forest). SILVA is an rRNA database established by the Max Planck Institute for Marine Microbiology. The database provides internet based, quality controlled aligned rRNA sequences from Bacteria, Archaea, and Eukarya (Pruesse, *et. al.*, 2007). The sequence information from

16S and 18S rRNA genes, extracted from environmental samples, can be used to identify sequence matches with known microorganisms in the SILVA database. If the 16S rRNA gene sequence is less than 97% similar to the nearest phylogenetic neighbor, a new species is identified. Conversely, sequences that are greater than 97% similar to the nearest phylogenetic neighbor are clustered within a previously defined taxon (Janda and Abbott, 2007).

DNA extracts were sent to Molecular Research (Mr. DNA) in Shallowater, TX for PCR amplification and 16S and 18S rRNA sequencing. PCR products, from DNA extracts with an A260/280 ratio less than 1.80 were cleaned using the PowerClean Pro DNA Clean-Up kit. The 16S and 18S rRNA gene primers, Eukarya A7F and 570R, Bacteria 1100F and 1492R, Archaea 344F and 915R, and universal 515F and 806R, after Boyd, 2010 were used (Table VI). Reagents used for PCR were from the HotStarTaq Plus Master Mix Kit. Step-down PCR cycles were conducted under the following conditions: an initial denaturation at 94°C for 4 minutes, followed by 35 cycles of denaturation at 94°C for 1 minute, annealing at 42, 51, 53, 55, and 61 °C for 1 minute and primer extension at 72°C for 1.5 minutes, and an elongation step of 72°C for 20 minutes. The results of the PCR amplification were verified with a 2% agarose gel. Samples were separated by molecular weight and DNA concentrations and then purified using Ampure XP beads. These data were used to prepare the Illumina DNA library. Sequencing was performed on a MiSeq. The data were processed using the Mr. DNA analysis pipeline. The pipeline joined sequences, removed barcodes and sequences <150bp, and deleted ambiguous base calls. The sequences were cleaned for background noise, operational taxonomic units (OTUs) were clustered at 3% divergence (97% similarity), and chimeras were removed. OTUs were taxonomically classified using nucleotide Basic Local Alignment Search Tool (BLASTn) against a curated database from the Ribosomal Database Project (RDP-II) and National Center for

Biotechnology Information (NCBI) (Dowd, 2017; Schmidt, 2017). Results were provided in the form of relative abundance and sequence count.

The primers used (Table VI) had sequences with incompletely specified nucleobases. Letter codes for bases other than guanine, adenine, thymine, and cytosine are used in designing PCR primers. Some designations, such as R and Y, are generalizations that code for a purine (adenine or guanine) and a pyrimidine (thymine and cytosine), respectively. Other codes, such as W, S, M, K, H, B, V, D, and N, are used to describe a broad characteristic needed in the sequence, like M for an amino group (adenine or cytosine) or K for a keto group (guanine or thymine). The universal reverse primer, Universal 806 R, uses the codes, W and H, which denote weak interactions of 2 hydrogen bonds (adenine or thymine) and a non-guanine base (adenine or cytosine or thymine, respectively (Table IV) (IUPAC-IUB, 1992).

Table VI: 16S and 18S rRNA gene primer sequences and melting temperatures.

Primer code	Sequence	Melting Temp (°C)
Euk – A7F	5' AAC CTG GTT GAT CCT GCC AGT 3'	58.9
Euk – 570R	5' GCT ATT GGA GCT GGA ATT AC 3'	50.8
Bacteria – 1100F	5' YAA CGA GCG CAA CCC 3'	53.5
Bacteria – 1492R	5' GGT TAC CTT ACG ACT T 3'	49.4
Archaea – 344F	5' ACG GGG YGC AGC AGG CGC GA 3'	71.2
Archaea – 915R	5' GTG CTC CCC CGC CAA TTC CT 3'	62.9
Universal – 515F	5' GTG CCA GCM GCC GCG GTA A 3'	65.2
Universal – 806 R	5' GGA CTA CHV GGG TWT CTA AT 3'	50.7

Primers were purchased from Integrated DNA Technologies. Bolded portions of sequences highlight incompletely specified nucleobases.

2.3.2. Chemical Analysis

Analytical Laboratory using EPA Method 200.7. The ICP-OES was calibrated with every sample run and quality control samples were analyzed according to EPA Method 200.7. Instrumental errors were determined to be 10% for all concentrations $\geq 144 \mu\text{mol/kg}$ lithium, $\geq 43 \mu\text{mol/kg}$ sodium, $\geq 26 \mu\text{mol/kg}$ potassium, $\geq 41 \mu\text{mol/kg}$ magnesium, and $\geq 14 \mu\text{mol/kg}$ calcium. For lithium and sodium concentrations $< 144 \mu\text{mol/kg}$ and $< 43 \mu\text{mol/kg}$, respectively, the instrumental errors were $29 \mu\text{mol/kg}$ and $9 \mu\text{mol/kg}$, respectively. For potassium, magnesium, and calcium concentrations, $< 26 \mu\text{mol/kg}$, $< 41 \mu\text{mol/kg}$, and $< 14 \mu\text{mol/kg}$, respectively, the instrumental errors were $5 \mu\text{mol/kg}$, $8 \mu\text{mol/kg}$ and $3 \mu\text{mol/kg}$, respectively.

2.3.2.3. Ion Chromatography

Samples, $0.2 \mu\text{m}$ filtered and stored in trace metal cleaned 30 mL HDPE bottles at -4°C , were analyzed with a Metrohm Compact IC Plus at the MBMG Analytical Laboratory using EPA Method 300.0 for total dissolved anion concentrations (Fig. 7). The ion chromatograph was calibrated with every sample run and quality control samples were analyzed according to EPA Method 300.0. Instrumental errors were determined to be 11.9% for fluoride concentrations, 0.92% for chloride concentration, 3.4% for nitrite concentrations, 1.1% for bromide concentrations, 0.87% for nitrate concentrations, 10.8% for phosphate concentrations, and 1.9% for sulfate concentrations.

2.3.2.4. Carbon Analysis (Dissolved Organic and Inorganic Carbon)

Samples, $0.2 \mu\text{m}$ filtered and stored in muffled 60 mL glass vials with no headspace at 4°C , were analyzed with a Picarro Carbon Analyzer Model 1030 in the MBMG Analytical Laboratory by Renee Schmidt for dissolved organic and inorganic carbon and $\delta^{13}\text{C}$ vs PDB. The Carbon Analyzer was calibrated before each run. Due to issues with long sample runs and

limited gaseous reagents, mudpot samples were unable to be analyzed therefore results have been omitted from this work.

2.3.2.5. Inductively Coupled Plasma – Mass Spectrometry (ICP-MS)

Samples, 0.2 μm filtered and stored in trace metal cleaned 30 mL HDPE bottles and acidified with 300 μL concentrated nitric acid (Fisher TraceMetal Grade A505-P212) at room temperature, were analyzed with a Thermo Scientific iCAP Q inductively coupled plasma/mass spectrometer (ICP/MS) at the MBMG Analytical Laboratory using EPA method 200.8 for total dissolved trace element concentrations (Fig. 7). The ICP-MS was calibrated before each run and quality control samples were analyzed according to the EPA method. Errors represent instrumental error and are summarized in Appendix B.

2.3.3. Solid Preparation and Analysis

2.3.3.1. Desiccation

Field samples of mudpot solids were collected in pre-weighed trace metal clean certified polyethylene bottles and sealed with electrical tape. These samples were stored at room temperature until desiccation. An aliquot was poured into a pre-weighed trace metal clean petri dish (Pyrex 100 x 20 mm). After weighing, the samples were dried in a 30°C oven for 48 hours to dry but not cause low temperature metamorphism. The dried samples were weighed and transferred from the petri dishes to resealable plastic storage bags.

2.3.3.2. TerraSpec Halo Short Wave Infrared Portable Spectrometer

Dried sample chips were analyzed using the TerraSpec Halo short wave infrared (SWIR) portable spectrometer. The TerraSpec Halo SWIR portable spectrometer has a mineral spectra

library that matches spectra from contact measurements of mudpot solids with clay minerals. The software provided multi-mineral predictions, with associated spectral scalar values (Table VII).

2.3.3.3. Scanning Electron Microscopy

The LEO 1430VP Scanning Electron Microscope (SEM) located at Montana Tech of the University of Montana's Center for Advanced Mineral and Metallurgical Processing (CAMP) was used in addition to the TerraSpec Halo SWIR to identify non-clay constituents of mudpot solids using Energy Dispersive X-ray (EDX) analysis. Dried mudpot solids were attached to pressure sensitive tape and coated with graphite. The samples were then visualized under the scanning electron microscope and particles of interest underwent elemental wt.% analysis via EDX.

3. Results

3.1. Geochemical

3.1.1. Background Context for Geochemical Observations

Mudpots in Yellowstone National Park exist in a relatively wide swath of geochemical space. *In situ* meter readings recorded a temperature range of 64.8 to 92.5°C, a pH range of 1.41 to 6.08, a measured conductivity range from 262 to 16380 $\mu\text{S}/\text{cm}$, and a dissolved oxygen range from below the detection limit of 0.47 to 22.5 μM (Figs. 8, 9, 10, 11). All data, unless specifically cited otherwise, are derived from analysis of samples collected by LEGEND in 2016. Given the gravity filtration method for obtaining filtered pore waters, mudpots amenable to sample collection had approximate viscosities (near *in situ* temperatures) ranging from 5 to 100 centipoise ($\text{mPa}\cdot\text{s}$). Mud from turbid features with viscosities incompatible with the current sampling setup was collected for solid composition analysis only.

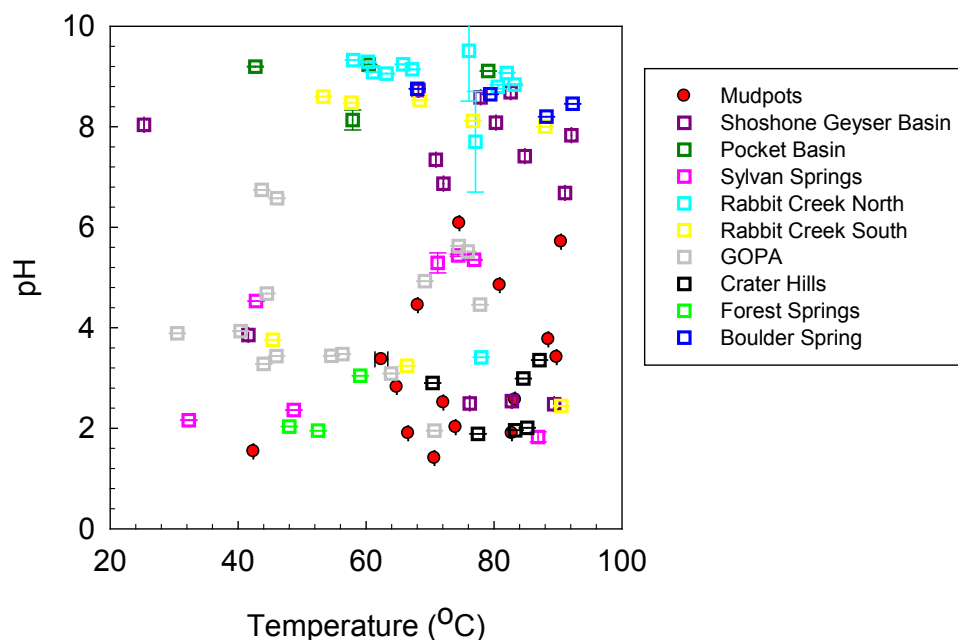


Figure 8: pH vs temperature. Mudpots (red circles) and hot springs (open colored squares) sorted by thermal area. Errors from field fluctuations are typically $\pm 0.1^\circ\text{C}$ and ± 0.003 pH units, which are smaller than the symbols.

In situ and temperature corrected conductivity values were measured at each hot spring and mudpot, however with further consideration, conductivity values may not reflect reality for mudpots. There were measured discrepancies between the conductivity of 1 mM NaCl solution and the conductivities of Na-montmorillonite suspended in 1 mM NaCl (Shainberg and Levy, 1975) (Fig. 9). The more clay suspended in 1 mM NaCl (expressed as grams of clay/100 mL of 1 mM NaCl solution at 25°C) resulted in greater measured differences between the conductivity of the 1 mM NaCl solution and the clay suspension. Additionally, charge balance for mudpots have been difficult to accurately calculate, possibly due to the cation and anion exchange properties of clays. Further work is needed to contextualize field conductivity measurements for mudpots.

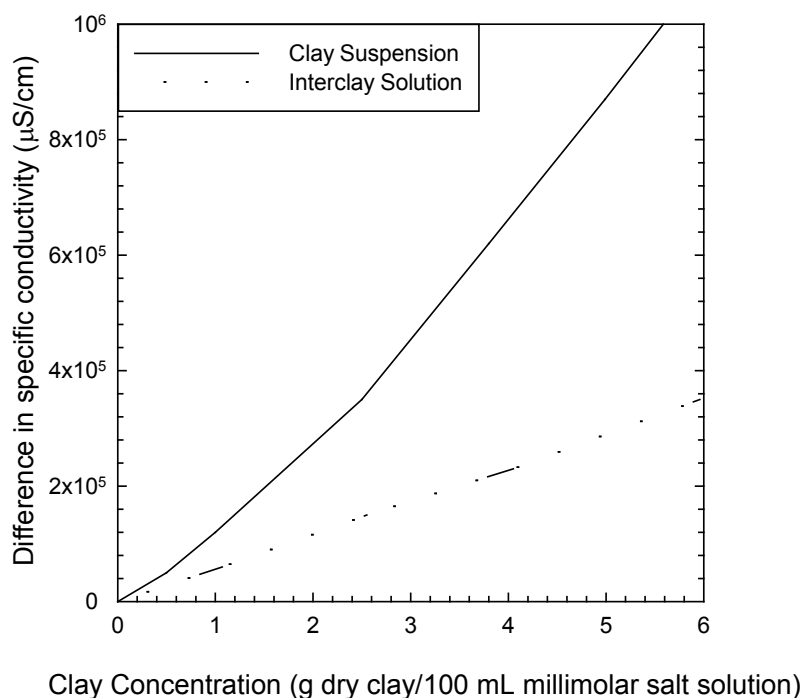


Figure 9: Difference in specific conductivity of clay suspensions vs clay concentration. Modified from Shainberg and Levy, 1975. The experiment conducted by Shainberg and Levy made comparisons of a reference 1 mM NaCl solution to Na-Monmorillonite suspensions and interclay solutions.

Conductivities ranged from 250 to 16380 $\mu\text{S}/\text{cm}$ (Fig. 10). The majority of sampled mudpots had measured non-temperature corrected conductivities between 530 and 2500 $\mu\text{S}/\text{cm}$ and temperatures between 60 and 90°C. Both outliers with conductivities greater than 10,000 $\mu\text{S}/\text{cm}$ are sampled mudpots from Forest Springs thermal area.

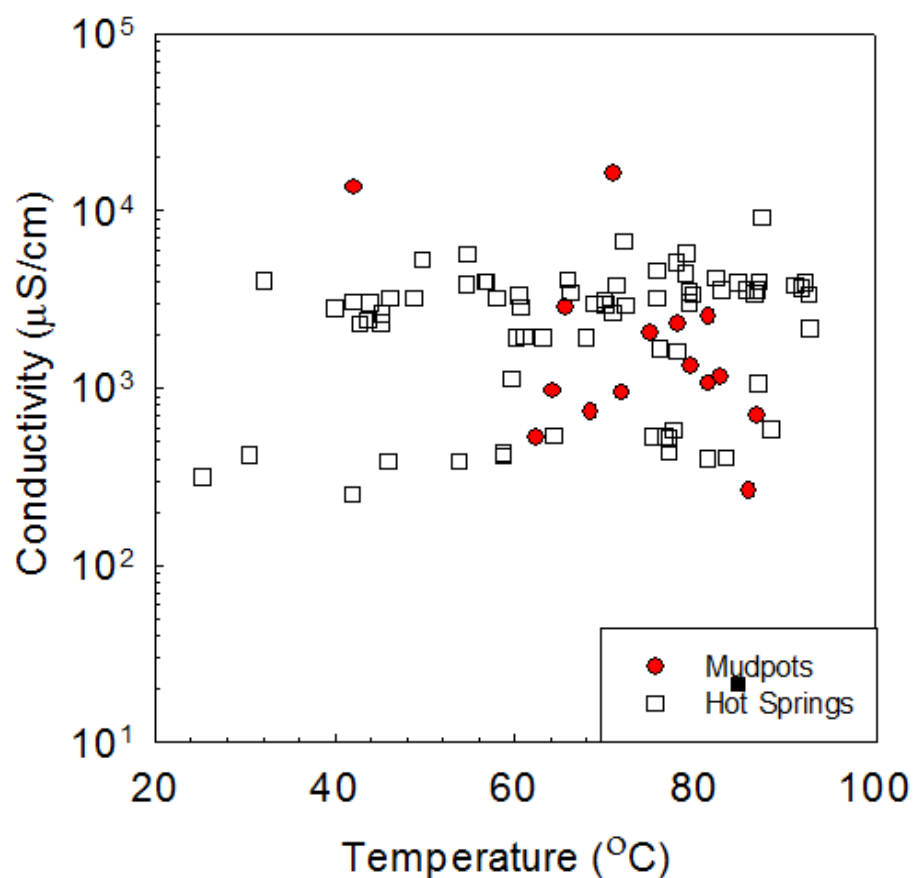


Figure 10: Conductivity vs temperature. Errors from field fluctuations are typically $\pm 0.1^{\circ}\text{C}$ and $\pm 5 \mu\text{S}/\text{cm}$, which are smaller than the symbols.

The majority of sampled hot springs and mudpots were undersaturated with respect to dissolved oxygen (Fig. 11). The sampled mudpots are divided into oxic and anoxic. Roughly half of the sampled mudpots have dissolved oxygen concentrations greater than 8.4 μM , the

other half of sampled mudpots have dissolved oxygen concentrations below $2.5 \mu\text{M}$. A working definition of suboxic is between 2 and $10 \mu\text{M}$ (del Giorgio, 2005).

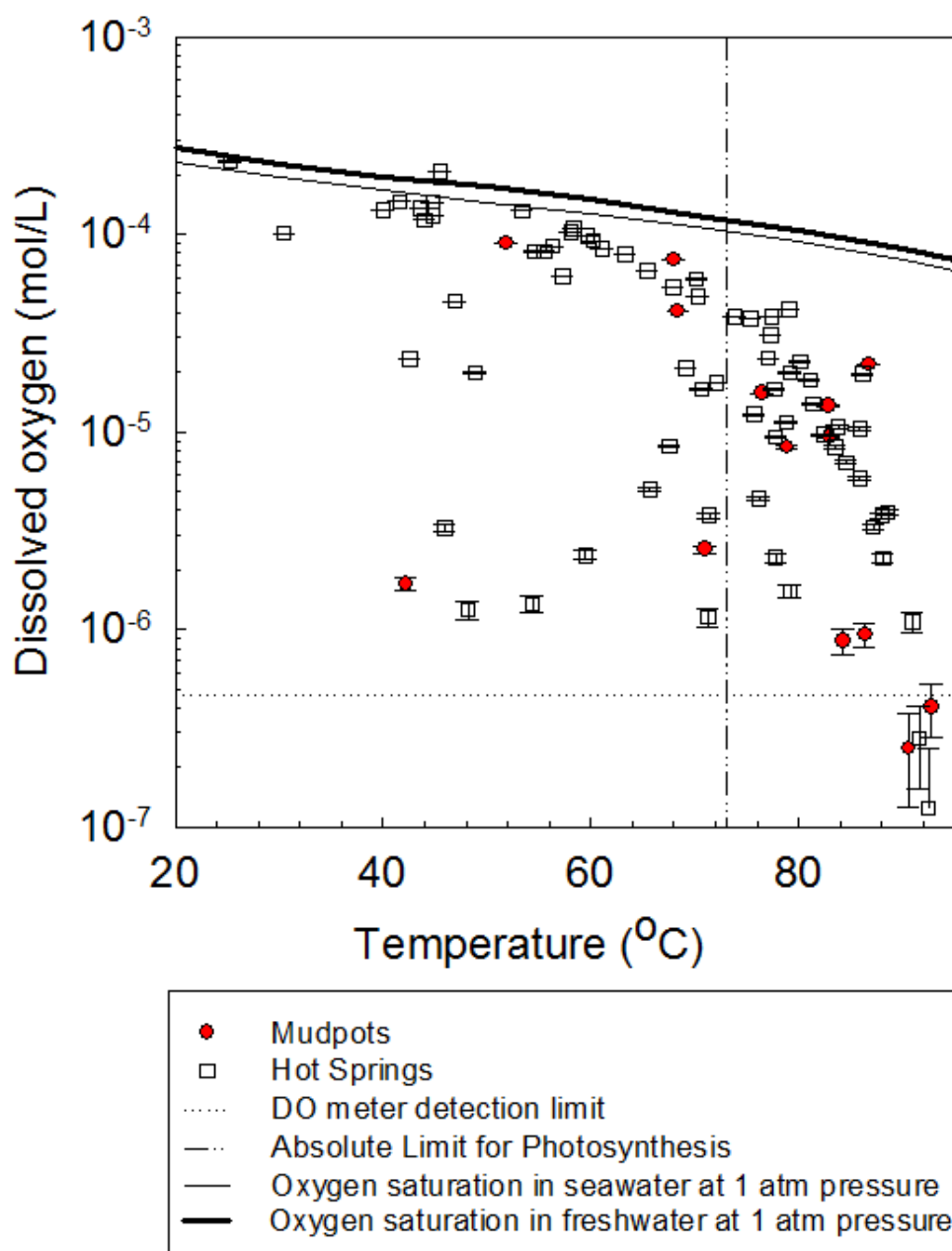


Figure 11: Dissolved oxygen concentration vs temperature. Oxygen solubility shown with both field data and theoretical calculations (Weiss, 1970; Gundersen and Ramsing, 2003). Photosynthesis absolute limit after Brock 1967a and Cox *et al.*, 2011. Errors from field fluctuations are typically $\pm 0.1^{\circ}\text{C}$ and $\pm 0.13 \mu\text{M}$ and temperature errors are smaller than the symbols.

3.1.2. Water Isotopes

Water isotope values for acidic features, including mudpots, are more enriched in δD relative to hydrogen and $\delta^{18}O$ relative to $\delta^{16}O$, than more alkaline hot springs, with some overlap (Fig. 12). Most sampled hot springs and mudpots are from more evaporated waters than meteoric water. The sampled mudpot outlier is from the Forest Springs thermal area.

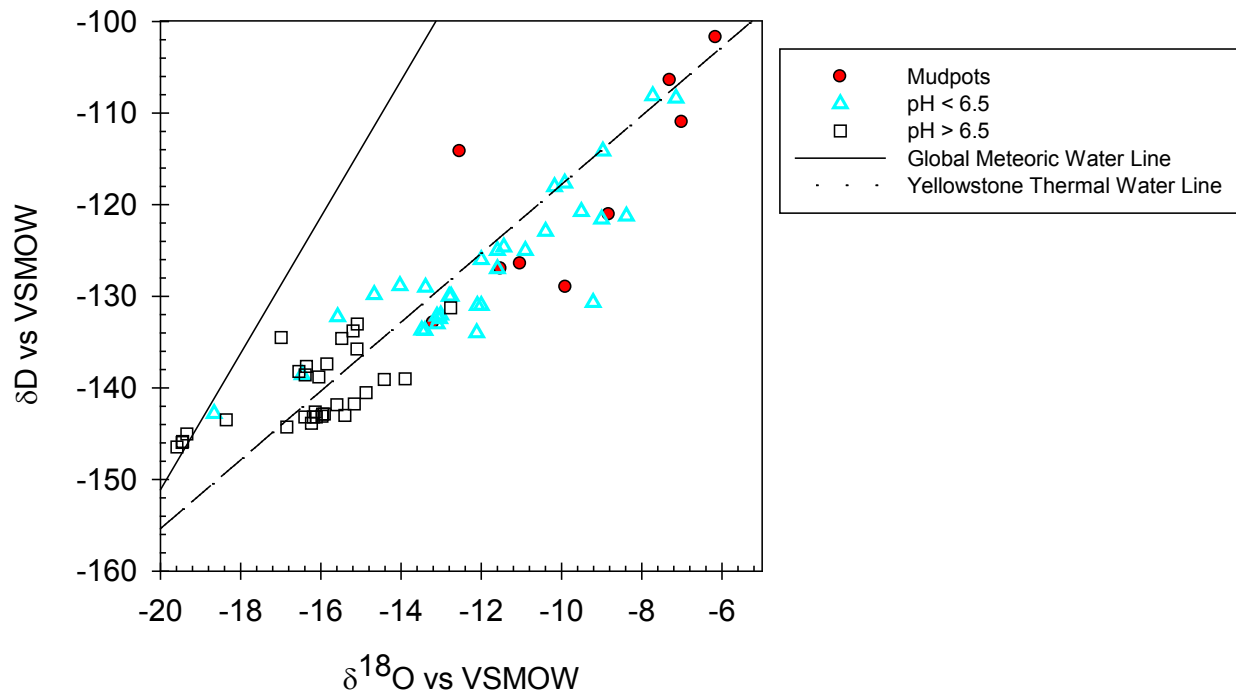


Figure 12: δD vs $\delta^{18}O$. This plot represents mudpots (red circles) and thermal springs (open squares). GMWL is $\delta D = 8.0 * \delta^{18}O + 10$ (Craig, 1961), YTWL = $\delta D = 3.7 * \delta^{18}O - 80.2$ (this work). Instrumental errors are $\delta^{18}O = 0.1$ ‰ and $\delta D = 1$ ‰, which are smaller than the symbols on the plot.

The deuterium/hydrogen and oxygen-18/oxygen-16 values are reported as δ in the units of parts per thousand or per mille (‰) relative to Vienna Standard Mean Ocean Water (VSMOW). Smaller values are lower, lighter, or depleted with respect to another isotope. Different processes change water isotope values. For instance, the relative elevation at which precipitation falls can affect the isotopic composition of the water (Fig. 13) (Clark and Fritz, 1997).

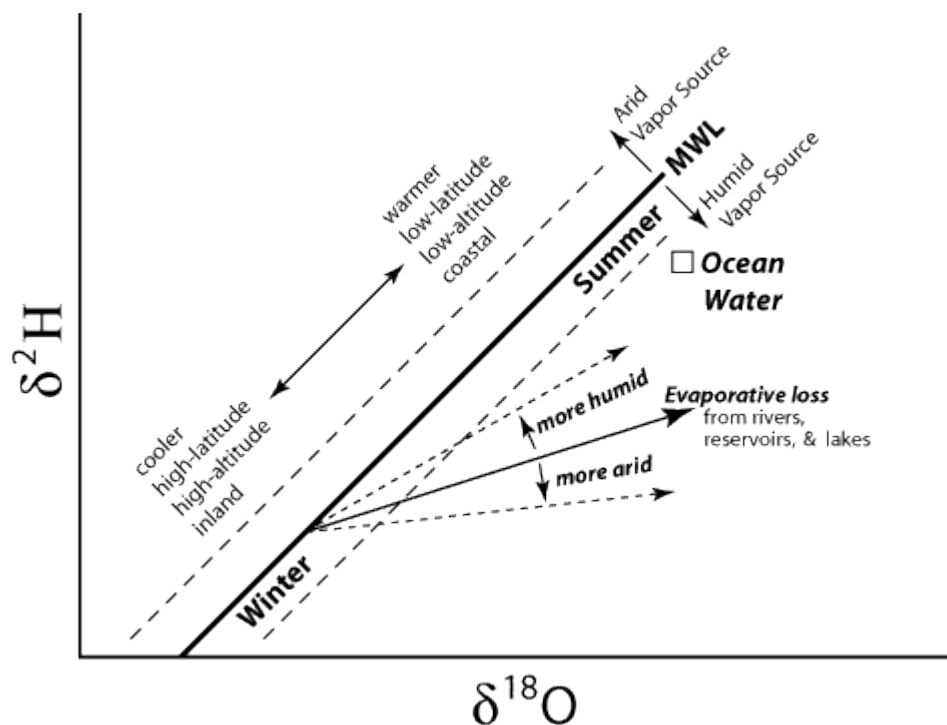


Figure 13: Changes in water isotope values based on environmental conditions (Clark and Fritz, 1997).

A water isotope trend can be considered in the context of steam-water fractionation activities at depth. Hot springs in YNP can be characterized by how the hydrothermal fluids fractionate at depth; single stage steam separation, continuous stage steam separation, or a mix of the two (Truesdell, 1977). The samples are grouped into regions by deep hydrothermal fluid behavior (Fig. 14).

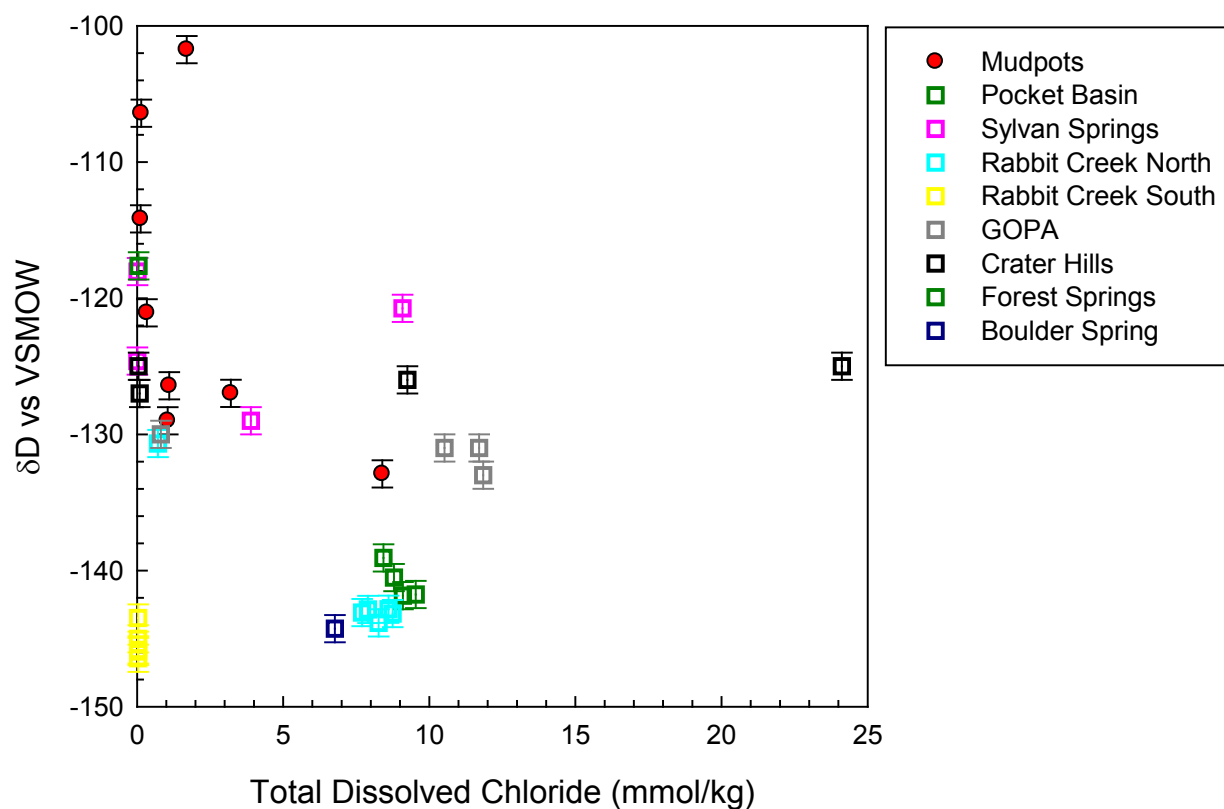


Figure 14: δD vs total dissolved chloride. This plot represents : δD and chloride in mudpots (red circles) and hot springs (open colored squares). Instrumental error for δD is 1 ‰ and chloride is 0.92‰ and δD errors are smaller than the symbols.

3.1.3. Major Cation and Anion Concentrations

Major cation and anion concentrations for mudpots can be one to two orders of magnitude different from nearby hot springs. Mudpots often have higher concentrations of sulfate (Fig. 15, 18, 19, 20) and consistently have lower concentrations of sodium. Bromide, phosphate, nitrite, and nitrate are generally near or below detection limit (Table XXII). All chloride concentrations were corrected for high blank concentrations (Fig. 6a), however chloride contributions from the blank were typically about 5% of total dissolved chloride measured (Tables XXII and XLI).

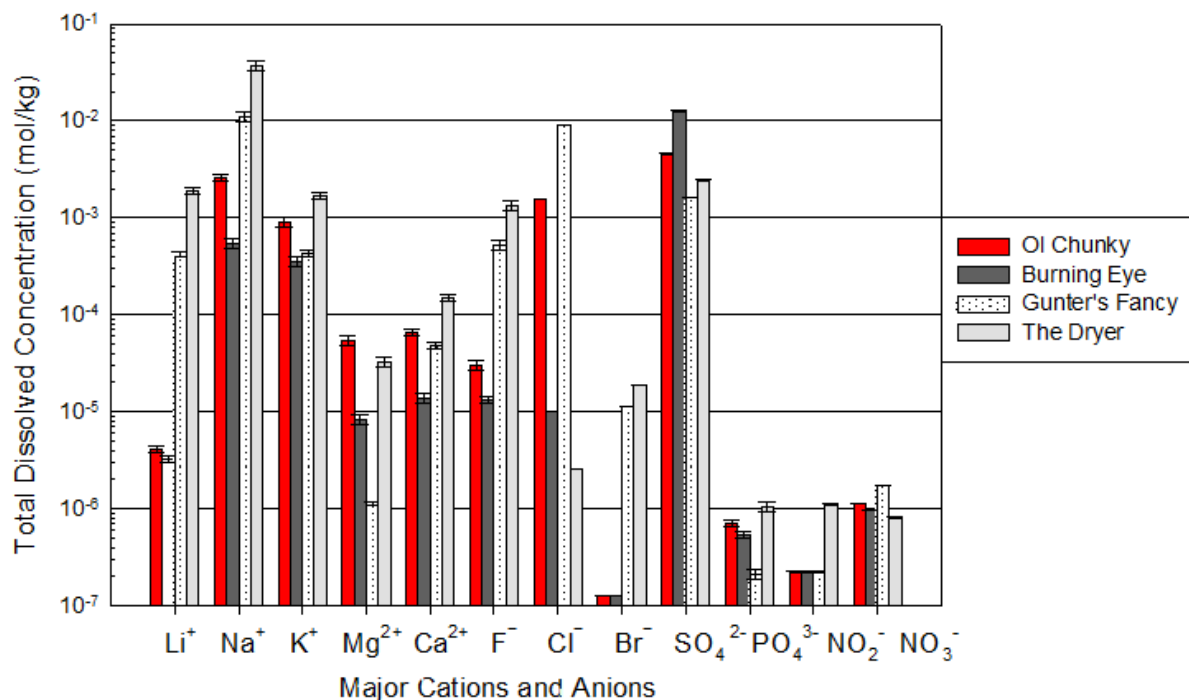


Figure 15: Major cation and anion concentrations for Sylvan Springs. This plot depicts mudpots (red) and hot springs (white and gray). Instrumental errors are $\pm 10\%$ for all cation values and are $\pm 11.9\%$ for fluoride, $\pm 0.92\%$ for chloride, $\pm 1.1\%$ for bromide, $\pm 1.9\%$ for sulfate, $\pm 10.8\%$ for phosphate, and $\pm 0.87\%$ for nitrate.

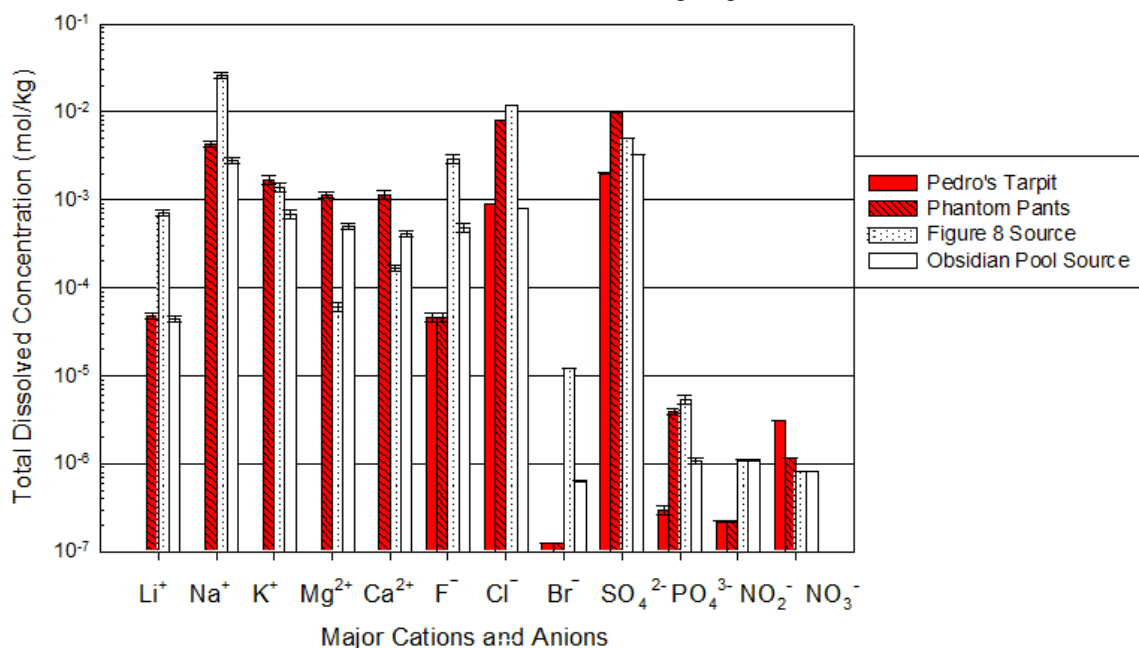


Figure 16: Major cation and anion concentrations for GOPA. Instrumental errors are $\pm 10\%$ for all cation values and are $\pm 11.9\%$ for fluoride, $\pm 0.92\%$ for chloride, $\pm 1.1\%$ for bromide, $\pm 1.9\%$ for sulfate, $\pm 10.8\%$ for phosphate, and $\pm 0.87\%$ for nitrate. This plot represents mudpots (red) and hot springs (white).

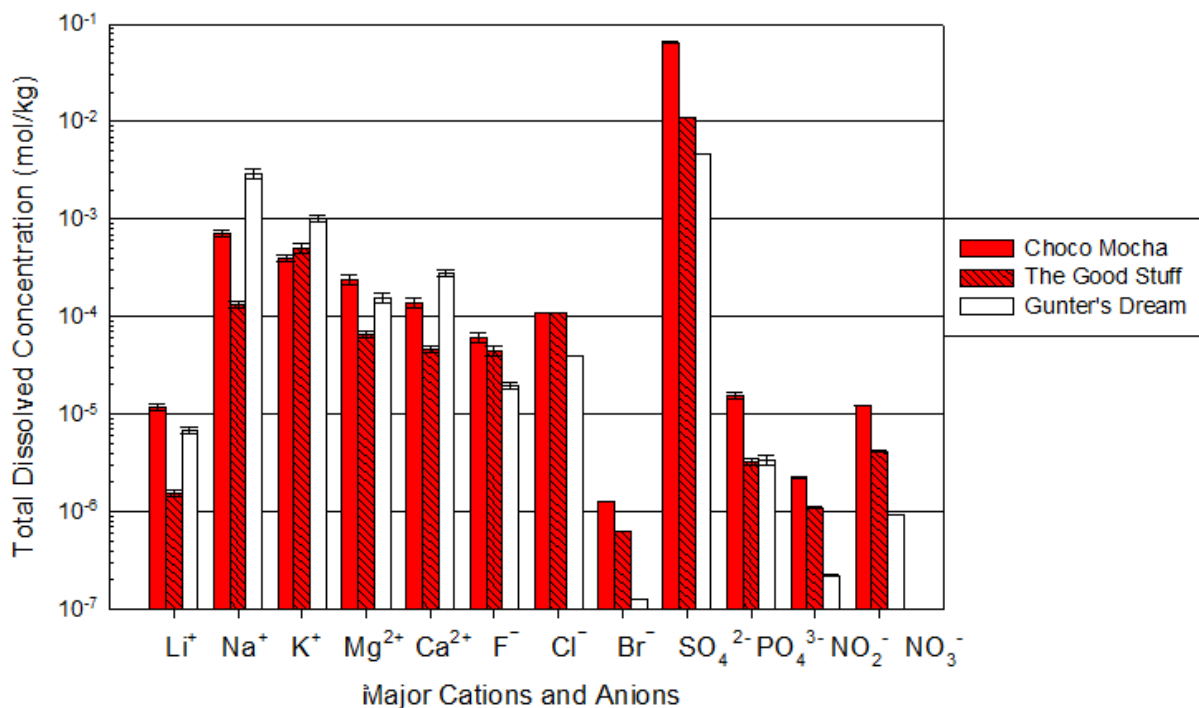


Figure 17: Major cation and anion concentrations for Forest Springs. Instrumental errors are $\pm 10\%$ for all cation values and are $\pm 11.9\%$ for fluoride, $\pm 0.92\%$ for chloride, $\pm 1.1\%$ for bromide, $\pm 1.9\%$ for sulfate, $\pm 10.8\%$ for phosphate, and $\pm 0.87\%$ for nitrate. This plot represents mudpots (red) and hot springs (white).

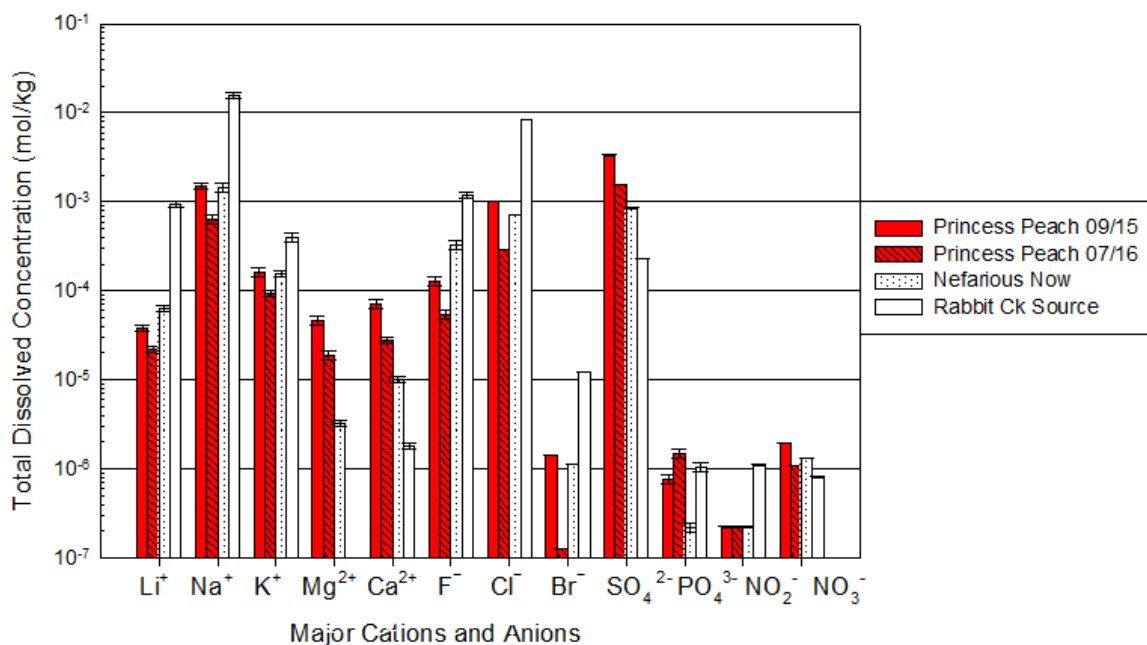


Figure 18: Major cation and anion concentrations for Rabbit Creek North. Instrumental errors are $\pm 10\%$ for all cation values and are $\pm 11.9\%$ for fluoride, $\pm 0.92\%$ for chloride, $\pm 1.1\%$ for bromide, $\pm 1.9\%$ for sulfate, $\pm 10.8\%$ for phosphate, and $\pm 0.87\%$ for nitrate. This plot represents mudpots (red) and hot springs (white).

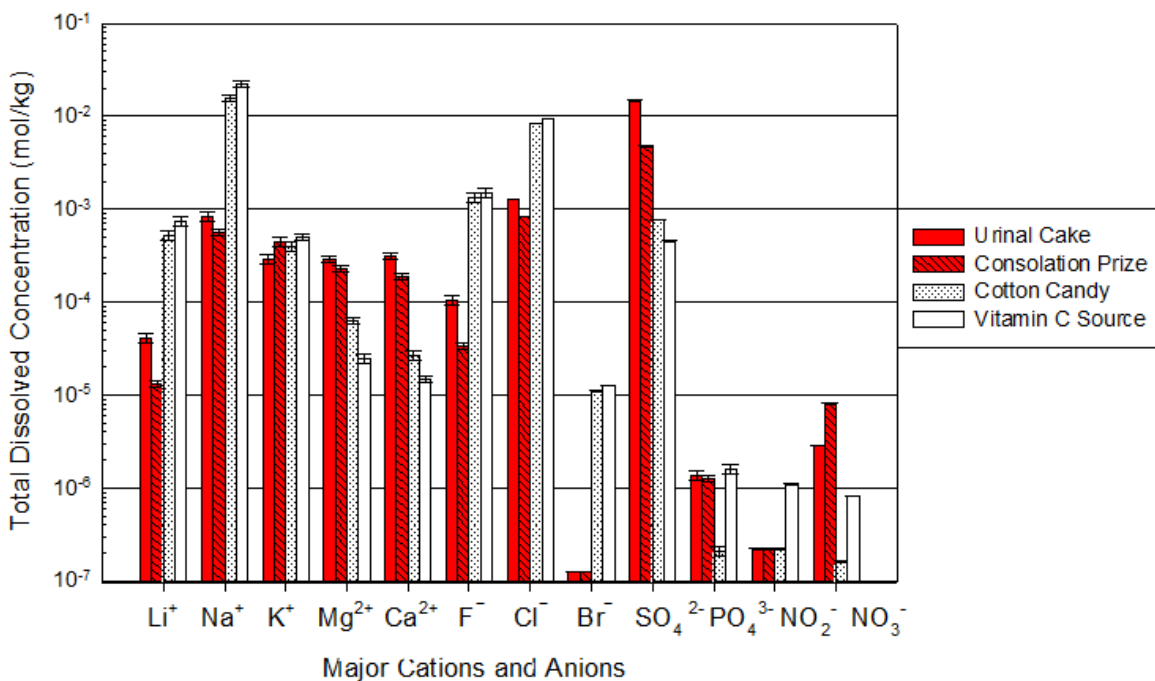


Figure 19: Major cation and anion concentrations for Pocket Basin. Instrumental errors are $\pm 10\%$ for all cation values and are $\pm 11.9\%$ for fluoride, $\pm 0.92\%$ for chloride, $\pm 1.1\%$ for bromide, $\pm 1.9\%$ for sulfate, $\pm 10.8\%$ for phosphate, and $\pm 0.87\%$ for nitrate. This plot represents mudpots (red) and hot springs (white).

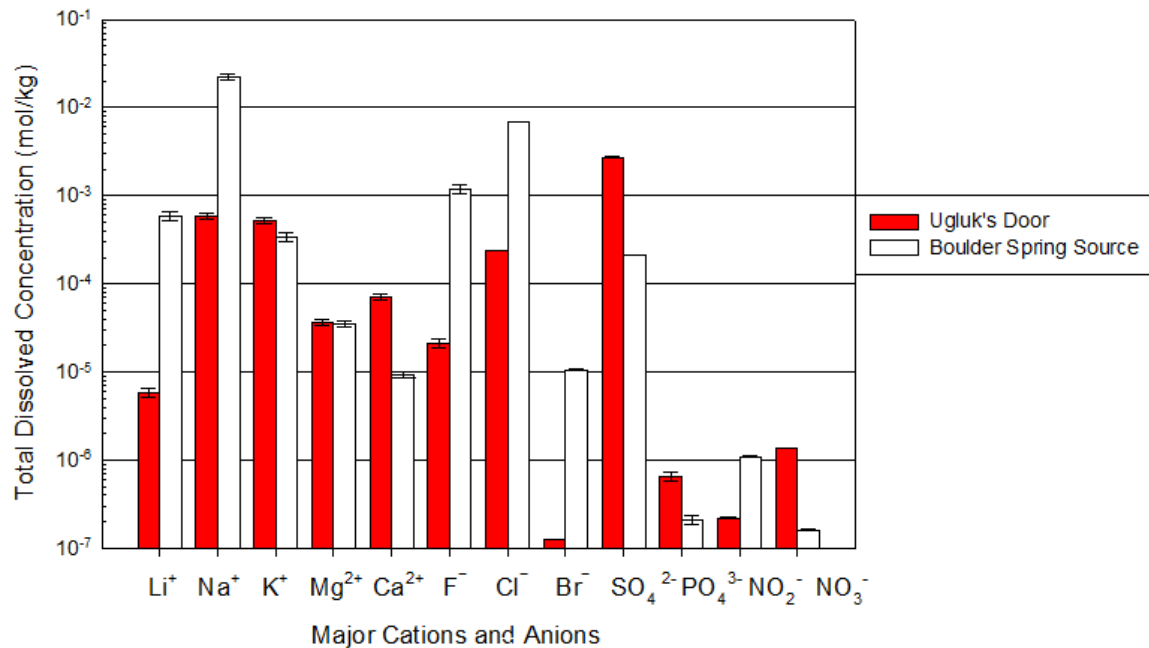


Figure 20: Major cation and anion concentrations for Boulder Spring. Instrumental errors are $\pm 10\%$ for all cation values and are $\pm 11.9\%$ for fluoride, $\pm 0.92\%$ for chloride, $\pm 1.1\%$ for bromide, $\pm 1.9\%$ for sulfate, $\pm 10.8\%$ for phosphate, and $\pm 0.87\%$ for nitrate. This plot represents mudpots (red) and hot springs (white).

Sulfate concentrations decrease with increasing pH. Higher pH thermal features (alkaline chloride hot springs) have lower sulfate concentrations than lower pH features (mudpots and acid sulfate hot springs) (Fig. 21). Mudpots and hot springs, with similar linear regressions, show pH dependent sulfate concentrations.

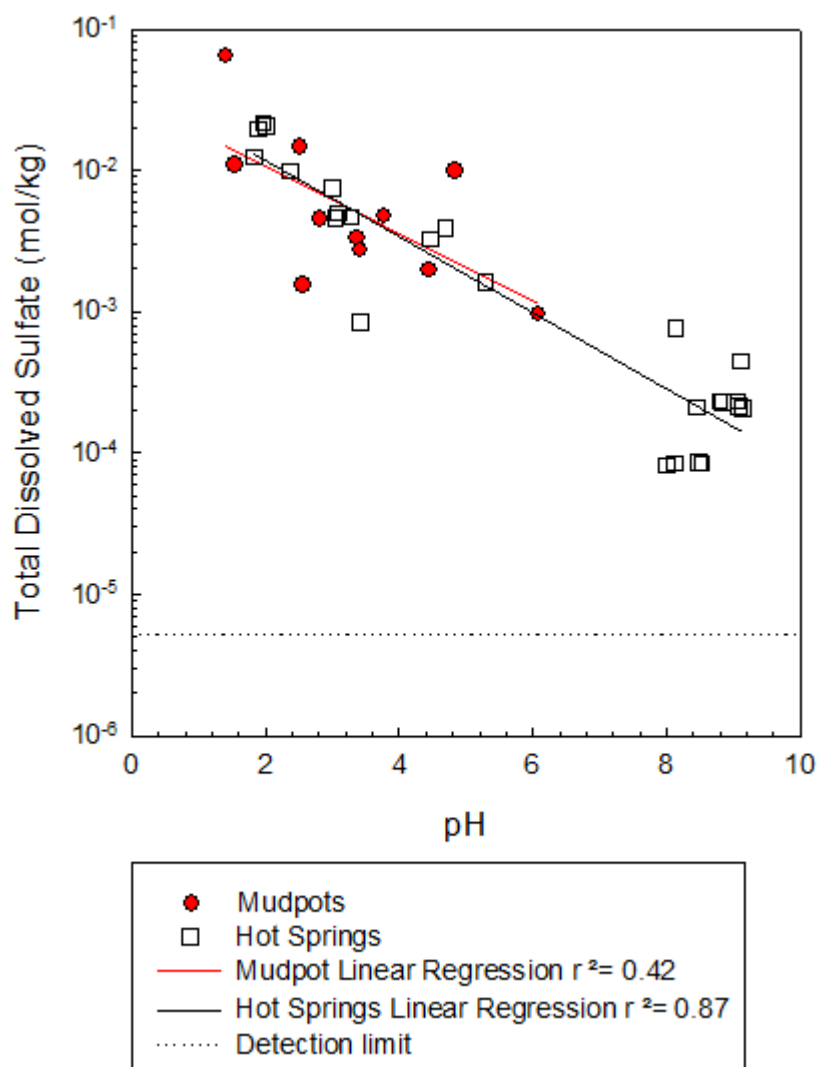


Figure 21: Dissolved sulfate vs pH. Instrumental and field fluctuation errors are typically $\pm 1.9\%$ total dissolved sulfate and ± 0.003 pH units, which are smaller than the symbols (Table XXII).

Chloride concentrations in mudpots and the majority of sampled mudpots increase with pH. Outliers are alkaline hot springs in Rabbit Creek South with low chloride and acidic hot springs in eastern YNP with high chloride (Fig. 22).

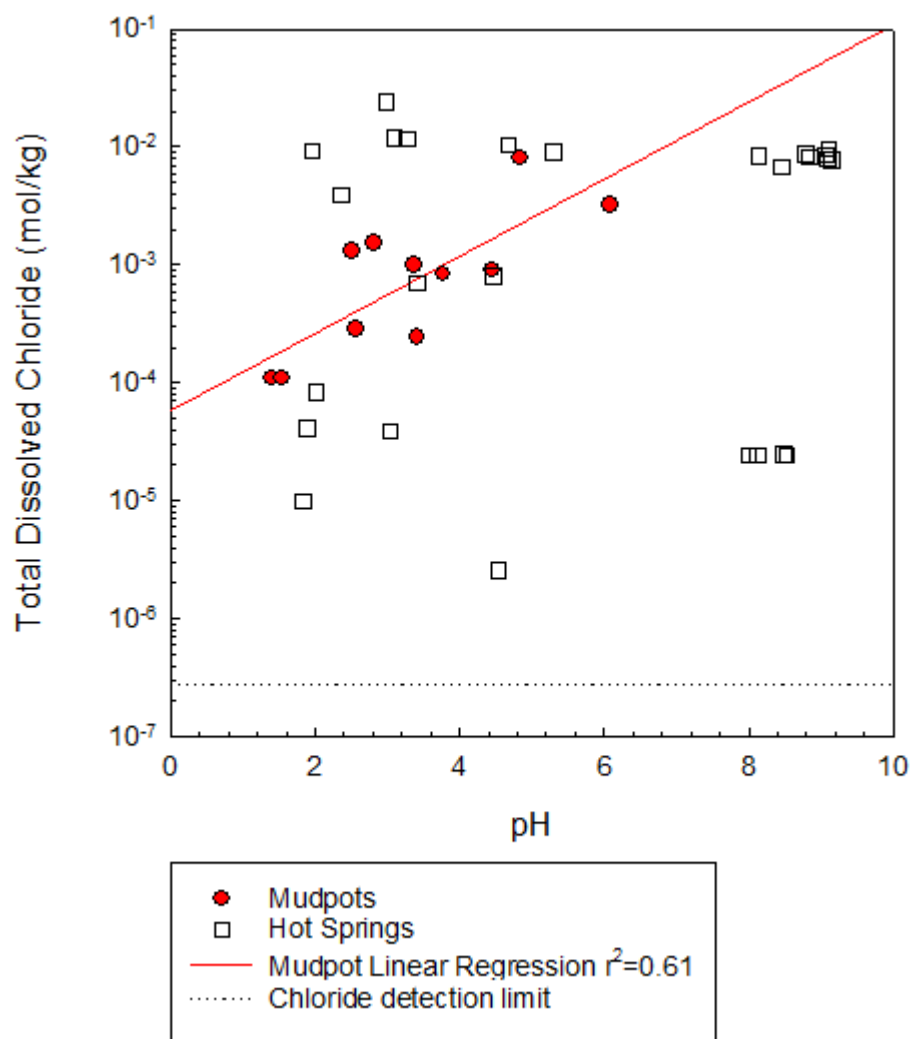


Figure 22: Chloride concentrations vs pH. Instrumental and field fluctuation errors are typically $\pm 0.92\%$ total dissolved chloride and ± 0.003 pH units, which are smaller than the symbols (Table XXII).

3.1.4. Trace Element Concentrations

Total dissolved trace element concentrations for sampled mudpots and hot springs can be grouped into elements in which mudpots are deficient or enriched, relative to hot springs, and

elements which decrease with pH, regardless of thermal feature. Lithium, boron, arsenic, rubidium, molybdenum, and cesium have higher concentrations in hot springs than mudpots (Fig. 23). Iron, vanadium, cobalt, nickel, zinc, and cerium have lower concentrations in hot springs than mudpots (Fig. 24). Aluminum, titanium, manganese, barium, beryllium, and strontium decrease with pH (Fig. 25). Chromium, copper, gallium, selenium, zirconium, niobium, palladium, silver, cadmium, tin, antimony, lanthanum, praseodymium, neodymium, tungsten, thallium, lead, thorium, and uranium were either below or close to their respective detection limits, ranging from 0.85 to 29 nmol/kg (Tables XXXVI and XXXIX). While these three main groupings were observed, the concentration ranges for sampled mudpots and hot springs varies by element. Field fluctuation errors are typically ± 0.003 pH units, which are smaller than the symbols (Fig. 8). Instrumental and field fluctuation errors fell within the symbol (Tables XX, XXIII to XXX). Lithium (a) and boron (b) range from 10^{-8} to 10^{-2} mol/kg and arsenic (c), rubidium (d), cesium (e), and molybdenum (f) range from 10^{-9} to 10^{-4} mol/kg (Fig. 23). Iron (a) and zinc (b) range from 10^{-8} to 10^{-2} mol/kg and nickel (c), cobalt (d), cerium (e), and vanadium (f) range from 10^{-9} to 10^{-5} mol/kg. Lines represent detection limit (Fig. 24). Aluminum (a) ranges from 10^{-8} to 10^{-2} mol/kg and titanium (b), manganese (c), barium (d), beryllium (e), and strontium (f) range from 10^{-8} to 10^{-4} mol/kg (Fig. 25). The outlier mudpot, Mudder's Milk, was omitted from linear regression calculations.

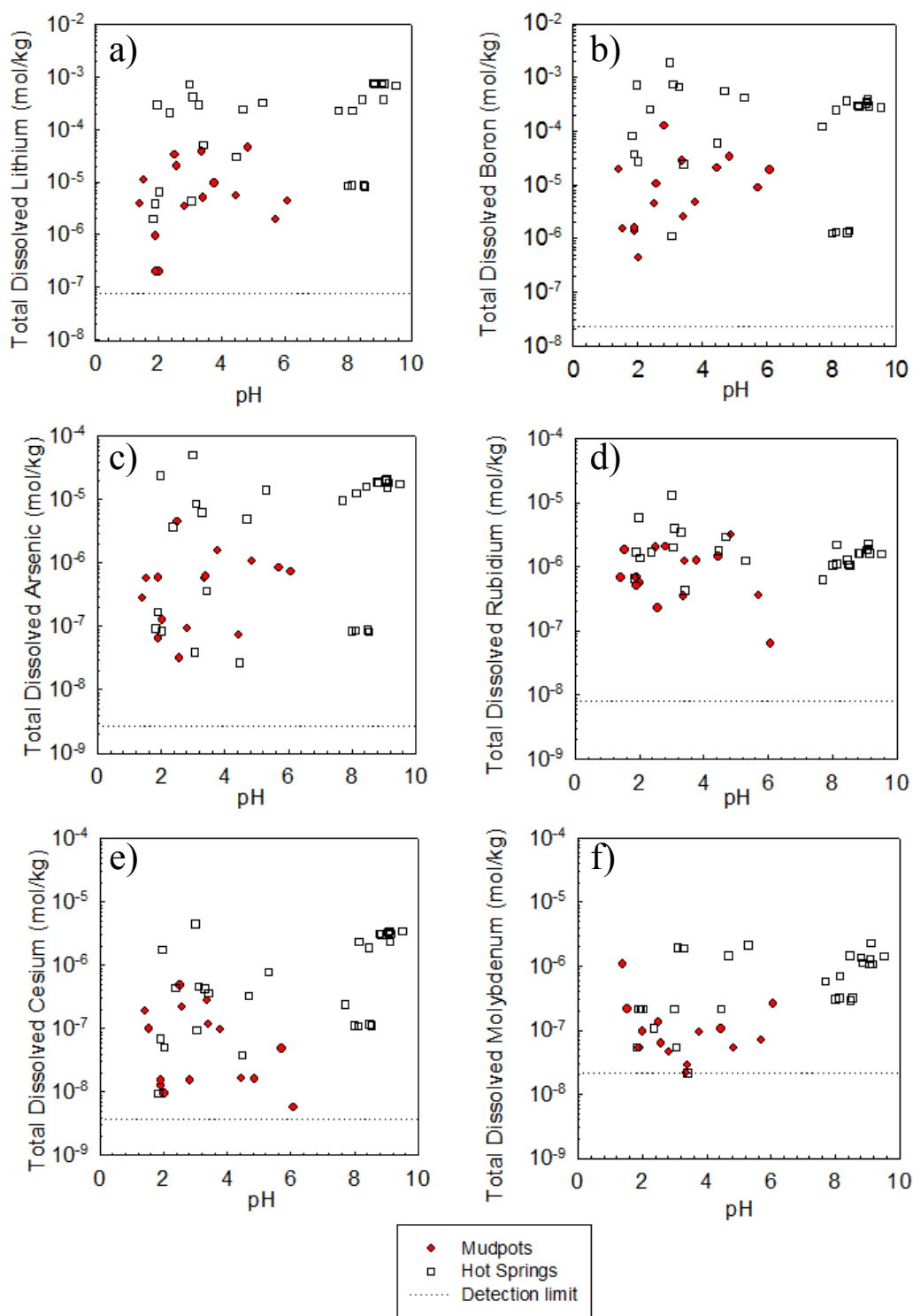


Figure 23: Total dissolved trace elements vs pH for elements in which hot springs have concentrations higher than mudpots. This plot represents lithium (a), boron (b), arsenic (c), rubidium (d), cesium (e), molybdenum (f) for mudpots (red circles) and hot springs (open squares). Dotted line represents detection limits for each element.

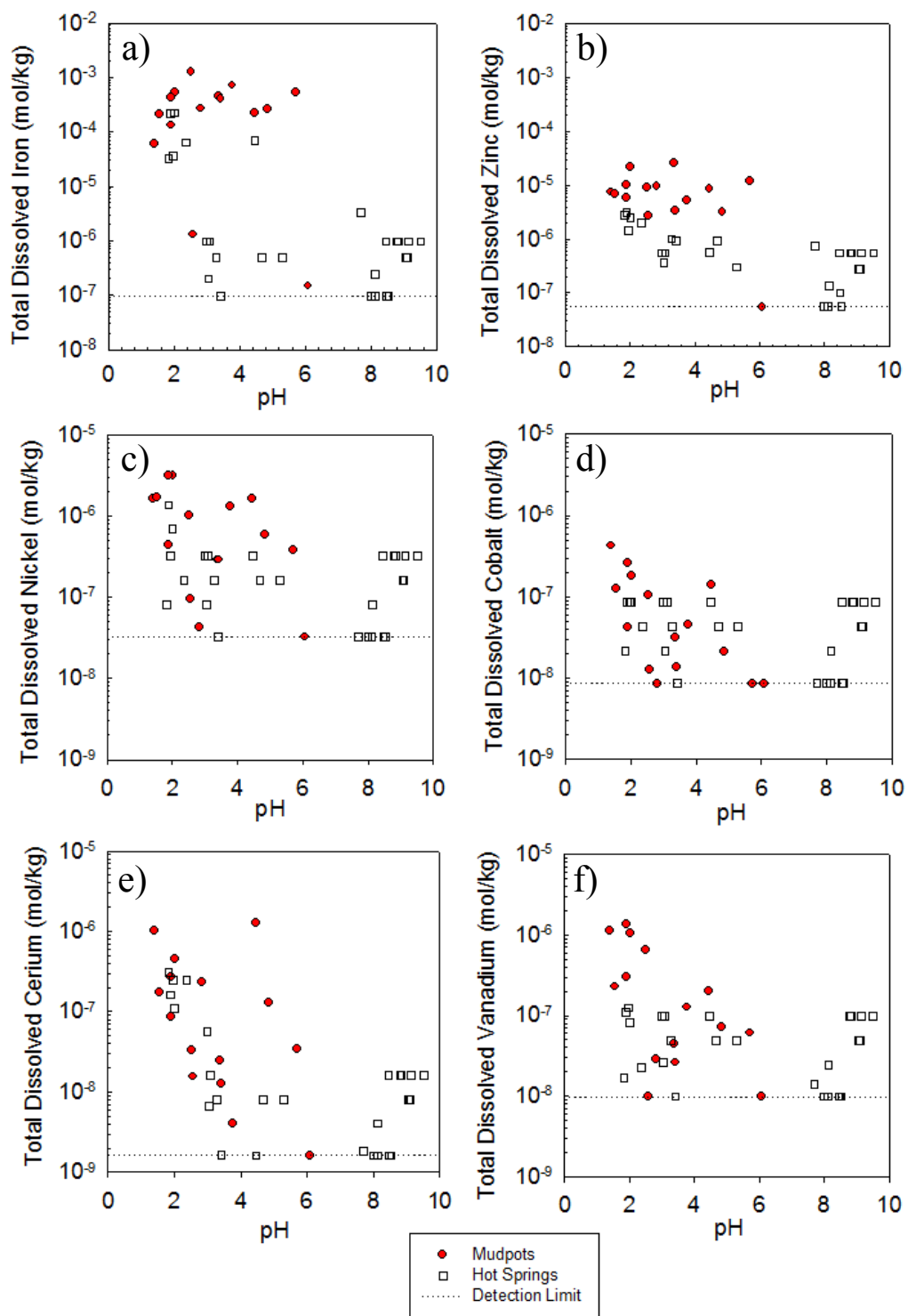


Figure 24: Total dissolved trace elements vs pH for elements in which hot springs have concentrations lower than mudpots. This plot represents iron (a), zinc (b), nickel (c), cobalt (d), cerium (e), vanadium (f) for mudpots (red circles) and hot springs (open squares). Dotted line represents respective detection limits for each element.

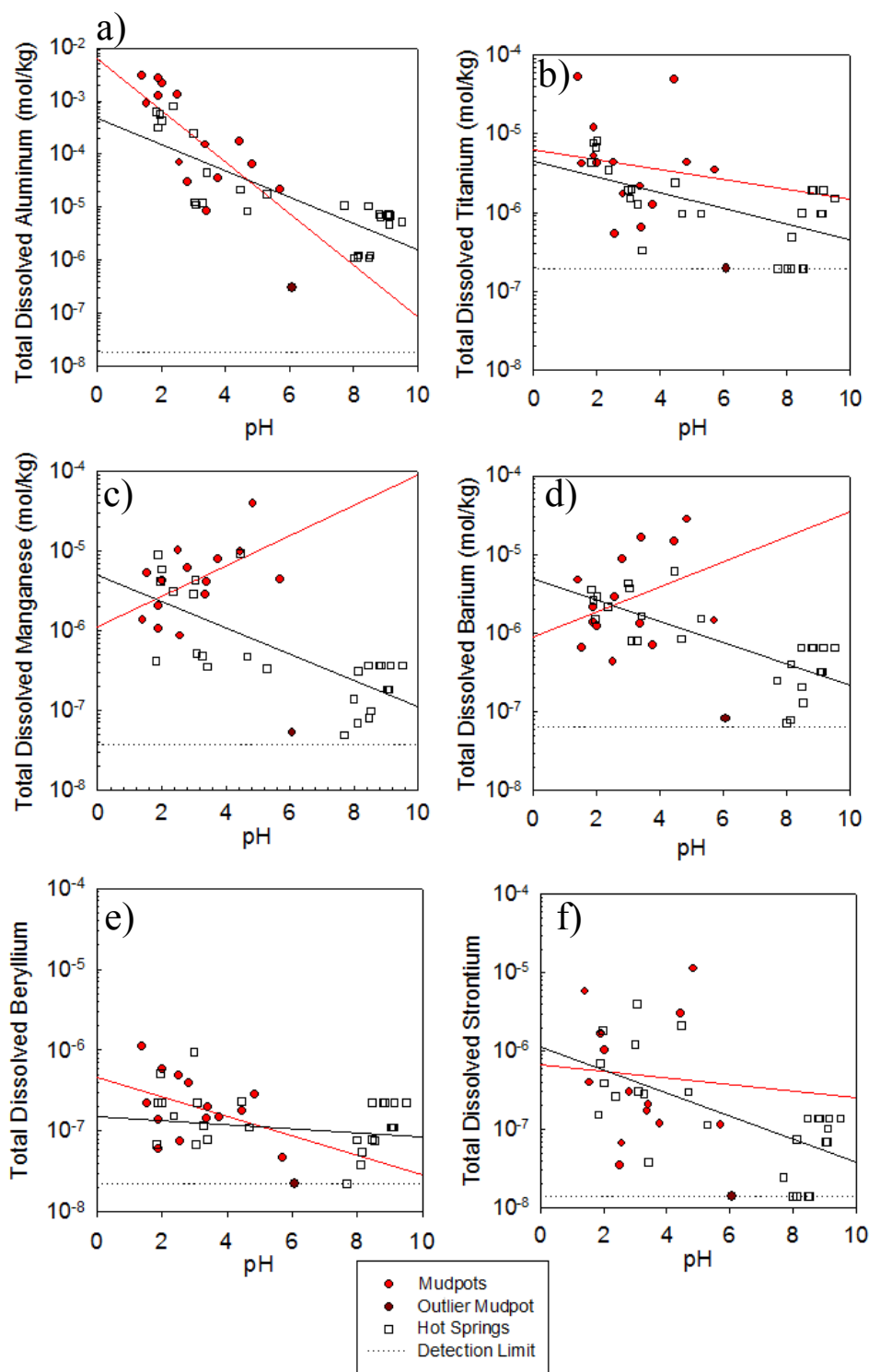


Figure 25: Total dissolved trace elements vs pH for elements that trend with pH in both mudpots and hot springs. This plot represents aluminum (a), titanium (b), manganese (c), barium (d), beryllium (e), strontium (f) for mudpots (red circles) and hot springs (open squares). Dotted line represents respective detection limits for each element.

Mudpots are categorized as acid sulfate features with lower chloride concentrations, relative to alkaline chloride hot springs. Mudpots have intermediate to low concentrations of arsenic, compared to sampled hot springs (Fig. 23c). There is a positive relationship in hot spring geochemistry between total dissolved arsenic and chloride concentrations (Fig. 26).

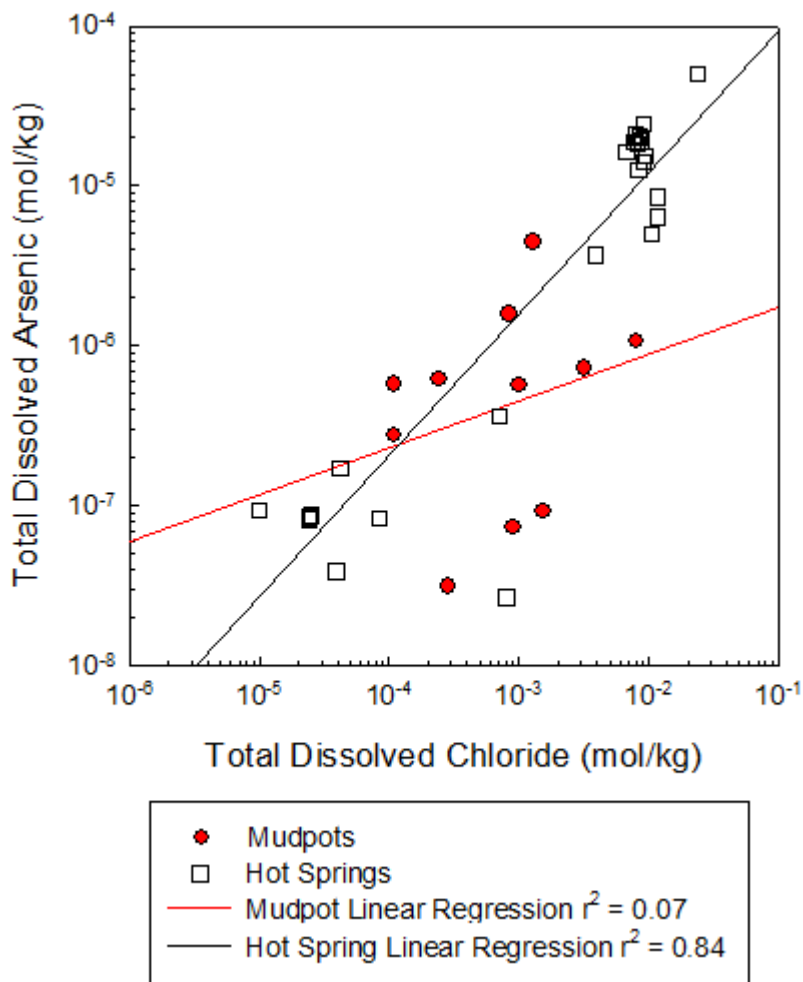


Figure 26: Arsenic vs chloride concentrations. This plot represents arsenic vs chloride for mudpots (red circles) and hot springs (open squares). Instrumental errors are $\pm 0.92\%$ total dissolved chloride and $\pm 2.3\%$ total dissolved arsenic, which are smaller than the symbols.

Mudpots have lower boron concentrations than hot springs with a similar pH value (Fig. 27). The large pH error values are from a sampling trip in which the WTW Profi-Line pH meter

and probe were unable to be used, resulting in collection of samples for later pH analysis at 25°C. Notable outliers are the Rabbit Creek mudpot and its adjacent hot spring, which have similar boron concentrations and the Forest Springs reference hot spring, which has a lower boron concentration than expected for a hot spring at that pH. Sampled mudpots have pH values of 1.4 to 5.7 and boron concentrations between 1.2×10^{-4} and 4.4×10^{-7} M.

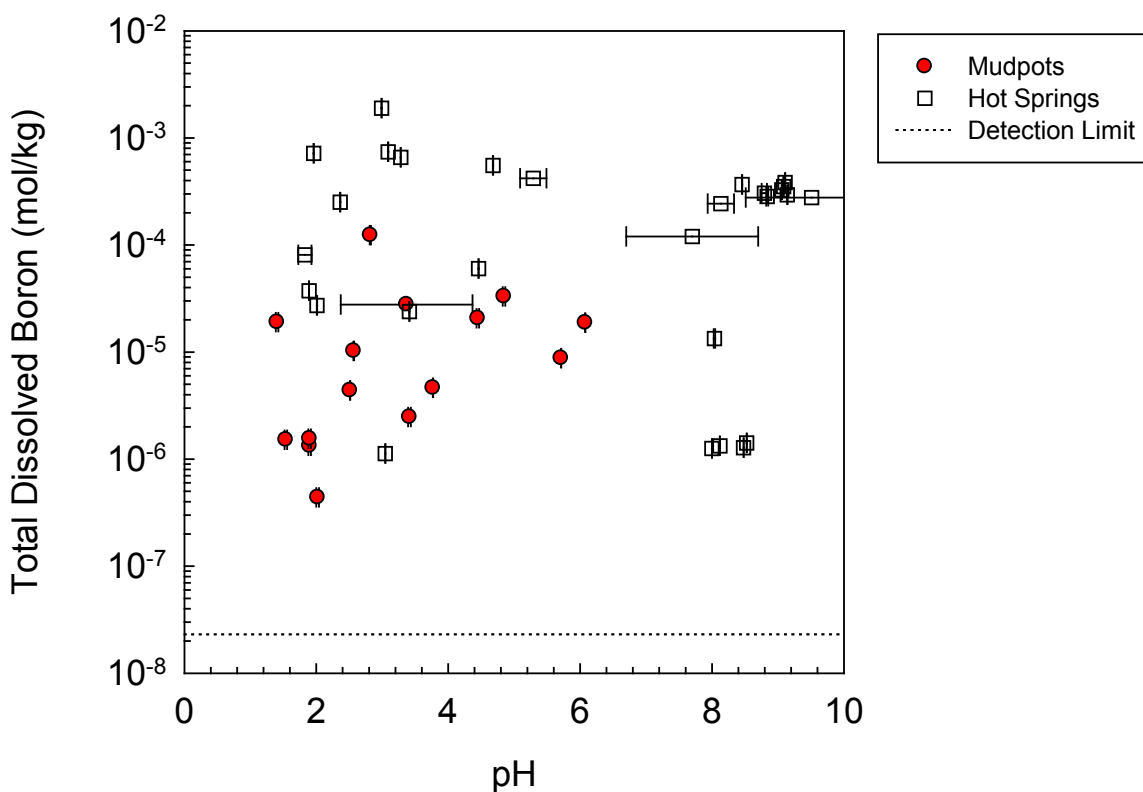


Figure 27: Boron concentration vs pH in hot springs and mudpots. This plot represents hot springs as open squares and mudpots as red circles. Field fluctuation errors range from 0.002 to 1.0 for pH and instrumental errors are $\pm 3.4\%$ total dissolved boron. Instrumental errors for boron are smaller than the symbols.

Boron speciates primarily as boric acid ($B(OH)_3$) at pH values less than 9 (Fig. 28). For more alkaline pH values, the speciation becomes less straightforward with boric acid, triborate, and pentaborate all contributing (Schott *et al.*, 2015). In sampled YNP hot springs, pH values range from 1.83 to 9.51 (Fig. 8), making boron speciation less pertinent.

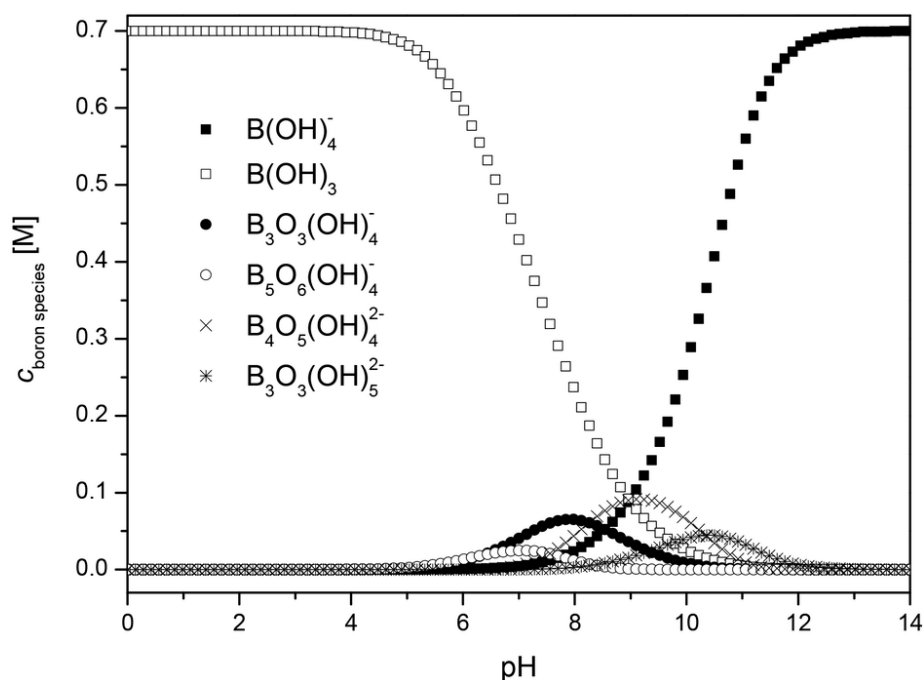


Figure 28: Boron speciation with pH (Schott *et al.*, 2015).

Considering differences in trace metal relative abundances, it is possible to tentatively assign chemical “fingerprints” to each thermal region. Cerium and neodymium have greater relative abundances in eastern YNP mudpots than western YNP mudpots. Furthermore, eastern mudpots generally have more titanium, nickel, and strontium than western mudpots. Boron and lithium are more dominant in western mudpots. Arsenic is also more abundant in the Pocket Basin thermal area in the western region of the park (Fig. 29). The mudpot from the Shoshone Geyser Basin contained 74.40% boron, 17.03% lithium, 1.23% aluminum, and 0.57% iron (Table XXXI), and therefore was not feasible for fingerprint comparisons to mudpots with

compositions greater than 50% iron or aluminum.

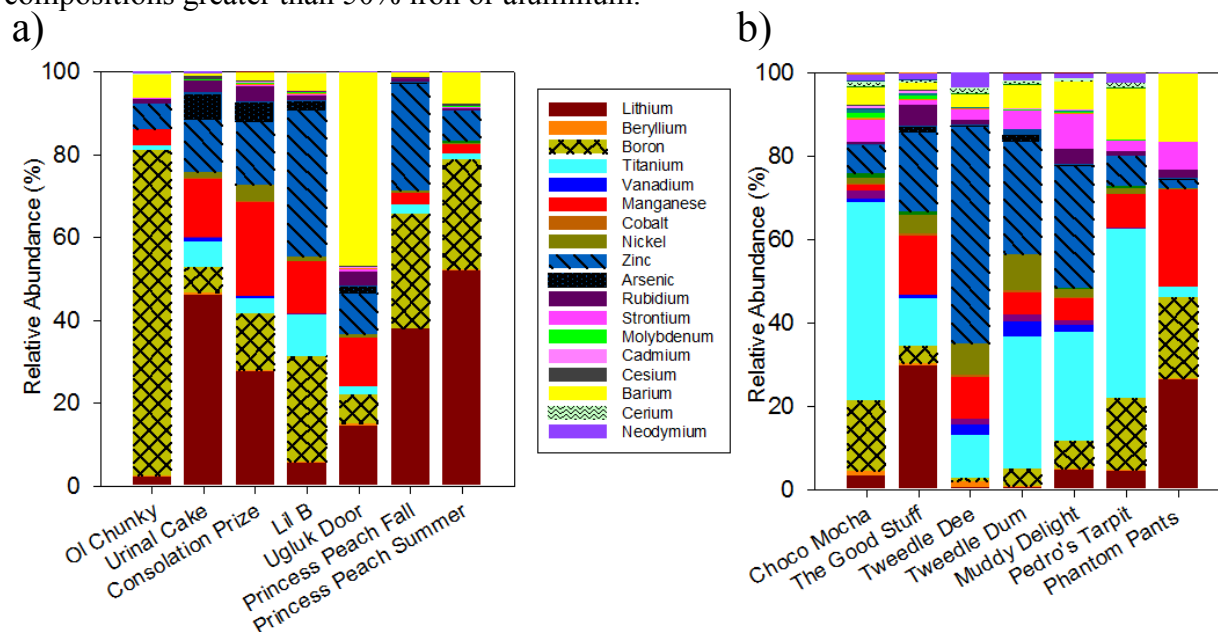


Figure 29: Trace element relative abundances in YNP mudpots. Western Mudpots (a) and Eastern Mudpots (b). Aluminum and iron concentrations have been excluded from Figure 29, as they comprise 70% – 95% of the relative abundance for the trace elements. Plots a) and b) represent 5 – 30% of total dissolved trace metals. Plot legend only lists elements with visible portions (> 1%), however all elements analyzed are included, except aluminum and iron.

Mudpots can be grouped by whether iron or aluminum dominates and this division is reflected in the relative abundance of the elements. Most of the aluminum dominated mudpots are also located in the eastern half of the park with the exception of the July sample of the Rabbit Creek mudpot (Princess Peach Summer) (Fig. 30). The iron dominated mudpots are in the western half of the park, with the exception of Phantom Pants, a mudpot from GOPA that formed over the course of two weeks.

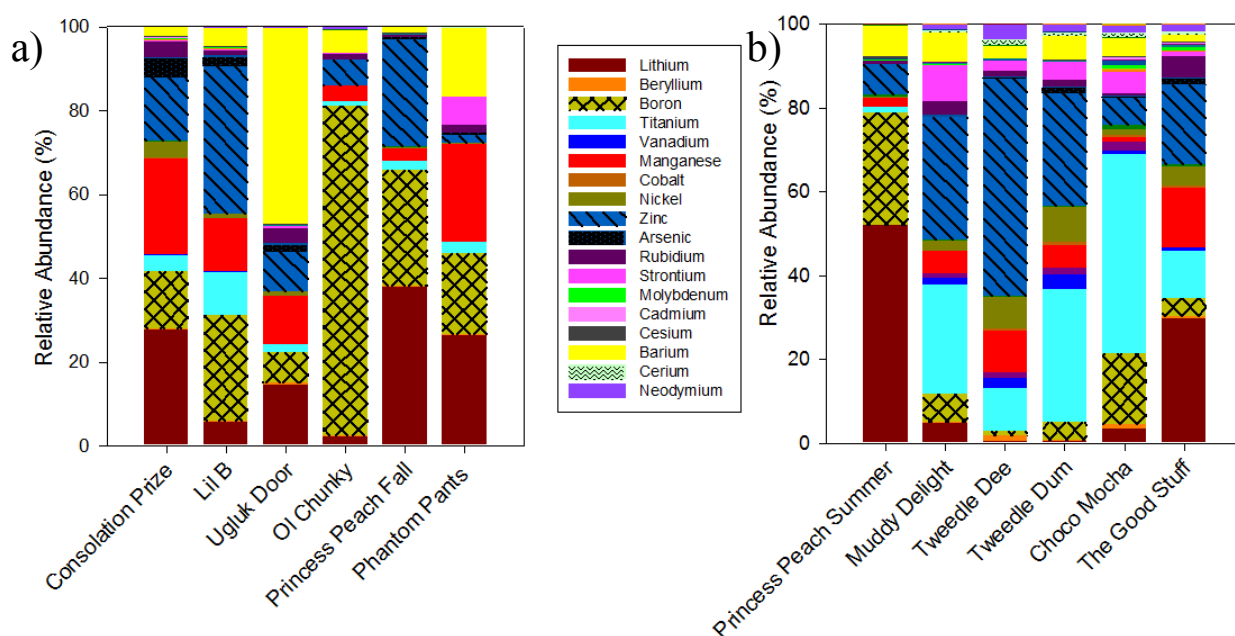


Figure 30: Trace element relative abundance grouped by (a) iron or (b) aluminum dominance.

3.2. Geologic

3.2.1. Clay Compositions

Clay minerals were identified from desiccated mudpot samples using the TerraSpec Halo portable near infrared spectrometer. Mudpots in eastern YNP are primarily composed of montmorillonite, halloysite, and alunite, whereas mudpots in western YNP are primarily kaolinite and alunite (Table VII). A limitation of the TerraSpec Halo portable near infrared spectrometer is that it does not recognize quartz.

Table VII: TerraSpec Halo portable Near Infrared Spectrometer and mudpot solids calculations.

Thermal Area	Mudpot Code	Composition	Al-OH Scalar Value	Kx Scalar Value	Bulk Density (g/cm³)	% Water (and volatiles)
Sylvan Springs	160716E Ol Chunky	kaolinite PX Mg-illite	2206.7	1.008	1.43	72.4
Pocket Basin	160715V Consolation Prize	kaolinite PX sepiolite K-alunite	2207.8	0.961	1.33	51.2
	160715Y Hide and Seek	halloysite K-alunite	2207.2	0.996	1.66	53.2
	160715 Lil B	kaolinite PX K-alunite	2207.7	0.934	1.74	36.9
	160715T Urinal Cake	kaolinite WX K-alunite dickite	2207.5	9.976	1.47	68.4
Boulder Spring	160726S Ugluk's Door	kaolinite WX halloysite	2207.1	0.936	1.26	88.0
Rabbit Creek	150919B Princess Peach	hematite kaolinite PX	2207.5	0.977	ND	ND
Shoshone	160712D Mudder's Milk	kaolinite PX	2207.2	0.994	1.24	94.6
Forest Springs	160719W Choco Mocha	No match found	NA	NA	1.39	87.9
	160719Z The Good Stuff	K-alunite kaolinite WX	2207.8	0.995	1.56	65.7
Crater Hills	160518N Tweedle Dum	halloysite K-alunite magnesite gypsum	2207.7	1.000	ND	ND
	160518L Tweedle Dee	halloysite K-alunite montmorillonite	2207.8	1.001	ND	ND
GOPA	The Gomper	halloysite Mg-illite	2207.0	1.003	1.71	43.6
	160720 Phantom Pants	montmorillonite vermiculite	2205.8	NA	2.23	21.3
	160724 Phantom Pants	montmorillonite kaolinite PX	2205.6	1.014	1.78	46.7
	160517E Pedro's Tarpit	montmorillonite Fe-smectite	2209.0	NA	2.01	ND
	160517C Muddy Delight	halloysite K-alunite beidellite kaolinite PX	2207.9	1.003	ND	ND

ND = not determined, NA = not applicable, PX = poorly crystallized and likely formed at a low temperature, WX = well crystalized and likely formed at a high temperature.

The bulk density was calculated by subtracting the mass of the full polyethylene bottle of mud from the pre-weighed mass of the empty bottle and dividing by the volume of the bottle. This bulk density cannot be related to individual minerals because it includes water and any detritus in the sample and the dry density is also not related to minerals because the desiccation process may dehydrate minerals. Water content was calculated by subtracting the mass of dried sample from the mass of wet sample and dividing by the mass of wet sample. It is most analogous to a relative measure of *in situ* viscosities. Higher water contents indicate less viscous samples. The Kx value is a scalar value inversely related with the temperature at which the mineral formed.

In addition to preliminary identification of clay minerals in desiccated samples, scanning electron microscopy (SEM) images were taken for Muddy Delight in GOPA, Princess Peach in Rabbit Creek, and Tweedle Dee in Crater Hills. In these images, brightness indicates heavier elements. Possible identifications of minerals were created using the weight percent elemental data provided with Energy Dispersive X-Ray Spectroscopy (EDX). In addition to lithic fragments found in all SEM images, pyrite (Fig. 31b), elemental sulfur (Fig. 31c), and barite (Fig. 31d) were observed. Felsic rock fragments and clay minerals were also observed (Fig. 32b).

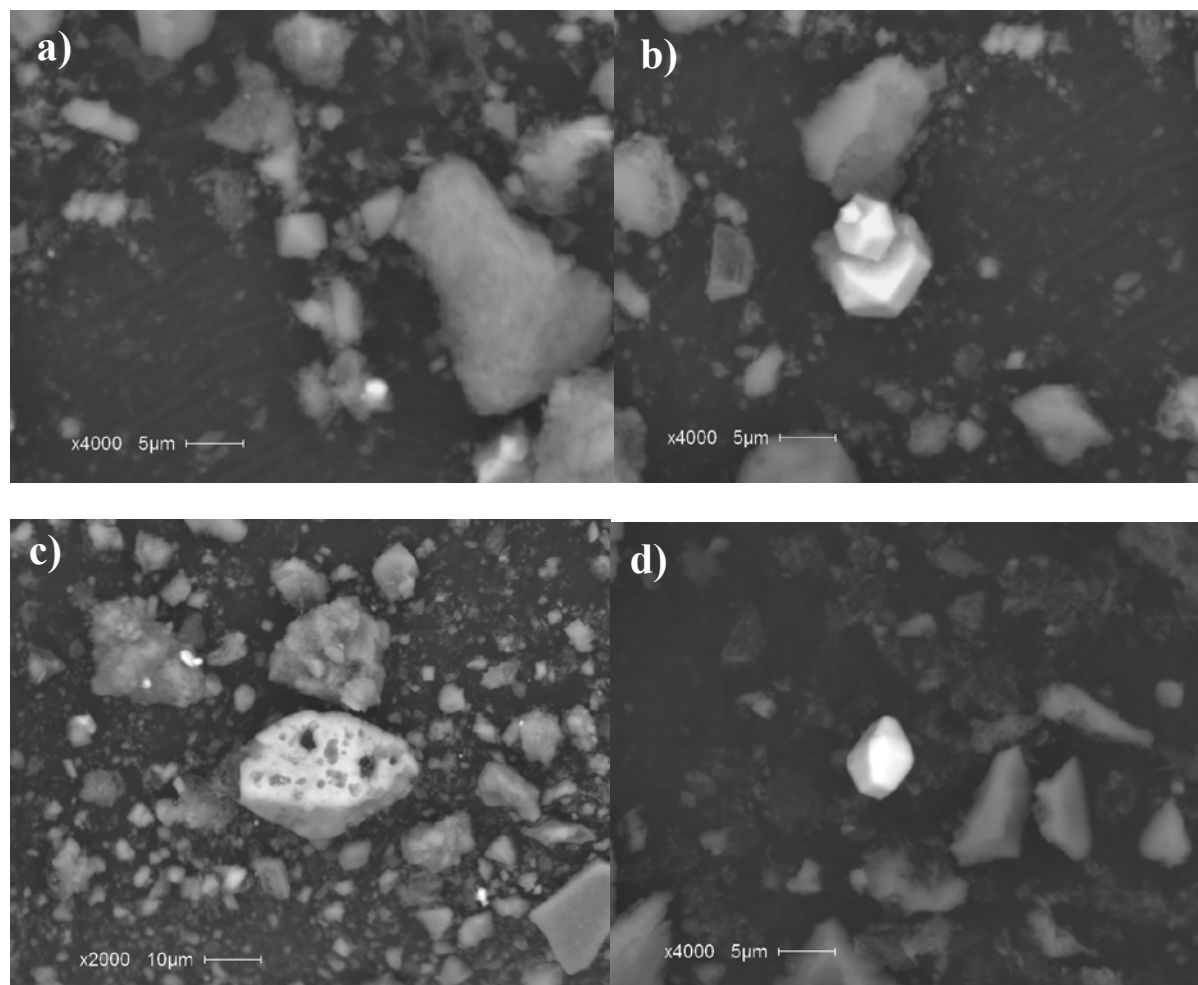


Figure 31: SEM images from mudpots in the Crater Hills Area. (a) clay minerals, (b) cluster of pyrite crystals, (c) elemental sulfur in a field of amorphous silica and clay mineral fragments, and (d) barite crystal.

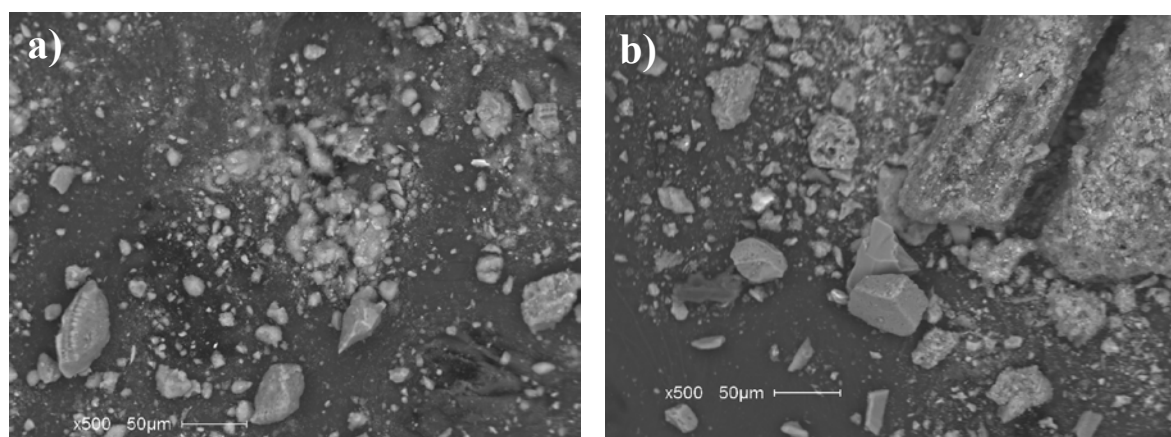


Figure 32: SEM images: (a) Princess Peach and (b) Muddy Delight. Unaltered and partially altered felsic rock debris can be seen alongside clay mineral fragments and amorphous silica.

3.2.2. Mineral Solubility Predictions via EQ3 and CHNOSZ

EQ3 is software from the Lawrence Livermore National Laboratory that is designed for geochemical modelling of aqueous systems (Wollery, 1992). The fundamental purpose of EQ3 is to make speciation-solubility calculations (Wollery and Jarek, 2003), using thermodynamic database consistent with Helgeson *et al.* (1978), Shock *et al.* (1989, 1997), Shock and McKinnon (1993), Sverjensky *et al.* (1997), standard Gibbs energies for metabolic reactions summarized by Amend and Shock (2001), and an extended Debye-Hückel equation for activity coefficients (Helgeson, 1969). Equilibrium constants for reactions involving aqueous species were calculated with the revised Helgeson-Kirkham-Flowers equation of state (Shock *et al.*, 1992) using the SUPCRT92 computer code (Johnson *et al.*, 1992). Mudpots that had measurements for pH, conductivity, temperature, dissolved oxygen, anion, cation, and trace elements were modelled in EQ3 (Wollery, 1992). These calculations resulted in predictions regarding the supersaturation of minerals and the speciation of basis species (Table VIII). All mudpots model results contained alunite as a supersaturated phase.

Table VIII: Mineral supersaturation in mudpots (modelled predictions).

Sample Code	Thermal Area	Supersaturated Minerals
160715T	Pocket Basin	alunite, hematite, magnetite, goethite
160716E	Sylvan Springs	alunite, hematite, goethite
160717M	Rabbit Creek North	alunite, hematite, goethite
160726S	Boulder Spring	alunite, diaspore, gibbsite, hematite, magnetite, goethite

3.2.3. Clay Stability Plots

CHNOSZ is a package for the open source programming language, R (Dick, 2008). CHNOSZ was designed to perform thermodynamic calculations for geochemistry and compositional biology. EQ3 and CHNOSZ use information from databases of thermodynamic

properties of minerals, and aqueous chemical species and can also makes calculations using thermodynamic properties of organic chemical species and amino acids, provided those species are in the database. An Eh-pH plot was created in CHNOSZ for probable clay species in YNP. A recurring issue was the lack of smectite group minerals in thermodynamic databases used by modeling programs (Dick, 2008). The Eh-pH diagram shows that the stability of these clays is pH dependent and that kaolinite dominates. These predictions disagree with field data because of the absence of smectite group minerals in thermodynamic databases.

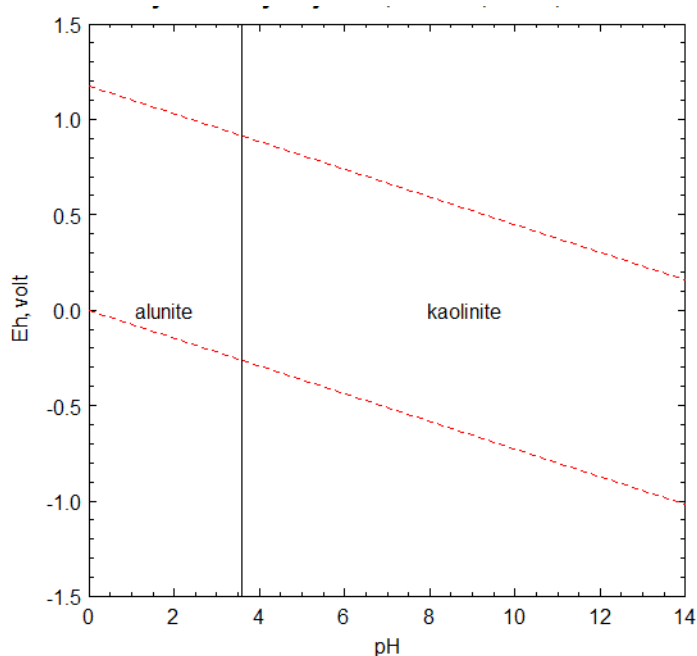


Figure 33: Eh-pH diagram generated in CHNOSZ for alunite and kaolinite at 92.5°C, 1 bar, and assuming all species have millimolar concentrations.

3.3. Microbial Diversity

Four mudpots were selected for microbial diversity analysis (16S and 18S rRNA gene amplification and sequencing). Phantom Pants and Pedro's Tarpit are found on the eastern side of YNP at the GOPA thermal area. Consolation Prize and Urinal Cake are found on the western side of YNP at the Pocket Basin thermal area. Phantom Pants formed over a two week period in

a)

Region	Count	Percentage
Phantom Pants only	1	(4%)
Consolation Prize only	5	(20%)
Pedro's Tarpit only	7	(28%)
Urinal Cake only	4	(16%)
Phantom Pants & Consolation Prize	0	(0%)
Phantom Pants & Pedro's Tarpit	0	(0%)
Phantom Pants & Urinal Cake	2	(8%)
Consolation Prize & Pedro's Tarpit	0	(0%)
Consolation Prize & Urinal Cake	1	(4%)
Pedro's Tarpit & Urinal Cake	0	(0%)
Phantom Pants & Consolation Prize & Pedro's Tarpit	0	(0%)
Phantom Pants & Consolation Prize & Urinal Cake	0	(0%)
Phantom Pants & Pedro's Tarpit & Urinal Cake	4	(16%)
Consolation Prize & Pedro's Tarpit & Urinal Cake	0	(0%)
All four categories	1	(4%)

b)

Region	Count	Percentage
Phantom Pants only	3	(33.3%)
Consolation Prize only	2	(22.2%)
Pedro's Tarpit only	1	(11.1%)
Urinal Cake only	0	(0%)
Phantom Pants & Consolation Prize	0	(0%)
Phantom Pants & Pedro's Tarpit	0	(0%)
Phantom Pants & Urinal Cake	0	(0%)
Consolation Prize & Pedro's Tarpit	0	(0%)
Consolation Prize & Urinal Cake	2	(22.2%)
Pedro's Tarpit & Urinal Cake	0	(0%)
Phantom Pants & Consolation Prize & Pedro's Tarpit	0	(0%)
Phantom Pants & Consolation Prize & Urinal Cake	0	(0%)
Phantom Pants & Pedro's Tarpit & Urinal Cake	1	(11.1%)
Consolation Prize & Pedro's Tarpit & Urinal Cake	0	(0%)
All four categories	0	(0%)

While no definitive statements may be made regarding metabolic processes that are actually occurring, the table below lists the genera and any known associated metabolisms (Table IX). This foundational work provides a basis to make general hypotheses about possible microbial contributions to mudpot geochemistry, which will be tested using proteomic analyses.

Table IX: Archaeal and bacterial genera.

Mudpot	Kingdom	Genus	Known metabolism(s)
Pedro's Tarpit pH 4.45 ± 0.02 Temp $68.1 \pm 0.1^\circ\text{C}$ $8.4 \mu\text{M DO}$ (0.268 ppm)	Archaea	Nitrososphaera Methanobrevibacter Methanosaeta Candidatus Nitrosocaldus Thermoplasma Methanomicrobium Methanobacterium Methanocorpusculum Candidatus Nitrosotalea Aciduliprofundum Thermogymnomonas	ammonia oxidizing gram +, methanogen, strict anaerobe gram –, obligate anaerobe, methanogen acetate to CH_4 thermophilic, ammonia oxidizing, autotroph, aerobic, nitrification facultative anaerobe, pH 1 to 2, obligate heterotroph strict anaerobe, need acetate, pH 5.9 – 7.7, 25 – 45°C strict anaerobe, $\text{CO}_2 + \text{H}^+$ to CH_4 needs NH_4 & sulfide strict anaerobe, likes $[\text{NaCl}] = 0.1 - 0.25\text{M}$ ammonia oxidizing, obligate acidophile, fix HCO_3^- thermoacidophile strict aerobic, acidophilic, thermophilic, heterotrophic
	Bacteria	<u>Ralstonia</u> Caenimonas	aerobe, can use NO_2^- as e^- acceptor in anaerobic conditions, some species metal resistant, facultative chemolithoautotroph
Phantom Pants pH 4.85 ± 0.02 Temp $80.9 \pm 0.1^\circ\text{C}$ $0.88 \mu\text{M DO}$ (0.028 ppm)	Archaea	Caldococcus Vulcanisaeta	strict anaerobe, hyperthermophile, reduce sulfide anaerobic, heterotrophic, hyperthermophilic archaeon, grows best 85 – 90°C, pH 4 – 4.5
	Bacteria	<u>Ralstonia</u> Curvibacter Corynebacterium Brevundimonas Geobacillus	aerobe, can use NO_2^- as e^- acceptor in anaerobic conditions, some species metal resistant, facultative chemolithoautotroph aerobic/anaerobic, heterotrophic, grows best in a metal doped media (Mg, Cu, Mn, Fe, Mo, Li) aerobic, found in soils, plants, and human microbiota resistant to radiation, thermophile, spore-forming, aerobic/facultative anaerobe, can use NO_3 as e^- acceptor
Consolation Prize pH 3.77 ± 0.003 Temp $88.5 \pm 0.1^\circ\text{C}$ $13 \mu\text{M DO}$ (0.431 ppm)	Archaea	Methanosaeta Nitrososphaera Methanobrevibacter Methanobacterium <u>Methanococcus</u> Desulfurococcus Methanospirillum Methanomassiliicoccus Methanosphaerula Cenarchaeum	gram – obligate anaerobe, methanogen acetate to CH_4 ammonia oxidizing gram + methanogen, strict anaerobe strict anaerobe, $\text{CO}_2 + \text{H}^+$ to CH_4 needs NH_4 & sulfide obligate anaerobe and methanogenic, grow autotrophically on mineral material anaerobe with sulfur metabolism strict anaerobe, fixes nitrogen obligate anaerobe, methanogen, obligately methylotrophic (uses Me-OH or mono, di, or trimethylamine. Obligately H_2 dependent, chemoorganotrophic, can't use CO_2 or NO_3 , tungstate and selenite can stimulate growth
	Bacteria	Caulobacter Caenimonas Rhodoplanes	stalk forming alphaproteobacteria gram – alphaproteobacteria, some have photosynthetic pigments

Table IX: Archaeal and bacterial genera, continued.

Mudpot	Kingdom	Genus	Known metabolism(s)
Urinal Cake pH 2.51 ± 0.002 Temp 72.1 ± 0.1°C 22 µM DO (0.698ppm)	Archaea	Caldococcus Methanosaeta <u>Methanococcus</u> Nitrososphaera Methanobrevibacter Methanobacterium Thermoplasma Methanosarcina Ferroplasma Methanolinea Methanolobus	strict anaerobe, hyperthermophilic, reduces sulfide gram – obligate anaerobe, methanogen acetate to CH ₄ obligate anaerobe and methanogenic, grow autotrophically on mineral material ammonia oxidizing gram + methanogen, strict anaerobe strict anaerobe, CO ₂ + H ⁺ to CH ₄ needs NH ₄ & sulfide facultative aerobe, obligate thermoacidophile, grows best 55 – 59°C, pH 1 – 2, can anaerobically reduce S ⁰ to H ₂ S, cells undergo lysis near neutrality strict anaerobe, methanogen, can fix nitrogen, acidophile, mesophile, aerobic to facultative anaerobe, Fe oxidizing chemolitho or chemoorganotroph. Common in ARD waste strict anaerobe, methanogen, grows best 37 to 50°C, H ₂ and CO ₂ or formate used to form methane strict anaerobe, Tungstate req'd for CH ₄ production
	Bacteria	Corynebacterium Curvibacter	aerobic, found in soils, plants, and human microbiota aerobic/µaerobic, heterotrophic, grows best in a metal doped media (Mg, Cu, Mn, Fe, Mo, Li)

Bolded genera were unique to that particular mudpot. Underlined genera are unique to GOPA and double underlined genera are unique to Pocket Basin. All metabolic information summarized from Bergey, 2015.

DNA extracts from sampled mudpots show 16S rRNA genes associated with methanogens and microbes known to participate in the nitrogen cycle. Relative abundance plots at the class and genus levels show broadly similar microbial classes, with much variation in genera. The newest mudpot, Phantom Pants, has less diversity than more established mudpots (Fig. 35). Due to omission of several bacterial genera that were 0.05% or less, the relative abundance falls short of 100% (Fig. 38).

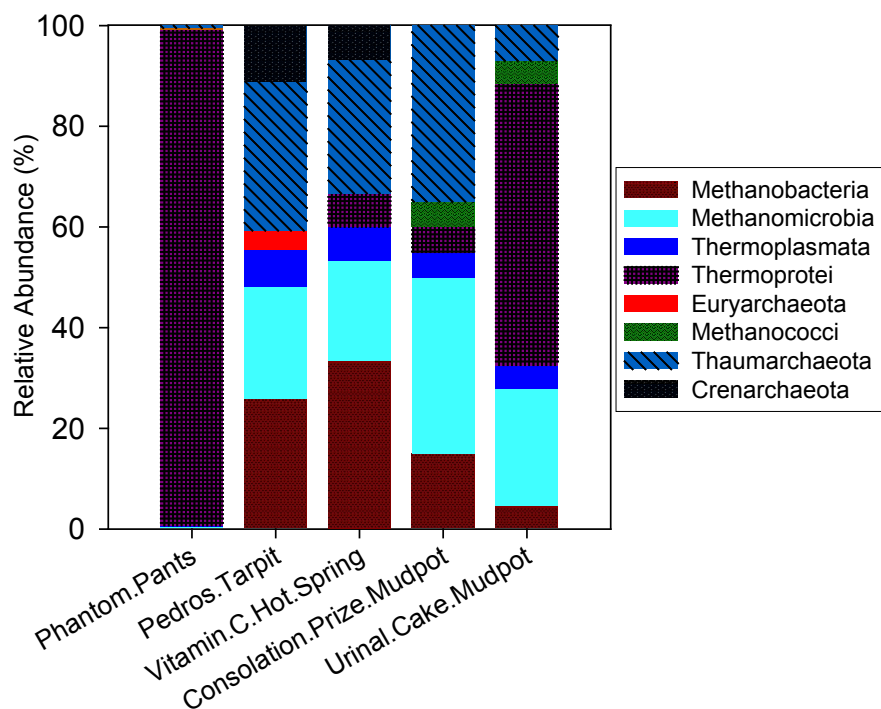


Figure 35: Archaeal classes identified with 16S rRNA gene diversity.

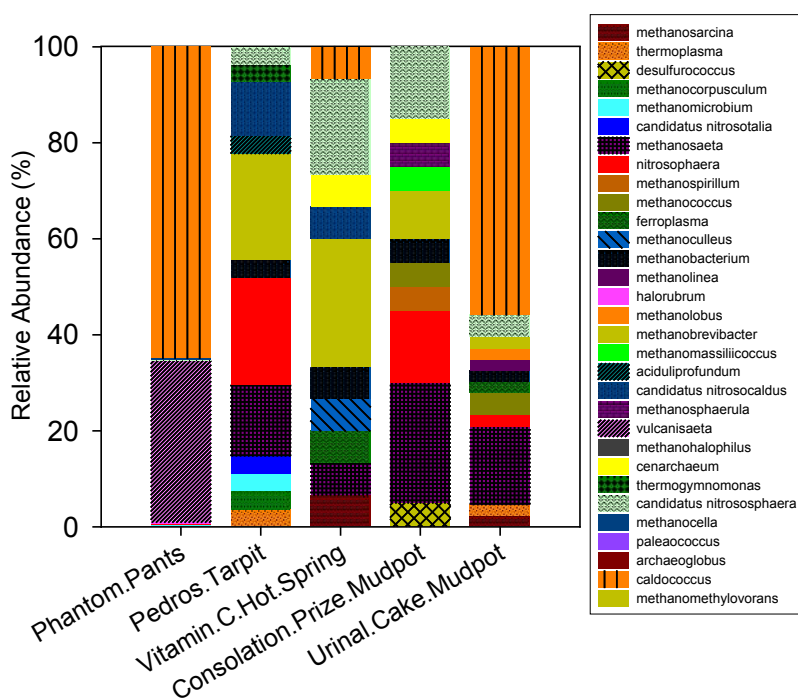


Figure 36: Archaeal genera identified with 16S rRNA gene diversity.

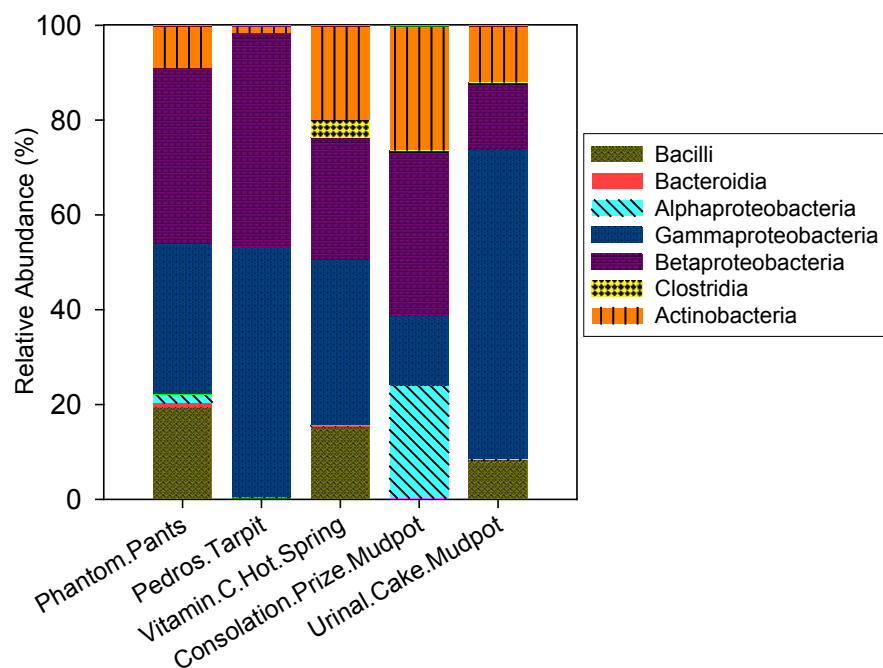


Figure 37: Bacterial classes identified with 16S rRNA diversity.

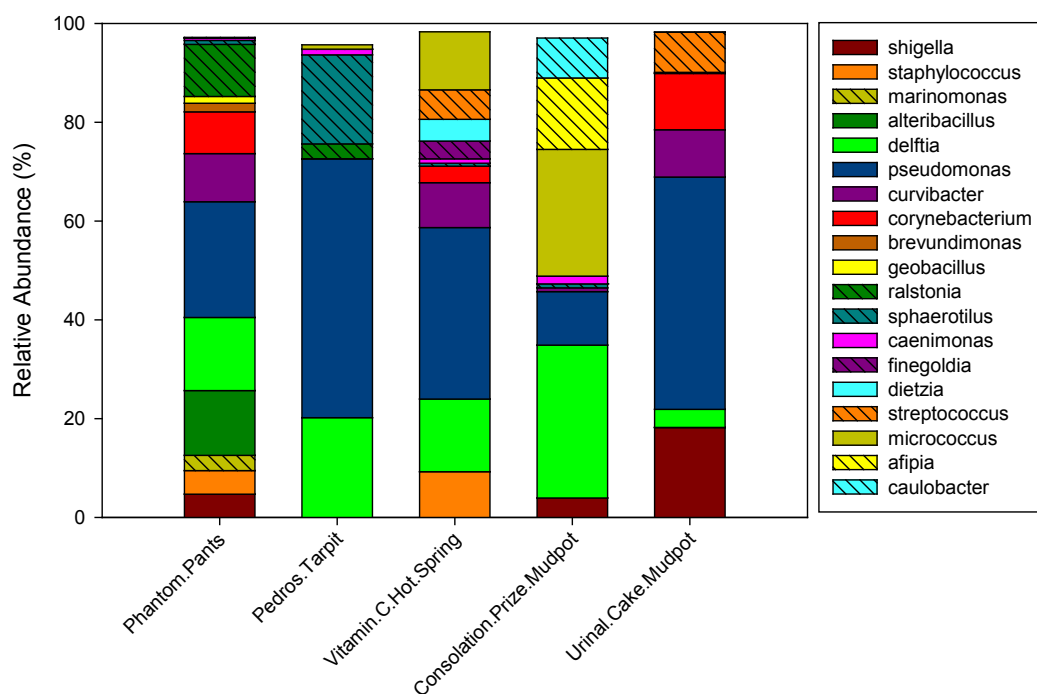


Figure 38: Bacterial genera identified with 16S rRNA gene diversity.

4. Discussion

4.1. Thermal Source Waters

Hot springs and mudpots may be grouped by source water-steam behavior (Fig. 39). This behavior can be inferred from chloride concentrations and δD values. The blank corrected chloride concentrations of some sampled hot springs do not show as clear of a trend due to low chloride alkaline thermal waters with shallow recharge circulation, which lends meteoric input. There are four main domains for thermal features with respect to chloride; low chloride indicative of large meteoric input, high chloride and alkaline pH indicative of a liquid water fraction at depth, high chloride and acidic pH indicative of some steam separation at depth, and mudpots with a moderate amount of chloride. For instance, in the Rabbit Creek thermal area, the source and its photosynthetic outflow have a mixed composition; influenced by both single stage steam separation and continuous steam separation, both representing source waters that were boiling at 93°C, whereas a mudpot and its nearby analog hot spring are acid sulfate springs. These acid sulfate springs have a source that undergoes single stage steam separation and mixing of 93°C evaporated acid sulfate waters and much hotter 360°C deep source waters. In the more southern part of the Rabbit Creek thermal area, a hot spring and its photosynthetic outflow are boiling meteoric water as demonstrated by δD vs $\delta^{18}O$ and [B] vs pH (Fig. 12 and 27). This diversity within a thermal area suggests a source that splits off into a steam (and volatiles) portion and a more continuously separated steam portion of more chloride rich waters. The southern region is likely comprised of shallow recirculated rain and snow runoff based on both low chloride concentrations (Fig. 39) and δD and $\delta^{18}O$ values (Fig. 12).

The same process can be observed in the Pocket Basin/Boulder Spring thermal areas. Sources and photosynthetic outflows are similar, with regard to chloride concentrations and δD

values, to the Rabbit Creek Source and outflow and the corresponding mudpots. This could suggest a local reservoir for the Lower Geyser Basin, which is distinct from the source waters that feed the more northern Sylvan Springs. Sylvan Springs is a thermal area which is now primarily populated by acid sulfate features. These acid sulfate features, which are generally characterized as low chloride waters that undergo long term evaporation, have similar chloride concentrations and δD values, with the exception of a turbid pH 5 hot spring with a higher chloride concentration (Fig. 39).

Given that Sylvan Spring was once described as clear with alkaline chloride waters (Allen and Day, 1935), it is possible that the same thermal source waters that feed the Lower Geyser Basin once fed the Sylvan Springs thermal area. This could be explained as either the thermal source water reservoir decreased in size, now excluding Sylvan Springs thermal area, or seismic activity may have changed pathways in which thermal waters travelled, causing the Sylvan Springs thermal area to be more steam fed. Changes from alkaline chloride source waters to more evaporated acid sulfate waters could be traced with long term collection of filtered samples for water isotopes and chloride.

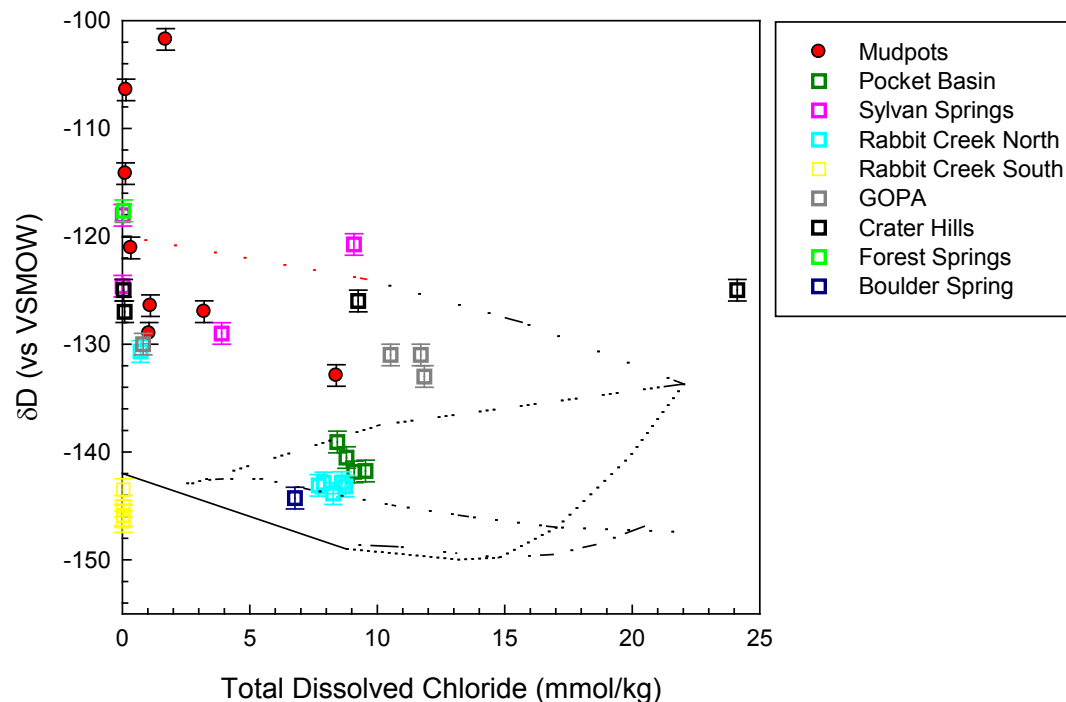


Figure 39: δD vs chloride concentrations of mudpots (red circles) and hot springs (open squares) from Fig. 14. The lines represent steam fractionation conditions at depth. Instrumental errors are $\pm 1\%$ and $\pm 0.92\%$ total dissolved chloride, which are smaller than the symbols.

Table X: Explanation of lines from Truesdell plot.

Line A	single stage steam separation between deep thermal fluids with a temperature of 360°C and evaporated acid sulfate fluids with a temperature of 93°C. The red portion of line A has been extrapolated for completeness.		terminate in a mixing point of 360°C thermal water with 93°C acid sulfate waters
Line B	boiling at 93°C and single stage steam separation	different processes calculated for the waters plotted on line E	terminate in a mixing point of 360°C thermal water with 93°C acid sulfate waters
Line C	boiling at 93°C and continuous steam separation		
Line D	liquid water in equilibrium with steam along the same temperature gradient, with the intersection at 238°C, the temperature of the fluid found in drill core hole Y12		terminate in a mixing point of 360°C thermal water with 93°C acid sulfate waters
Line E	mixing compositions between meteoric water assumed to be 5°C and a temperature gradient of thermal fluids		
Line F	continuous steam separation along a temperature gradient		

There are different hydrothermal fractionation processes that occur at depth and influence the surface chemistries (Table X). The low chloride springs with δD less than -140 ‰ are heated meteoric waters; whereas the low chloride features with δD greater than -130 ‰ are acid sulfate springs. Truedell offers little explanation regarding water isotopes in acid sulfate springs, however it is noted that in some acidic thermal areas evaporation can cause variable δD over time, while leaving the already low chloride concentrations relatively unchanged. With the exception of low chloride acid sulfate springs and mudpots, the majority of the samples group in the regions partitioned by Truesdell (Fig. 39 and Table X), which allows for predictions to be made regarding the steam separation occurring at depth.

Hot spring blank corrected chloride concentrations do not exhibit as clear a trend with pH (Fig. 22). The boiling meteoric waters from Rabbit Creek South which are high pH, low chloride, also do not neatly fall into the category of alkaline chloride. The low pH, high chloride springs are from the eastern half of the park, specifically from GOPA and Crater Hills. These eastern thermal areas do not exhibit any trends in Figure 22, however there is a documented inverse correlation between radon isotopes and carbon dioxide flux to assign relative depths of thermal water sources (Moloney, 2011). The $^{220}\text{Rn}/^{222}\text{Rn}$ vs CO_2 concentrations show a relationship that can be interpreted as Rabbit Creek Source Hot Spring which has a relatively shallow thermal source and the Mud Volcano area (an area near GOPA) that has a deeper source in relation to Rabbit Creek. However, there was variation between samples suggesting the possibility of multiple sources.

δD and $\delta^{18}\text{O}$ relative to VSMOW can provide information regarding the general and recent origin of water (Fig. 12). For example, meteoric source water will be relatively enriched in

δD relative to hydrogen and $\delta^{18}\text{O}$ relative to $\delta^{16}\text{O}$, whereas hydrothermal waters will be relatively depleted, with respect to δD vs VSMOW.

While mudpots do not cluster separately from acidic thermal features, there is a relationship in this data set between pH of thermal features and the isotopic composition (Fig. 12). It has been noted, in personal communication with V. Debes, that in larger data sets of thermal feature water isotope data over roughly ten years, the relationship between pH and δD and $\delta^{18}\text{O}$ data space is less clearly defined and some thermal areas exhibit δD based fluctuations over time due to evaporation.

The Yellowstone Thermal Water Line (YTWL) shows an overall evaporative loss relative for the Global Meteoric Water Line (GMWL). The YTWL was determined using the line of best fit of the July 2016 water isotope data for samples collected from hot springs and mudpots, relative to VSMOW (Fig. 12). With the addition of more evaporated water isotope samples, such as those from Washburn Thermal Area, or more shallow recharge heated meteoric waters, such as those from Rabbit Creek South thermal area, the YTWL may shift slightly, but is expected to remain relatively stable. The YTWL would be more representative of the park if it included a greater variety of thermal features, more acid sulfate samples with substantial evaporation, and a better distribution of backcountry water isotope values from the central YNP in areas such as Joseph's Coat, Violet Springs, Turbid Creek, and the Mudkettles. Additionally, adding water isotope data for YNP rivers, snow, and rain to improve the Yellowstone Meteoric Water Line (YMWL) (Kharaka, 2002) would provide another comparison, other than the GMWL. The YMWL, derived from snow, rain, and surficial water samples from the Greater Yellowstone area, is similar to the GMWL and may be essentially the same line when considering measurement error (Kharaka, 2002). The GMWL was determined from a much

larger data set of meteoric water samples with limited evaporation, relative to standard mean ocean water (Craig, 1961). The mudpot with water isotope values between the GMWL and the YTWL (-12.5, -114) is a turbid briny hot spring, rather than a true mudpot in that it precipitates a salty sludge and does not appear to be viscous due to suspensions of clay minerals (Fig. 12).

4.2. Rock-Pore Water Chemistries

There are similarities in mudpot pore water chemistries of certain regions. The higher mudpot pH values were measured in mudpots from GOPA, the Pocket Basin, and Shoshone Geyser Basin (Fig. 8). Relative abundance trace element data from mudpots in the eastern half of the park (Forest Springs, Crater Hills, and GOPA) are more similar to each other than to relative abundance trace element data from mudpots in the western half of the park (Sylvan Springs, Pocket Basin, Boulder Spring, and Rabbit Creek) (Fig. 29). Furthermore, the eastern thermal areas appear on the 1972 surficial geology of Yellowstone National Park map to be underlain by the Hayden Valley Flow in the Pleistocene Central Plateau Member (Fig. 3 and Table I). Forest Springs, Crater Hills and GOPA are underlain by the Hayden Valley Flow (Morgan *et al.*, 2005). The eastern mudpots have relatively more titanium, nickel, strontium, cerium, and neodymium than the western mudpots (Fig. 29) and have aluminum dominated chemistries (Fig. 30).

Boron, which has been used as an indication of the degree to which water interacts with rock (Palmer and Sturchio, 1990), is uncharacteristically low in mudpot samples, relative to pH (Fig. 27). This boron deficiency in filtered mudpot fluids could be explained by the ion exchange layer in clay minerals and the anion and cation exchange capacities of clays, in which boron is either intercalated or possibly adsorbed to the surface of clay particles in suspension (Fleet, 1965; Williams and Hervig, 2002; Williams, *et al.*, 2011). Either of these possibilities would give lower than expected boron concentrations for the pore fluids in mudpots. Boric acid has the

ability to adsorb to clay minerals, oxyhydroxides of aluminum, iron, and magnesium, as well as organic matter because of its capacity for hydrogen bonding (Huang and Li, 2012). Its adsorption can be described with a Langmuir isotherm.

Boron has been observed to be volatile in sulfuric acid, dilute and concentrated, however this occurs around 75°C and is minor (< 3%) in substantial solution volumes (Feldman, 1961). Boron is much more volatile in organic rich solutions, such as methanol, ethanol, or swamp water (Gaillardet *et al.*, 2001), however there is little indication at this time that mudpots are primarily comprised of organics. Volatilization of boron from mudpots is not a likely explanation for the uncharacteristic decrease, because the mudpot fluid volume is much larger than those described in earlier works and roughly half of all mudpots are cooler than temperatures described by Feldman (Feldman, 1961) (Fig. 8).

The general mudpot formation model is that mudpots are formed by processes of separation of steam at depth and oxidation of hydrogen sulfide to sulfuric acid (Allen and Day, 1935; Xu, 1998). Differences in mudpot clay composition support the assertion that differences in surficial geochemistry reflect regional variations in geology. Regional differences between the thermal areas in Yellowstone National Park become apparent when examining trace element distributions (Fig. 29). The dominant trace metals show chemical differences between the eastern and western thermal areas, with an anomaly found in the Greater Obsidian Pool Area, presumably because a mudpot was newly formed. Bulk composition concentrations from XRF and ICP-MS for several Pleistocene aged rhyolitic flows in YNP show chemical variations between the Hayden Valley Flow and the more western rhyolites in the Central Plateau Member (Girard, 2010). Within the multiple eruption cycle of the Yellowstone supervolcano, the magma, which formed the Hayden Valley Flow and Solfatara Plateau Flow, was geochemically distinct

from all other Central Plateau Member rhyolites. While no definitive connection exists at this time, there are relationships between the observed chemical variations in eastern mudpots and underlying Hayden Valley Flow.

Western mudpots contained higher percentages of arsenic, however this may be a subregional characteristic as it is mostly found in the Pocket Basin/Boulder Spring mudpots and notably absent from the more northern Sylvan Spring mudpot and the more southern Rabbit Creek mudpot (Fig. 29). Arsenic concentrations in sampled YNP hot springs may be partially related to the fractionation of thermal fluids at depth (Fig. 23c), whereas mudpot arsenic concentrations may be related to bulk composition of underlying rock units (Fig. 29). Crater Hills Geyser and a nearby hot spring, as well as sampled hot springs from Sylvan Springs thermal area, have uncharacteristically high chloride and arsenic concentration for acid sulfate features (Fig. 26).

Drill core log information from cores Y-3 (near Ojo Caliente hot spring) and Y-5 (near Rabbit Creek source hot spring) suggest that geology varies drastically between Rabbit Creek and Pocket Basin, with Rabbit Creek being underlain by older Lava Creek Tuff and Pocket Basin being underlain by younger Nez Perce Creek Flow in the Central Plateau Member rhyolite (Table I) (Keith and Muffler, 1978; Barger and Muffler, 1982; Barger and Beeson, 1985). There are noted differences between the composition of the Hayden Valley Flow and the Nez Perce Creek Flow due to age of eruption and changing melt composition (Girard, 2010).

4.3. Mudpots are Dynamic (Resurgent Domes/Seasonal Variations)

Allen and Day first observed the seasonal variation of mudpots in 1935. Seasonal variation was observed in chemical concentrations in a mudpot from the Rabbit Creek thermal area, sampled in September 2016 and July 2016. The concentration changes consisted of heavier

δD and $\delta^{18}O$ values, lower major anion and cation concentrations, and lower trace element concentrations in the July sample (Figures 12, 18, 29, 30). These changes indicate a concentration of the September sample, relative to the July sample. This concentration is most likely due to a decrease in shallow groundwater recharge as summer and fall in YNP can be drier than spring. The more apparent result of mudpot concentration is an increase in viscosity during the fall, when precipitation is less. This increase in viscosity was visually observed at seasonally sampled sites, such as GOPA, Crater Hills, and Rabbit Creek North (Table VII and Figure D-10)

In addition to the seasonal cycle of concentration and dilution, the mudpot at Rabbit Creek thermal area changed overall composition from ~21% aluminum and ~64% iron in September to ~64% aluminum and ~1% iron in July (Table XXXI). This dramatic shift in composition from fall to summer may be related to the change in pH from 3.4 in the fall to 2.6 in the summer (Fig. 8). Given that EQ3 predicted iron minerals to be supersaturated in mudpots and the pink color of the mudpot is assumed to be from iron minerals, it could be that the composition change of the filtered mudpot fluid reflects a change in equilibrium between solids and dissolved species. This pH, dissolved aluminum, and iron relationship is counterintuitive. The September pH measurement was taken hours later at 25°C, due to equipment issues in the field, therefore if the September pH measurement was ≥ 0.5 units less, pH could not explain the change. Additional seasonal sampling is required to better quantify the geochemical variations of mudpots.

In addition to seasonal variation due to cycles of concentration and dilution, a mudpot formed during the two week July sampling season (Fig. D - 9). Phantom Pants, which had historically been a “frying pan” (an area with more water than a fumarole, but less water than a hot spring), became more hydrated and developed viscosity. Over the course of two weeks in

July 2016, Phantom Pants decreased in viscosity and bulk density. The mud had coarser particulates than the nearby Pedro's Tar pit, but resulted in similar mineral identification (Table VII). This regional level dynamism likely results from close proximity to the Sour Creek resurgent dome, which may alter flow paths for thermal waters and steam.

4.4. Preliminary Summary of Mudpot Characteristics

Allen and Day in 1935 defined mudpots as, "hot springs carrying very little water and a large amount of fine grained mineral matter". Allen and Day did not consider viscosity a defining characteristic and note that there is, "no dividing line between mud pots and muddy springs". This characterization of an acid sulfate spring that just happens to be incredibly turbid is limited and does not accurately reflect the chemical characteristics nor the diversity within mudpots as a whole. While mudpots share similarities in formation with acidic hot springs, the increased water rock interaction and formation of clays alters trace element composition of filtered mudpot fluids, setting them apart from acidic hot springs. Mudpots were found to be acidic to circumneutral (Fig. 8), have no characteristic dissolved oxygen concentrations (Fig. 11), have steam based source waters (Fig. 39), have millimolal concentrations of sulfate (Fig. 21), have pore fluids either dominated by dissolved iron or aluminium (Fig.30), and have unique chemical "fingerprints" that can be used to identify underlying geology (Fig. 29). Dissolved oxygen measurements of mudpots and hot springs corresponded with the dissolved oxygen vs temperature equilibrium curve (Fig. 11). Above 60°C, field measurements are undersaturated and may be undergoing deaeration (Fig. 11). Solubility properties controlled oxygen concentrations in both mudpots and hot springs, rather than other environmental factors. Due to higher conductivities found in thermal features than freshwater, the field measured dissolved oxygen concentrations drop precipitously with temperature more so than expected in freshwater. The

absolute temperature boundary for photosynthesis is included to provide context for the sample set (Brock, 1967a; Cox *et. al.*, 2011). While Allen and Day's definition is still essentially correct, it is a gross oversimplification that ignores the key characteristics found to be common amongst all mudpots sampled in this study.

The conductivity of salt solutions with suspended clays can be different by a factor of five when compared to similar non-turbid salt solutions (Shainberg and Levy, 1975). Higher clay concentrations, in grams of clay/100mL of solution, have greater differences in conductivity between the suspended clay solution and the standard salt solution (Fig. 9). This documented discrepancy in conductivities likely arises from the charged nature of clay minerals, as well as the ion exchange layer. In addition to skewing individual measurements, this discrepancy suggests that conductivities would be variable within a single mudpot if there was heterogeneity in clay concentrations, as visually observed in several large mudpots. Additionally, mudpot sample clay concentrations fall within a range of 9 grams dry clay/100 mL salt solution to 60 grams dry clay/100 mL salt solution, the clay compositions of sampled mudpots are mostly alunite, kaolinite, and montmorillonite (Table VII), and the conductivities are lower than the reference salt solution, all of which is outside the scope of the Shainberg and Levy study. Additional work is needed to investigate the relationship between higher clay concentrations and a standard salt solution, in order to give context to field measurements.

In Yellowstone National Park, mudpots are primarily comprised of kaolinite, K-alunite, and montmorillonite, however variations arise based on regional underlying geology (Table VII). These clays are the product of argillic and sericitic alteration (equations from Section 1.1). Mudpots form when acidic steam alters surficial geology which create suspensions of clay minerals. SEM images may present a snapshot into different stages of hydrothermal alteration

occurring in mudpots over time. Although pyrite, barite, and elemental sulfur have been seen in SEM images of dried mud sample, these minerals may not all exist in *in situ* mudpot conditions. However, they may be found in clay deposits formed by mudpots (Figures 31 and 32). Given the samples collected from the newly formed mudpot in GOPA in addition to samples collected from an established mudpot in GOPA, it is likely that in addition to chemical changes that alter minerals in rhyolite to form clays, there is “weathering” that occurs in mudpots to decrease particulate size and increase homogeneity over time.

Speciation calculations with EQ3 and the *jus* database predicted supersaturation of alunite and ferric iron minerals in mudpots, which is logical considering mudpots are acidic and have millimolar concentrations of sulfate; however, this may not represent a complete prediction of the mineral composition of mudpots because HaloSpec Near Infrared analysis of dried mud samples gives readings of kaolinite, illite, halloysite, iron montmorillonite as well as alunite (Table VII). The database file used for EQ3 models only includes kaolinite and alunite, which accounts for some of the discrepancy, however the model may be lacking a key parameter to fully represent observed mudpot conditions. The iron minerals modeled seem realistic because observed rust colored precipitates in suboxic, sealed clay composition bottles stored at room temperature before desiccation and analysis may show the presence of iron in the stored sample, which is consistent with the EQ3 prediction of iron containing minerals.

The TerraSpec Halo Near Infrared portable spectrometer does not recognize quartz. Given that YNP drill core mineralogies contain polymorphs of silica, as well as silica being a side product of argillic and sericitic alteration (Eqn 4 and 5), it is expected that mudpots contain amorphous silica or a polymorph of silica. Further mineralogical work with dried clay samples

should include x-ray diffraction (XRD), with and without glycolation, to better identify minerals present.

Mudpots and acid sulfate hot springs are chemically similar, with respect to major anions and cations, however most sampled mudpots fluids contain $\geq 65\%$ iron and aluminum, whereas most sampled acid sulfate hot springs contain $\leq 45\%$ iron and aluminum, with many containing $\leq 10\%$ iron and aluminum (Table XXXI). This distinction in relative abundances of trace elements in mudpots and acid sulfate hot springs allows for mudpot chemistries to be divided by underlying rock unit (Fig. 29), however, hot springs trace element concentrations are often thermal region specific and broad relationships are not possible. Additionally, mudpots are likely to host unique microbial ecosystems that vary within the mudpot due to visually observed heterogeneous viscosity and likely dissolved oxygen. This variation would be unlike hot springs of similar pH and temperature.

4.5. Comparison of Mudpots and Adjacent Hot Springs by Thermal Area

When considering the cation and anion concentrations of mudpots and their adjacent hot springs, if the thermal area has an overall trend towards acid sulfate springs, then the major anions and cations may have similar concentrations (within an order of magnitude). However, if the nearby hot spring is an alkaline chloride feature, the mudpot will have lower concentrations of lithium, sodium, fluoride, and chloride (more than an order of magnitude). For instance, in the Pocket Basin thermal area the sampled alkaline chloride hot springs have pH values of 7.3 and 9.1 and the sampled mudpots have pH values ranging from 2.5 to 5.7. In Sylvan Springs thermal area, pH values of the mudpot and two of the acid sulfate hot springs sampled ranged from 1.8 to 2.8, and the other three sampled turbid hot springs ranged from pH 4.5 to 5.4. The more acidic

features had more similar chemistries, and the turbid, less acidic features had similar chemistries (Fig. 17). This grouping by pH rather than turbidity may suggest that steam fractionation of thermal source waters and meteoric input are a better indication of similar chemistries than turbidity. In Rabbit Creek North, there are acidic turbid hot springs and mudpots in one area and an alkaline hot spring with a photosynthetic outflow in another area, however the cations and anions for the area present similarly to a mixing of thermal waters (Fig. 18), with the mudpot and the alkaline chloride source as endmembers and a pH 3.4 turbid spring as a mixed thermal feature.

4.6. Comparisons with Known International Mudpot Geochemistry

A few issues exist in drawing comparisons between this work and the published mudpot data. Most limiting is inconsistency in sampling and sample processing methods. This study used gravity and syringe filtration to reach a final pore size of 0.2 μm . Filtration generally occurred for 30 to 45 minutes before preserving samples. Some studies chose to collect mudpot suspended sediments and digest in a laboratory setting (Kanellopoulos, 2016), while others let samples settle and decanted off supernatant for filtration (Allen and Day, 1935). For highly weathered mudpots with clay sized particulate, settling and decantation can be a lengthy process during which redox chemistries can change. Another filtration method is high pressure field extraction of pore fluids (Bortnikova, 2010). While this process is much faster, there is potential for any biological material to disproportionately contribute to total dissolved concentrations in the filtered fluids via cell lysis.

Aside from drastically differing methods, limited sampling parameters can make comparisons beyond *in situ* meters troubling. Aside from geochemical work with Siberian mudpots, most mudpot research is limited to a single geochemical facet, such as δD and $\delta^{18}\text{O}$

values (Shevenell, *et al.*, 1987). This is likely due to the difficulties that arise in filtering a sufficient volume of mudpot fluid for preservation and lab analysis. Pairing methodological difficulties with limited existing mudpot research means few comparisons can be made.

Much existing mudpot research focuses on mineralogy, which is inherently specific to field areas due to underlying rock units. However, it was found in Nicaraguan mudpots with a similar near infrared portable spectrometer, the TerraSpec 4, that hydrothermal alteration from Nicaraguan mudpots yield clay minerals and iron oxides (Marcucci *et al.*, 2013). XRD analysis of dried solids from Greek mudpots found clay minerals, elemental sulfur, quartz, barite, and halite (Kanellopoulos *et al.*, 2016). SEM and TerraSpec Halo results of dried solids from YNP mudpots have similar findings, with the exception of halite (Table VII). Two inherent possible issues with mineralogical analysis of slowly dried mudpot solids are loss of hydrated minerals and crystallization of species not found *in situ*. It is suspected that results such as barite crystals may not reflect realistic mineralogy of active mudpots, however could be found in clay deposits formed by now inactive mudpots. Siberian mudpots hosted in basalt contained kaolinite, smectite, pyrite, marcasite, quartz, alunogene, and anhydrite (Bortnikova, 2009). Additional in depth study of clays from international mudpots is required to better understand variations in mudpot hydrothermal alteration in relation to host rock.

4.7. Microbial Communities in YNP Mudpots

From the four analyzed mudpots, there were no genera of Bacteria or Archaea that were found in all of them, however both sampled mudpots from the Pocket Basin and Pedro's Tarpit from GOPA shared the archaeal genera, *Methanosaeta*, *Methanobrevibacter*, *Methanobacterium*, and *Candidatus Nitrososphaera* (Fig. 34). Additionally, *Nitrososphaera* and *Methanococcus* were unique to the Pocket Basin. *Thermoplasma* was found in both Pedro's Tarpit and Urinal Cake.

Caldococcus was found in both Phantom Pants and Urinal Cake. Methanogenesis, ammonia oxidation, and sulfur reduction are known metabolisms associated with organisms in these genera (Table IX). This may suggest complex archaeal communities thriving in mudpots, with heterogeneous viscosities, temperatures, and concentrations of dissolved gases, such as oxygen, carbon dioxide, ammonia, and hydrogen sulfide.

There were far fewer bacterial genera found in mudpots than archaeal genera (Fig. 34 and Table IX). While there were no genera shared in all the samples, *Ralstonia* was found in both sampled mudpots in GOPA. *Caenimonas* was common to Pedro's Tarpit in GOPA and Consolation Prize in Pocket Basin. *Corynebacterium* and *Curvibacter* were common to Phantom Pants in GOPA and Urinal Cake in Pocket Basin. Many of the bacterial genera found in the mudpots are either resistant to metals in environmental samples, or grow better in the presence of metals (Table IX) (Bergey, 2015). Two genera of bacteria found in the mudpot Phantom Pants, *Ralstonia* and *Geobacillus*, are known to use nitrite and nitrate, respectively, as electron acceptors (Table IX). These could hint towards microbial nitrogen cycling in mudpots. Pedro's Tarpit is a well-established mudpot near the newer mudpot Phantom Pants in GOPA. Pedro's Tarpit hosts a more diverse microbial community with a greater number of known metabolisms than Phantom Pants. It is possible that the relative lack of microbial diversity in Phantom Pants may be attributed to its recent formation.

In addition to 16S rRNA gene diversity information from four sampled mudpots, one Pocket Basin hot spring was sequenced. Unfortunately, this pH 9.1 hot spring with a source temperature of 79.1°C is a less than ideal microbial comparison with mudpots. Both Pocket Basin mudpots had been hotter but were within an order of magnitude with respect to dissolved oxygen concentrations. The archaeal and bacterial class relative abundances between the hot

spring and most sampled mudpots, excluding the new GOPA mudpot, were similar, however archaeal and bacterial genera relative abundances were different between mudpots and the hot spring. The hot spring shared more archaeal genera with the nearby sampled mudpots (Fig. 36), than the eastern mudpots, which may suggest a microbial ecosystem that is somewhat specific to Pocket Basin, or the larger Midway Geyser Basin, which share some geochemical characteristics.

However, it is ill advised to make broad statements regarding similarity of microbial communities based on one alkaline chloride hot spring. The Pocket Basin hot spring is chemically dissimilar from the nearby mudpots with respect to major cations and anions (Fig. 19) and likely formed from boiling alkaline chloride waters undergoing nearly continuous steam separation (Fig. 39) whereas the mudpots are formed from highly evaporated acid sulfate steams. Good chemical analogs would be acid sulfate hot springs with high total dissolved aluminum and iron, relative to other trace elements.

4.8. Future Work

In order to continue mudpot studies, the sampling regimen needs to be expanded, lab experiments and geochemical modelling needs to be done to provide context for the datasets, and additional types of analyses need to be explored to better understand the samples that already have been collected. An expanded sampling regimen would sample more mudpots and maintain long-term sampling of previously sampled mudpots, sample more eastern features to provide a more balanced picture of YNP, aim to measure nearby freshwater whenever possible to provide a contrast from meteoric waters, and incorporate field spectrophotometric values for dissolved silica and ammonium concentrations. The field spectrophotometric values lend greater accuracy

to geochemical models of water-rock interactions because they would supplement existing major anions and cations data.

An experiment replicating the Shainberg *et al.* conductivity of clays work, but expanding the experiment to include montmorillonite, K-alunite, illite, and kaolinite and much higher clay concentrations, would provide framework for considering field conductivity readings of mudpots. It would likely allow for a correction factor to account for different clay minerals and different in situ viscosities of mudpots.

An assessment of volatility of boron in mudpots that considers pH, temperature, dissolved organic carbon, and salinity would be useful in determining the quantity of boron that is sorbing to clay particles, intercalating into the structure of expanding clays, and volatilizing into the atmosphere. Understanding how boron partitions in environmental samples would allow for more accurate models to be made for mudpot systems.

Additional analysis should include detailed scanning electron microscopy (SEM) investigation of the mudpots, Phantom Pants and Pedro's Tarpit, to see if the secondary alteration or weathering occurs after a mudpot is formed. X-ray powder diffraction should be run on glycolated and non-glycolated samples that have been separated in sedimentation columns to confirm the TerraHalo Spec near infrared handheld spectrometer's identification of clay minerals. DNA extractions for 16S rRNA gene diversity and potentially metagenomics should be done for Jabberwocky, Alice, and Burning Eye, as they are the closest chemical analogs to mudpots.

Additional 16S rRNA microbial gene diversity analyses should be completed to determine if there are any genera common to all mudpots. In addition to diversity studies, proteins should be extracted and analyzed to determine microbial metabolic processes occurring

at the time of sampling. Mudpot culture experiments should be devised to better understand nutrient demands and microbe-metal interactions.

5. Conclusion

The effort to create a geobiochemical characterization of YNP mudpots has yielded both straightforward, expected findings, as well as insight into the nature of thermal aquifers in Yellowstone and the interconnected nature of all thermal features in the park. From the initial observations and preliminary field measurements from Allen and Day in 1935, it comes as no surprise that mudpots exhibit chemical changes seasonally which follow the visually observed change to viscosity. The striking color variation in YNP mudpots observed by Raymahashay as well as Allen and Day is due to supersaturation of iron minerals in mudpot fluids. Boron, a measure of the degree of water-rock interaction, is uncharacteristically low in mudpots compared to other thermal features of similar pH and temperatures. The excess boron that is expected to exist in mudpots may be sorbing to kaolinite surfaces and intercalating into ion exchange layers of smectites.

Given the evaporated nature of the sampled thermal features, it was expected that hot springs and mudpots are isotopically distinct from meteoric water, indicating that the thermal fluids undergo more evaporation than meteoric water. Acid sulfate features are fed by more evaporated thermal fluids and show a relationship between pH and degree of evaporative loss. Conversely, alkaline chloride features show less evaporated thermal fluids. Somewhat less expected, but nonetheless straightforward, is the finding that mudpots are not anoxic, but follow a deaeration curve, and can be just as oxic as hot springs of similar temperatures. Field measurements of mudpots were problematic because concentrated clay suspensions affect conductivity.

Prior to this work, little was known about microbial communities in YNP mudpots. Sampled mudpots are likely complex mesotrophic ecosystems of biotic methanogenesis, nitrogen

cycling, and heterotrophy. While no evidence for methanotrophy was found in the four sampled mudpots, methanotrophic *Verrucomicrobia methylacidiphilum* has been found in the Italian mudpot, Campi Flegrei.

Very few general statements can be made regarding the flow of thermal source waters in Yellowstone National Park, because the thermal aquifer material is likely highly faulted and comprised of heterogeneous volcanic pseudo-stratification. However, Sylvan Springs thermal area was likely historically fed by the same thermal aquifer that today sources thermal features in the Midway Geyser Basin. At some point, the thermal fluid pathways changed, and Sylvan Springs thermal area is now sourced with different thermal fluids than the Midway Geyser Basin. In addition to this example of changing thermal water pathways, the resurgent domes have been observed to cause thermal areas, such as GOPA, to be more dynamic, and dome activity may indirectly cause mudpot formation.

Contrary to predictions by Allen and Day, neither turbidity nor proximity are reliable indicators of chemical similarity between mudpot and adjacent hot springs. Instead, the formation mechanism serves to determine the major chemistry, such as pH and total dissolved major cations and anions. These thermal source fluids, with their often non-intuitive subsurface pathways and separation of steam from briny liquid, better determine the chemical composition of a feature than physical characteristics.

The importance of this work with mudpots is derived from the unexpected conclusions regarding the interconnectedness of the geology, microbiology, and geochemistry of thermal features in the park. Mudpots in Yellowstone National Park can be used to determine underlying rock units through trace metal relative abundances, if total dissolved iron and aluminum comprise at least 65% of the mudpot's trace metal chemistry. This "fingerprinting" capability is

not feasible for hot spring trace metal chemistry because the composition is variable and rarely is at least 65% iron and aluminum (Table XXXI). Biotic processes in mudpots are likely to affect the surficial chemical concentrations of filtered mudpot fluid, especially nitrogenous species and dissolved organic carbon. Each thermal area is likely a unique microbial niche that has adjusted to the chemical parameters of that subregion.

6. References Cited

- Allen E. T. and Day A. L. (1935) *Hot Springs of the Yellowstone National Park*, Carnegie Institution of Washington, Washington, D.C.
- Amend J. P. and Shock E. L. (2001) Energetics of overall metabolic reactions in thermophilic and hyperthermophilic Archaea and bacteria. *FEMS Microbiol. Rev.* **25**, 175–243.
- Bargar K. E. and Beeson M. H. (1985) Hydrothermal Alteration in Research Drill Hole Y-3, Lower Geyser Basin, Yellowstone National Park, Wyoming. *U.S. Geological Survey Professional Paper* 1054–C.
- Bessonova E. P., Bortnikova S. B., Gora M. P., Manstein Y. A., Shevko A. Y., Panin G. L. and Manstein A. K. (2012) Geochemical and geo-electrical study of mud pools at the Mutnovsky volcano (South Kamchatka, Russia): Behavior of elements, structures of feeding channels and a model of origin. *Appl. Geochemistry* **27**, 1829–1843.
- Bortnikova S. B., Bortnikova S. P., A M. Y., Kiryuhin A. V, Vernikovskaya I. V and Palchik N. A. (2009) Thermal Springs Hydrogeochemistry and Structure At North- Mutnovskoe Fumarole Field (South Kamchatka , Russia). *Proceedings of the 34th Workshop on Geothermal Reservoir Engineering*. Stanford, CA: Stanford University.
- Bortnikova S. B. ., Gavrilenko G. M. ., Bessonova E. P. . and Lapukhov A. S. . (2010) The hydrogeochemistry of thermal springs on Mutnovskii Volcano, southern Kamchatka. *J. Volcanol. Seismol.* **3**, 388–404.
- Boyd E. S., Hamilton T. L., Spear J. R., Lavin M. and Peters J. W. (2010) [FeFe]-hydrogenase in Yellowstone National Park: evidence for dispersal limitation and phylogenetic niche conservatism. *ISME J.* **4**, 1485–1495.
- Bradley A. S., Love G. D., Brocks J. J., Sievert S. M. and Summons R. E. (2005) Branched Alkanes with Quaternary Carbon Centers (BAQCs): Biomarkers for Ancient Microbes or Recent Microbial Activity? *American Geophysical Union Fall Meeting Eos Trans.* San Francisco, CA: AGU.
- Brazelton, W.J., Ludwig, K.A., Sogin, M.L., Andreishcheva, E.N., Kelley, D.S., Shen, C., Edwards, R. L., Baross, J.A., Colwell, R.R. (2010) Archaea and Bacteria with Surprising Microdiversity Show Shifts in Dominance over 1000 year Time Scales in Hydrothermal Chimneys. *Proceedings of the National Academy of Sciences USA.* **107**. 1612 – 1617.
- Brock T. D. (1967a) Microorganisms adapted to high temperatures. *Nature* **214**, 882 - 885.
- Christiansen R. L. and Blank H. R. (1972) Volcanic Stratigraphy of the Quaternary Rhyolite Plateau in Yellowstone National Park. *U.S. Geological Survey Professional Paper* 729–B.

- Clark, I., and P. Fritz, (1997) *Environmental Isotopes in Hydrogeology*, Lewis Publishers, Boca Raton.
- Cline J. D. (1969). Spectrophotometric determination of hydrogen sulfide in natural waters. *Anal. Chem.* **21**, 1005-1009.
- Cox A., Shock E. L. and Havig J. R. (2011) The transition to microbial photosynthesis in hot spring ecosystems. *Chem. Geol.* **280**, 344–351.
- Craig H. (1961) Isotopic Variations in Meteoric. *Science (80)*. **133**, 1702–1703.
- Deamer D., Singaram S., Rajamani S., Kompanichenko V. and Guggenheim S. (2006) Self-assembly processes in the prebiotic environment. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* **361**, 1809–18.
- del Giorgio P. and Williams P. J. le B. (2005) *Respiration in Aquatic Ecosystems.*, Oxford University Press, New York.
- Dick J. M. (2008) Geochemical Transactions Calculation of the relative metastabilities of proteins using the CHNOSZ software package. *Geochem. Trans.* **9**, 1467–4866.
- Dowd, S. E. (2017) Methods and Overview Readme. *Mr. DNA Molecular Research LP*. Version 213.
- Feldman C. (1961) Evaporation of Boron from Acid Solutions and Residues. *Anal. Chem.* **33**, 1916–1920.
- Fleet M. E. L. (1965) Preliminary Investigations into the Sorption of Boron by Clay Minerals. *Clay Miner.* **6**, 3–16.
- Fournier R. O. (1989) Geochemistry and Dynamics of the Yellowstone National Park Hydrothermal System. *Ann. Rev. Earth Planet. Sci* **17**, 13–53.
- Gaillardet J., Lemarchand D., Gopel C. and Manhès G. (2001) Evaporation and Sublimation of Boric Acid: Application for Boron Purification from Organic Rich Solutions. *J. Geostand. Geoanalysis* **25**, 67–75.
- Girard G. and Stix J. (2010) Rapid extraction of discrete magma batches from a large differentiating magma chamber: The Central Plateau Member rhyolites, Yellowstone caldera, Wyoming. *Contrib. to Mineral. Petrol.* **160**, 441–465.
- Gundersen, J., Ramsing, N., (2003) Seawater and Gases: Tabulated Physical Parameters of Interest to People Working With Microsensors in Marine Systems. *Unisense*.
<http://www.unisense.com/files/PDF/Diverse/Seawater%20&%20Gases%20table.pdf>

- Hach Company. (2014) Silicomolybdate EPA Method 8185. 1 – 5.
- Havig J. R., Raymond J., Meyer-Dombard D. R., Zolotova N. and Shock E. L. (2011) Merging isotopes and community genomics in a siliceous sinter-depositing hot spring. *J. Geophys. Res.* **116**, G01005.
- Held P. G. (2001) Nucleic Acid Purity Assessment using A 260 /A 280 Ratios. Available at: https://www.biotek.com/resources/docs/PowerWave200_Nucleic_Acid_Purity_Assessment.pdf
- Helgeson H. C. (1969) Thermodynamics of hydrothermal systems at elevated temperatures and pressures. *Am. J. Sci.* **267**, 729– 804.
- Helgeson H. C., Delany J. M., Nesbitt H. W. and Bird D. K. (1978) Summary and critique of the thermodynamic properties of rock-forming minerals. *Am. J. Sci.* **278A**, 1–229.
- Huang P. M., Li Y. and Sumner M. E. (2012) Handbook of soil sciences: Properties and Processes, CRC Press, Boca Raton.
- Huber J. A., Butterfield D. A. and Baross J. A. (2002) Temporal changes in archaeal diversity and chemistry in a mid-ocean ridge seafloor habitat. *Appl. Environ. Microbiol.* **68**, 1585–1594.
- IUPAC – IUB Commission on Biochemical Nomenclature (CBN), (1992) *Biochemical Nomenclature and Related Documents*. Portland Press, 122 – 126.
- Janda, J.M., Abbott, S.L. (2007) 16S rRNA Gene Sequencing for Bacterial Identification in the Diagnostic Laboratory: Pluses, Perils, and Pitfalls. *J. Clin. Microbiol.* **45**, 2761 – 2764.
- Johnson J. W., Oelkers E. H. and Helgeson H. C. (1992) SUPCRT92: a software package for calculating the standard molal thermodynamic properties of minerals, gases, aqueous species, and reactions from 1 to 5000 bar and 0 to 1000 C. *Comput. Geosci.* **18**, 899–947.
- Johnston J. H. and Cardile C. M. (1987) Iron Substitution in Montmorillonite, Illite, and Glauconite by ^{57}Fe Mossbauer Spectroscopy. *Clays Clay Miner.* **35**, 170–176.
- Kanellopoulos C. and Xirokostas N. (2016) Mudpots At Stefanos Hydrothermal Crater of Nisyros Volcano: an Insight At the Hydrothermal Processes of an Active Volcano. *Bulletin of the Geological Society of Greece, Proceedings of the 14th Intern. Congress*. Thessaloniki, Greece.
- Keith T. E. C. and Muffler L. J. P. (1978) Minerals produced during cooling and hydrothermal alteration of ash flow tuff from Yellowstone drill hole Y-5. *J. Volcanol. Geotherm. Res.* **3**, 373–402.

- Keith E. Bargar L. J. P. M. (1982) Hydrothermal Alteration in Research Drill Hole Y-11 from a Vapor-Dominated Geothermal System at Mud Volcano, Yellowstone National Park, Wyoming. *Geology of Yellowstone Park Area; 33rd Annual Field Conference Guidebook*, 139–152.
- Kharaka Y. K., Thordsen J. J. and White L. D. (2002) Isotope and chemical Compositions of Meteoric and Thermal Waters and Snow from the Greater Yellowstone National Park Region. *U.S. Geological Survey Open-File Report* 2002-194.
- Levinson A. A. and Day J. J. (1968) Low temperature hydrothermal synthesis of montmorillonite. Ammonium-micas and ammonium-zeolites. *Earth Planet. Sci. Lett.* **5**, 52–54.
- Marcucci E. C., Hynek B. M., Kierein-Young K. S. and Rogers K. L. (2013) Visible-near-infrared reflectance spectroscopy of volcanic acid-sulfate alteration in Nicaragua: Analogs for early Mars. *J. Geophys. Res. Planets* **118**, 2213–2233.
- Mayer K., Scheu B., Montanaro C., Yilmaz T. I., Isaia R., Aßbichler D. and Dingwell D. B. (2016) Hydrothermal alteration of surficial rocks at Solfatara (Campi Flegrei): Petrophysical properties and implications for phreatic eruption processes. *J. Volcanol. Geotherm. Res.* **320**, 128–143.
- Moloney, Timothy P.; Sims, Kenneth W.; and Kaszuba, John P. (2011) Uranium and Thorium Decay Series Isotopic Constraints on the Source and Residence Time of Solutes in the Yellowstone Hydrothermal System. *University of Wyoming National Park Service Research Center Annual Report: 33*, Article 26.
- Morgan L. A. and Shanks W. C. P. I. (2005) Influences of Rhyolitic Lava Flows on Hydrothermal Processes in Yellowstone Lake and on the Yellowstone Plateau. *Geotherm. Biol. Geochemistry Yellowstone Natl. Park*, 32–52.
- Mulkidjanian a. Y., Bychkov a. Y., Dibrova D. V., Galperin M. Y. and Koonin E. V. (2012) PNAS Plus: Origin of first cells at terrestrial, anoxic geothermal fields. *Proc. Natl. Acad. Sci.* **109**, E821–E830.
- Oliveros, J.C. (2007-2015) Venny. An interactive tool for comparing lists with Venn's diagrams. <http://bioinfogp.cnb.csic.es/tools/venny/index.html>
- Palmer M. R. and Sturchio N. C. (1990) The Boron Isotope Systematics of the Yellowstone-National-Park (Wyoming) Hydrothermal System - a Reconnaissance. *Geochim. Cosmochim. Acta* **54**, 2811–2815.
- Pol A., Barends T. R. M., Dietl A., Khadem A. F., Eygensteyn J., Jetten M. S. M. and Op den Camp H. J. M. (2014) Rare earth metals are essential for methanotrophic life in volcanic mudpots. *Environ. Microbiol.* **16**, 255–264.

- Presens Precision Sensing GmbH. *User's Manual for DP – PSt3 oxygen dipping probe*, Regensburg, Germany.
- Prokofeva M. I., Rusanov I. I., Pimenov N. V and Bonch- E. A. (2006) Organotrophic Activity in Kamchatka Hot Springs with Low pH. *Microbiol. Orig. Russ. Text* **75**, 26–2617.
- Pruesse, E., Quast, C., Knittel, K., Fuchs, B.M., Ludwig, W., Peplies, J., Glöckner, F.O. (2007) SILVA: a comprehensive online resource for quality checked and aligned ribosomal RNA sequence data compatible with ARB. *Nucleic Acids Res.* **35**, 7188 – 7196.
- Raymahashay B. C. (1968) A geochemical study of rock alteration by hot springs in the Paint Pot Hill area, Yellowstone Park. *Geochim. Cosmochim. Acta* **32**, 499–522.
- Schott J., Kretzschmar J., Tsushima S., Drobot B., Acker M., Barkleit A., Taut S., Brendler V., Stumpf T., Cleve S. M. Van, Depmeier W., Gagliardi L., Shatruk M., Knappenberger K. L., Liu G., Skanthakumar S., Soderholm L., Dixon D. A. and Albrecht-Schmitt T. E. (2015) The interaction of Eu(iii) with organoborates – a further approach to understand the complexation in the An/Ln(iii)–borate system. *Dalt. Trans.* **44**, 11095–11108.
- Schmidt, R. (2017, in prep) Biogeochemical Interactions of Flooded Underground Mine Water. (Master Thesis)
- Shainberg I. and Levy R. (1975) Electrical Conductivity of Na-Montmorillonite Suspensions. *Clays Clay Miner.* **23**, 205–210.
- Shevenell L., Counce D., Goff F., Janik C. J., Vuataz F., Evans W., Trujillo P. E., Vuataz F., Janik C. J. and Evans W. (1987) Hydrogeochemical Data for Thermal and Nonthermal Waters and Gases of the Valles Caldera- Southern Jemez Mountains Region, New Mexico. *Los Alamos National Laboratory*. Technical Report LA – 10923 – OBES.
- Shock E. L., Helgeson H. C. and Sverjensky D. A. (1989) Calculation of the thermodynamic and transport properties of aqueous species at high pressures and temperatures: standard partial molal properties of inorganic neutral species. *Geochim. Cosmochim. Acta* **53**, 2157–2183.
- Shock E. L., Oelkers E. H., Johnson J. W., Sverjensky D. A. and Helgeson H. C. (1992) Calculation of the thermodynamic properties of aqueous species at high pressures and temperatures: effective electrostatic radii, dissociation constants, and standard partial molal properties to 1000 C and 5 kb. *J. Chem. Soc., Faraday Trans.* **88**, 803–826.
- Shock E. L. and McKinnon W. B. (1993) Hydrothermal processing of cometary volatiles – applications to triton. *Icarus* **106**, 464– 477.
- Shock E. L., Sassani D. C., Willis M. and Sverjensky D. A. (1997) Inorganic species in geologic fluids: correlations among standard molal thermodynamic properties of aqueous ions and hydroxide complexes. *Geochim. Cosmochim. Acta* **61**, 907–950.

- St. Clair, B. (2017) Kinetics, Thermodynamics, and Habitability of Microbial Iron Cycling. (Doctoral dissertation) Retrieved from ASU Library Digital Repository.
- Sverjensky D. A., Shock E. L. and Helgeson H. C. (1997) Prediction of the thermodynamic properties of aqueous metal complexes to 1000 C and 5 kb. *Geochim. Cosmochim. Acta* 61, 1359–1412.
- Szynkiewicz A., Johnson A. P. and Pratt L. M. (2012) Sulfur species and biosignatures in Sulphur Springs, Valles Caldera, New Mexico—Implications for Mars astrobiology. *Earth Planet. Sci. Lett.* **321**, 1 - 13.
- Tamura, H.; Goto, K.; Yotsuyanagi, T.; Nagayama, M. (1974) Spectrophotometric determination of iron (II) with 1,10-phenanthroline in the presence of large amounts of iron(III) *Talanta* **21**, 314 – 318.
- Trainer F. W., Rogers R. J. and Sorey M. L. (2000) Geothermal Hydrology of Valles Caldera and the Southwestern Jemez Mountains, New Mexico. *U.S. Geological Society Water-Resources Investigations Report* 00–4067.
- Truesdell A. H., Nathenson M. and Rye R. O. (1977) The effects of subsurface boiling and dilution on the isotopic compositions of Yellowstone thermal waters. *J. Geophys. Res.* **82**, 3694–3704.
- Richmond, G.L., Pierce, K.L., Waldrop, H.A., (1972) Surficial geologic map of Yellowstone National Park: U.S. Geological Survey Miscellaneous Geologic Investigations Map I-710, scale 1:125,000
- Weiss, R.F. (1970) The solubility of nitrogen, oxygen, and argon in water and seawater. *Deep Sea Research.* **17**, 721 – 735.
- Whitman, W. (editor). (2015) *Bergey's Manual of Systematics of Archaea and Bacteria*. Wiley Online Library, Bergey's Manual Trust.
- Williams L. B. and Hervig R. L. (2002) Exploring intra-crystalline B-isotope variations in mixed-layer illite-smectite. *Am. Mineral.* **87**.
- Williams, L. B., Holloway, J. R., Canfield, B., Glein, C. R., Dick, J. M., Hartnett, H. E., & Shock, E. L. (2011). Birth of biomolecules from the warm wet sheets of clays near spreading centers. In *Earliest Life on Earth: Habitats, Environments and Methods of Detection* (pp. 79-112). Springer Netherlands.
- Wilson J., Savage D., Cuadros J., Shibata M. and Ragnarsdottir K. V. (2006) The effect of iron on montmorillonite stability. (I) Background and thermodynamic considerations. *Geochim. Cosmochim. Acta* **70**, 306–322.

- Wilson J., Cressey G., Cressey B., Cuadros J., Ragnarsdottir K. V., Savage D. and Shibata M. (2006) The effect of iron on montmorillonite stability. (II) Experimental investigation. *Geochim. Cosmochim. Acta* **70**, 323–336.
- Woese, C.R., Fox, G.E. (1977) Phylogenetic structure of the prokaryotic domain: the primary kingdoms. *Proc. Natl. Acad. Sci. USA* **74**, 5088 – 5090.
- Wollery, T.J. (1992) EQ3NR, a computer program for geochemical aqueous speciation-solubility calculations: theoretical manual, user's guide, and related documentation (version 7.0). *Lawrence Livermore National Laboratory Report UCRL-MA-110662* **3**.
- Wollery T. W. and Jarek R. L. (2003) Software User's Manual EQ3/ 6, Version 8.0. U.S. Department of Energy, Office of Civilian Radioactive Waste Management, Office of Repository Development, Software Document Number 10813-UM-8.0-00, 376 pp.
- Xu Y., Schoonen M. A. A., Nordstrom D. K., Cunningham K. M. and Ball J. W. (1998) Sulfur geochemistry of hydrothermal waters in Yellowstone National Park: I. The origin of thiosulfate in hot spring waters. *Geochim. Cosmochim. Acta* **62**, 3729 - 3743.
- Yuan Y., Shi G., Yang M., Wu Y., Zhang Z., Huang A. and Zhang J. (2014) Formation of a Hydrothermal Kaolinite Deposit from Rhyolitic Tuff in Jiangxi, China. *J. Earth Sci.* **25**, 495–505.

7. Appendix A: Biological Data

Table XI: Relative abundance of bacterial classes.

Bacterial Class	160424L Mudpot	160517E Mudpot	160715X Hot Spring	160715V Mudpot	160715T Mudpot
Acidobacteriia	0.0	0.1	0.0	0.0	0.1
Actinobacteria	8.7	1.1	19.7	25.9	11.7
Alphaproteobacteria	1.9	0.2	0.1	23.8	0.1
Bacilli	19.5	0.1	15.2	0.0	8.1
Bacteroidia	0.7	0.0	0.1	0.0	0.1
Betaproteobacteria	36.9	45.0	25.7	34.6	14.1
Clostridia	0.1	0.1	3.7	0.1	0.1
Deltaproteobacteria	0.1	0.1	0.1	0.1	0.1
Gammaproteobacteria	31.7	52.7	34.9	14.9	65.4
Gemmatimonadetes	0.1	0.1	0.0	0.1	0.0
Sphingobacteriia	0.0	0.1	0.1	0.0	0.1

Relative abundances are percentages of operational taxonomic units (OTUs) for each bacterial class. The OTUs were clustered at 3% divergence (97% similarity).

Table XII: Total counts of bacterial classes.

Bacterial Class	160424L Mudpot	160517E Mudpot	160715X Hot Spring	160715V Mudpot	160715T Mudpot
Acidobacteriia	19	36	12	30	16
Actinobacteria	4990	517	6136	15724	3020
Alphaproteobacteria	1056	80	37	14450	31
Bacilli	11100	41	4747	30	2106
Bacteroidia	412	23	25	25	13
Betaproteobacteria	21071	20819	8025	21003	3640
Clostridia	62	56	1160	60	16
Deltaproteobacteria	46	61	27	67	21
Gammaproteobacteria	18070	24378	10881	9033	16899
Gemmatimonadetes	47	29	10	59	12
Sphingobacteriia	25	45	16	28	14

The counts are operational taxonomic units (OTUs), which were clustered at 3% divergence (97% similarity). The OTUs were taxonomically classified using nucleotide Basic Local Alignment Search Tool (BLASTn) against curated databases from the Ribosomal Database Project (RDP-II) and National Center for Biotechnology Information (NCBI).

Table XIII: Relative abundance bacterial genera.

Bacterial Genus	160424L Mudpot	160517E Mudpot	160715X Hot Spring	160715V Mudpot	160715T Mudpot
Acidobacterium	0.0	0.1	0.0	0.0	0.1
Acinetobacter	0.0	0.1	0.0	0.0	0.0
Afipia	0.0	0.0	0.0	14.5	0.0
Alteribacillus	13.1	0.0	0.0	0.0	0.0
Burkholderia	0.0	0.1	0.0	0.0	0.0
Caenimonas	0.4	1.1	0.9	1.6	0.2
Caulobacter	0.0	0.0	0.0	8.1	0.0
Corynebacterium	8.5	0.0	3.3	0.0	11.4
Curvibacter	9.7	0.0	9.1	0.8	9.6
Delftia	14.8	20.2	14.7	31.0	3.7
Finegoldia	0.0	0.0	3.6	0.0	0.0
Gallionella	0.2	0.4	0.1	0.1	0.1
Gemmatimonas	0.1	0.1	0.0	0.1	0.0
Marinomonas	3.1	0.0	0.0	0.0	0.0
Massilia	0.1	0.1	0.1	0.1	0.1
Methylibium	0.1	0.0	0.0	0.0	0.0
Micrococcus	0.0	0.9	11.8	25.7	0.0
Nitrospira	0.1	0.2	0.1	0.1	0.2
Prevotella	0.7	0.0	0.1	0.0	0.0
Pseudomonas	23.4	52.4	34.7	10.8	47.0
Psychrobacter	0.0	0.1	0.0	0.0	0.0
Ralstonia	10.5	3.0	0.0	0.0	0.0
Rhodoplanes	0.0	0.0	0.0	1.2	0.0
Shigella	4.7	0.0	0.0	3.9	18.2
Sphaerotilus	0.8	18.0	0.6	0.8	0.0
Sphingomonas	0.0	0.1	0.0	0.0	0.0
Staphylococcus	4.8	0.0	9.2	0.0	0.0
Streptococcus	0.1	0.0	6.0	0.0	8.1

Relative abundances are percentages of operational taxonomic units (OTUs) for each bacterial class. The OTUs were clustered at 3% divergence (97% similarity).

Table XIV: Total counts of bacterial genera.

Bacterial Genera	160424L Mudpot	160517E Mudpot	160715X Hot Spring	160715V Mudpot	160715T Mudpot
Acidobacterium	16	36	12	29	16
Acinetobacter	16	28	12	9	7
Afipia	4	0	0	8773	2
Alteribacillus	7484	0	0	3	0
Caenimonas	253	520	277	948	56
Caulobacter	0	2	0	4911	0
Corynebacterium	4827	3	1040	6	2946
Curvibacter	5553	12	2834	469	2475
Delftia	8445	9322	4581	18787	951
Finegoldia	1	1	1121	0	0
Gallionella	98	171	42	91	35
Gemmatimonas	47	29	10	59	12
Marinomonas	1756	1	1	1	0
Massilia	37	45	18	44	20
Methylibium	57	3	1	2	1
Micrococcus	15	408	3664	15569	9
Nitrospira	70	70	42	58	49
Prevotella	403	8	16	9	6
Pseudomonas	13377	24224	10824	6558	12160
Psychrobacter	4	40	2	4	1
Ralstonia	6018	1392	3	3	3
Rhodoplanes	1	8	6	704	5
Shigella	2677	10	2	2378	4706
Sphaerotilus	449	8334	185	483	2
Sphingomonas	21	27	5	16	4
Staphylococcus	2723	7	2881	3	2
Streptococcus	68	3	1855	4	2093

The counts are operational taxonomic units (OTUs), which were clustered at 3% divergence (97% similarity). The OTUs were taxonomically classified using nucleotide Basic Local Alignment Search Tool (BLASTn) against curated databases from the Ribosomal Database Project (RDP-II) and National Center for Biotechnology Information (NCBI).

Table XV: Relative abundance of archaeal classes.

Archaeal Class	160424L Mudpot	160517E Mudpot	160715X Hot Spring	160715V Mudpot	160715T Mudpot
Crenarchaeota	0.0	11.1	6.7	0.0	0.0
Euryarchaeota	0.0	3.7	0.0	0.0	0.0
Halobacteria	0.2	0.0	0.0	0.0	0.0
Methanobacteria	0.0	25.9	33.3	15.0	4.7
Methanococci	0.0	0.0	0.0	5.0	4.7
Methanomicrobia	0.5	22.2	20.0	35.0	23.3
Thaumarchaeota	0.5	29.6	26.7	35.0	7.0
Thermoplasmata	0.2	7.4	6.7	5.0	4.7
Thermoprotei	98.5	0.0	6.7	5.0	55.8

Relative abundances are percentages of operational taxonomic units (OTUs) for each bacterial class. The OTUs were clustered at 3% divergence (97% similarity).

Table XVI: Total counts of archaeal classes.

Archaeal Class	160424L Mudpot	160517E Mudpot	160715X Hot Spring	160715V Mudpot	160715T Mudpot
Crenarchaeota	0	3	1	0	0
Euryarchaeota	0	1	0	0	0
Halobacteria	1	0	0	0	0
Methanobacteria	0	7	5	3	2
Methanococci	0	0	0	1	2
Methanomicrobia	2	6	3	7	10
Thaumarchaeota	2	8	4	7	3
Thermoplasmata	1	2	1	1	2
Thermoprotei	404	0	1	1	24

The counts are operational taxonomic units (OTUs), which were clustered at 3% divergence (97% similarity). The OTUs were taxonomically classified using nucleotide Basic Local Alignment Search Tool (BLASTn) against curated databases from the Ribosomal Database Project (RDP-II) and National Center for Biotechnology Information (NCBI).

Table XVII: Relative abundance of archaeal genera.

Archaeal Genera	160424L Mudpot	160517E Mudpot	160715X Hot Spring	160715V Mudpot	160715T Mudpot
Aciduliprofundum	0.0	3.7	0.0	0.0	0.0
Caldococcus	64.9	0.0	6.7	0.0	55.8
Candidatus Nitrosocaldus	0.0	11.1	6.7	0.0	0.0
Candidatus Nitrososphaera	0.2	3.7	20.0	15.0	4.7
Candidatus Nitrosotalea	0.0	3.7	0.0	0.0	0.0
Cenarchaeum	0.0	0.0	6.7	5.0	0.0
Desulfurococcus	0.0	0.0	0.0	5.0	0.0
Ferroplasma	0.0	0.0	6.7	0.0	2.3
Halorubrum	0.2	0.0	0.0	0.0	0.0
Methanobacterium	0.0	3.7	6.7	5.0	2.3
Methanobrevibacter	0.0	22.2	26.7	10.0	2.3
Methanocella	0.2	0.0	0.0	0.0	0.0
Methanococcus	0.0	0.0	0.0	5.0	4.7
Methanocorpusculum	0.0	3.7	0.0	0.0	0.0
Methanoculleus	0.0	0.0	6.7	0.0	0.0
Methanolinea	0.0	0.0	0.0	0.0	2.3
Methanolobus	0.0	0.0	0.0	0.0	2.3
Methanomassiliicoccus	0.0	0.0	0.0	5.0	0.0
Methanomicrobium	0.2	3.7	0.0	0.0	0.0
Methanosaeta	0.0	14.8	6.7	25.0	16.3
Methanosarcina	0.0	0.0	6.7	0.0	2.3
Methanosphaerula	0.0	0.0	0.0	5.0	0.0
Methanospirillum	0.0	0.0	0.0	5.0	0.0
Nitrososphaera	0.2	22.2	0.0	15.0	2.3
Thermogymnomonas	0.0	3.7	0.0	0.0	0.0
Thermoplasma	0.2	3.7	0.0	0.0	2.3
Vulcanisaeta	33.7	0.0	0.0	0.0	0.0

Relative abundances are percentages of operational taxonomic units (OTUs) for each bacterial class. The OTUs were clustered at 3% divergence (97% similarity).

Table XVIII: Total counts of archaeal classes.

Archaeal Genera	160424L Mudpot	160517E Mudpot	160715X Hot Spring	160715V Mudpot	160715T Mudpot
Aciduliprofundum	0	1	0	0	0
Caldococcus	266	0	1	0	24
Candidatus nitrosocaldus	0	3	1	0	0
Candidatus nitrososphaera	1	1	3	3	2
Candidatus nitrosotalea	0	1	0	0	0
Cenarchaeum	0	0	1	1	0
Desulfurococcus	0	0	0	1	0
Ferroplasma	0	0	1	0	1
Halorubrum	1	0	0	0	0
Methanobacterium	0	1	1	1	1
Methanobrevibacter	0	6	4	2	1
Methanocella	1	0	0	0	0
Methanococcus	0	0	0	1	2
Methanocorpusculum	0	1	0	0	0
Methanoculleus	0	0	1	0	0
Methanolinea	0	0	0	0	1
Methanolobus	0	0	0	0	1
Methanomassiliicoccus	0	0	0	1	0
Methanomicrobium	1	1	0	0	0
Methanosaeta	0	4	1	5	7
Methanosarcina	0	0	1	0	1
Methanosphaerula	0	0	0	1	0
Methanospirillum	0	0	0	1	0
Nitrososphaera	1	6	0	3	1
Thermogymnomonas	0	1	0	0	0
Thermoplasma	1	1	0	0	1
Vulcanisaeta	138	0	0	0	0

The counts are operational taxonomic units (OTUs), which were clustered at 3% divergence (97% similarity). The OTUs were taxonomically classified using nucleotide Basic Local Alignment Search Tool (BLASTn) against curated databases from the Ribosomal Database Project (RDP-II) and National Center for Biotechnology Information (NCBI).

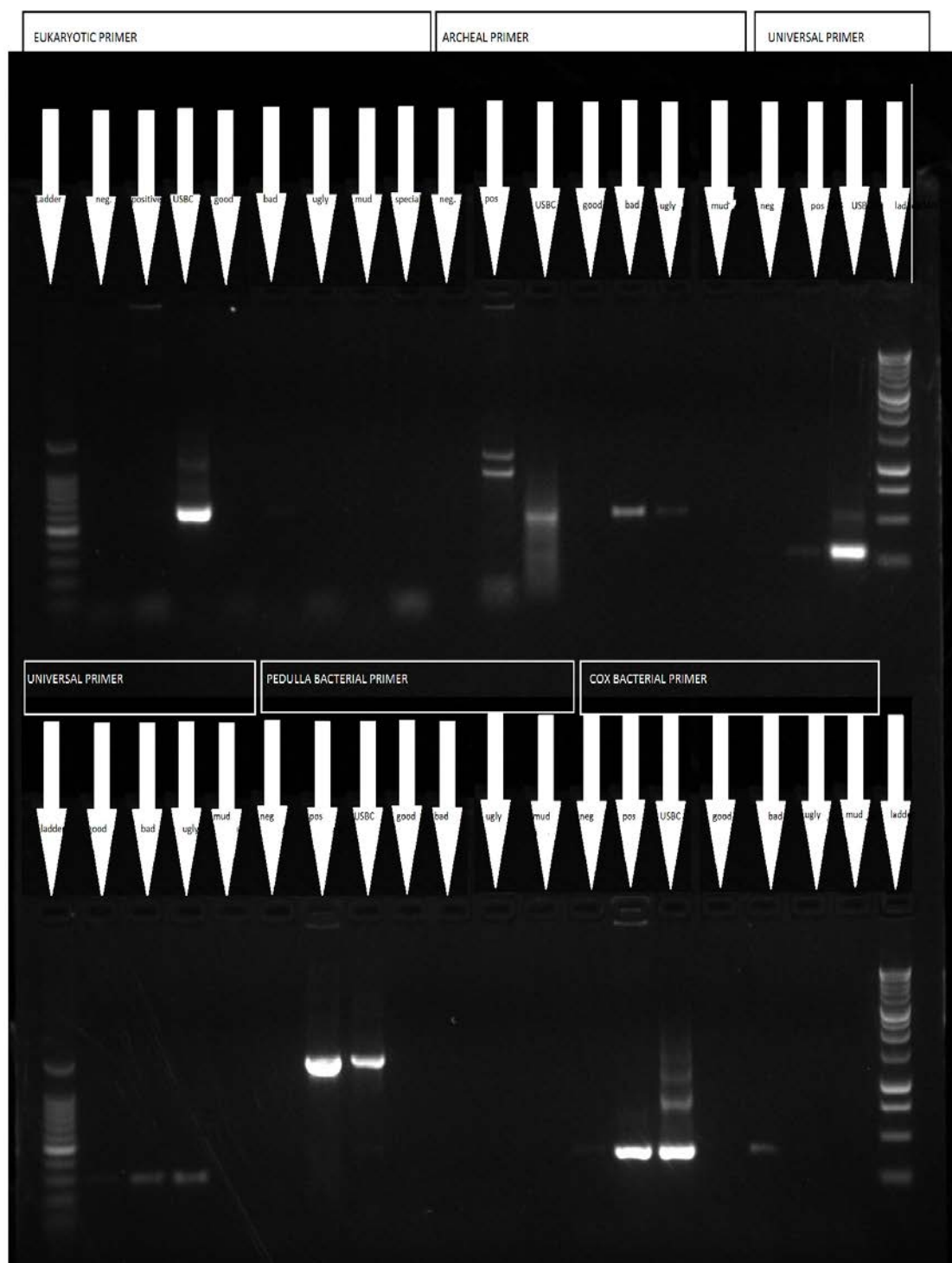


Figure 40: Gel electrophoresis image of PCR product from GOPA mudpot, as well as unrelated Silver Bow Creek samples.

8. Appendix B: Geochemical Data

Table XIX: *In situ* dissolved oxygen measurments.

Sample ID	Unofficial Sample Name	Temp. Dissolved Oxygen °C	Error Temp. °C	Dissolved Oxygen μmol/L	Error Dissolved Oxygen μmol/L
Mudpots					
160712D	Mudder's Milk	76.5	0.2	15.6	0.1
160715T	Urinal Cake	86.8	0.2	21.8	0.1
160715V	Consolation Prize	82.9	0.6	11.0	0.1
160715A	Lil' B	92.8	0.2	BDL	
160716E	Ol' Chunky	68.0	0.2	74.1	0.1
160717M	Princess Peach	90.6	0.2	BDL	
160726S	Ugluk's Door	92.5	0.2	BDL	
160517C	Muddy Delight	51.9	0.2	89.7	0.1
160517E	Pedro's Tarpit	78.9	0.2	8.4	0.1
160724L	Phantom Pants	84.3	0.2	0.9	0.9
160518L	Tweedle Dee	83.0	0.2	10.2	0.8
160518N	Tweedle Dum	86.4	0.2	0.9	0.1
160719W	Choco Mocha	71.0	0.2	2.5	0.1
Shoshone Geyser Basin					
160712C	Proudfeet	91.6	0.1	BDL	
160712E	Sarlace Pit	73.9	0.2	38.1	0.2
160712F	Amy Pohler	70.7	0.2	16.5	0.1
160712G	Anton Chekhov	84.6	0.1	6.6	0.1
160712H	Shoshone Creek	25.3	0.2	233.5	0.9
160713J	CB Outflow 1 (yellow mat)	70.3	0.2	48.1	0.1
160713K	CB Source	79.1	0.2	41.6	0.1
160713L	Gray Matter	42.6	0.2	23.4	0.1
160713M	Millenium Falcon	85.9	0.2	8.1	0.1
160713N	The Cooler Strikes Back OF 1	78.8	0.1	14.2	0.1
160713O	The Cooler Strikes Back Source	92.5	0.2		
160713P	Flame Leach	87.2	0.1	3.3	0.1
160713RSMR13	Gold Digger	76.2	0.1	4.6	0.1
160713Q	Redrum	91.0	0.1	0.9	0.1
Pocket Basin Thermal Area					
160715U	Vitamin C OF 2	41.6	0.1	146.3	0.1
160715W	Vitamin C OF 1	60.2	0.2	92.8	0.1
160715X	Vitamin C Pool Source	75.4	0.2	37.5	3.1
160715Z	Cotton Candy	56.3	0.2	86.6	0.1
Sylvan Springs Thermal Area					
160716D	Dryer	44.8	0.2	145.0	0.3
160716F	Burning Eye	88.0	0.9	3.8	0.3
160716G	Gunters Fancy	72.1	0.2	17.7	3.1
160716H	Goldilocks Source	48.9	0.2	19.8	0.1
160716I	Sylvan Spring	77.8	1.2	2.6	0.1
160716J	Evening Primrose	75.7	0.2	12.3	0.1

Table XIX: Field data collected with Presens PSt3 oxygen dipping probe, continued.

Sample ID	Unofficial Sample Name	Temp. Dissolved Oxygen °C	Error Temp. °C	Dissolved Oxygen µmol/L	Error Dissolved Oxygen µmol/L
Rabbit Creek North Thermal Area					
160717N	Pink Mat	81.4	0.1	13.9	6.3
160717O	Rabbit Creek Source	83.5	0.1	8.3	0.1
160717P	Rabbit Creek Yellow	67.9	0.2	54.1	0.9
160717Q	Rabbit Creek Orange	63.3	0.1	78.8	0.9
160717R	Rabbit Creek Green	61.1	0.2	84.7	0.3
160717S	Nefarious Now	77.4	0.2	38.4	0.1
160917B	Rabbit Creek Green	58.1	0.2	102.0	0.1
160917C	Rabbit Creek Orange	59.7	0.2	98.4	0.1
160917D	Rabbit Creek Yellow	65.5	0.2	65.6	0.1
160917E	Rabbit Creek Source	82.4	0.2	9.7	0.1
Rabbit Creek South Thermal Area					
160721M	Spitting Cobra Orange	53.4	0.2	131.0	0.1
160721N	Spitting Cobra Orange-Green	58.4	0.2	107.0	0.1
160721O	Spitting Cobra Green-Black	70.2	0.2	59.4	0.1
160721P	Spitting Cobra Black	77.3	0.2	30.9	0.1
160721Q	Spitting Cobra Source	88.6	0.2	3.9	0.1
160721R	Titiannia	45.5	0.2	206.9	0.1
160721S	Lil' Sebastian	67.6	0.2	8.4	0.1
160721T	Iron Fist	86.2	0.2	19.6	0.1
Greater Obsidian Pool Thermal Area					
160517D	Figure 8 Source	65.7	0.2	5.1	0.1
160517F	Obsidian Pool	77.1	0.2	23.5	0.1
160517G	Fig. 8 OF 2	44.8	0.2	123.4	0.1
160517H	Fig. 8 OF 1	44.0	0.2	117.8	0.1
160720F	HH/Fig. 8 Mix	40.0	0.2	132.0	0.1
160720J	Fig. 8 OF 1.5	54.6	0.2	81.6	0.1
160720C	Fig. 8 Source	57.3	0.2	60.9	0.1
160720E	Fig. 8 OF 1	55.5	0.2	81.9	0.1
160720H	Happy Harfer OF	46.0	0.2	3.3	0.1
160720G	Fig. 8 OF 2	43.7	0.2	135.0	0.1
160720I	OB1-Heim	77.8	0.2	9.4	0.1
160720MRRS13	Spotted Grizzly	30.5	0.2	101.0	0.1
160720MRRS14	Fig. 8 OF	46.9	0.2	45.6	0.1
160724	Moose Pool	71.3	0.2	1.2	0.1
160724I	Skippy's Bathtub	69.2	0.2	20.9	0.8
160724K	OB1 Hime	85.9	0.5	5.8	0.3
Crater Hills Thermal Area					
160518K	Alice	79.2	0.2	1.6	0.1
160518M	Jabberwocky	77.7	0.2	16.4	0.1
160518O	Delilah	80.2	0.2	22.5	0.1
160518P	Crater Hills Geyser	81.1	0.2	18.2	0.1
160518MR1	Crumpet	71.4	0.2	3.8	0.1
160724G	Crater Hills Geyser	83.8	0.2	7.4	0.9

Table XIX: Field data collected with Presens PSt3 oxygen dipping probe, continued.

Sample ID	Unofficial Sample Name	Temp.	Error	Dissolved Oxygen	Error
		Dissolved Oxygen	Temp.		Dissolved Oxygen
		°C	°C	μmol/L	μmol/L
Boulder Spring Thermal Area					
160726O	Boulder Spring OF 3	68.2	0.1	1.3	0.0
160726P	Boulder Spring OF 2	79.2	0.1	20.0	3.1
160726Q	Boulder Spring OF 1	88.1	0.2	0.1	0.1
160726R	Boulder Spring Source	92.5	0.2	BDL	
Forest Springs Thermal Area					
160719V	Yellow Sulfur Photosynthetic Cove	54.4	0.2	1.3	0.0
160719X	Purple Toad	48.2	0.2	1.3	0.0
160719Y	Gunter's Dream	59.5	0.2	2.4	0.1

Errors represent field fluctuations. Italicized error values were approximated because data were not collected at time of sampling. Detection limit for Presens DP – PSt3 is 0.47 µM (15 ppb) dissolved oxygen (Presens DP- PSt3 manual). BDL = below detection limit. OF = outflow.

Table XX: *In situ* temperature and pH measurements.

Sample ID	Unofficial Sample Name	Temp. pH meter	Error Temp.	pH	Error pH	Temp. conductivity	Error Temp.	Conductivity	Error Conductivity
		°C	°C			°C	°C	µS/cm	µS/cm
Mudpots									
160712D	Mudder's Milk	74.6	0.1	6.079	0.003	75.1	0.1	2032	2
160715T	Urinal Cake	72.1	0.1	2.513	0.002	79.6	0.1	1330	1
160715V	Consolation Prize	88.5	0.1	3.770	0.003	82.9	0.1	1164	2
160715A	Lil B	90.5	0.1	5.713	0.002	86.0	2.0	262	26
160716E	Ol' Chunky	64.8	0.1	2.820	0.010	64.2	0.1	967	10
150919B	Princess Peach	62.4	0.5	3.370	1.000	62.4	0.1	530	5
160717M	Princess Peach	83.3	0.2	2.569	0.006	87.0	0.1	699	1
160726S	Ugluk's Door	89.8	0.1	3.413	0.005	87.2	0.1	740	2
160517C	Muddy Delight	66.6	0.1	1.900	0.020	65.6	0.1	2845	5
160517E	Pedro's Tarpit	68.1	0.1	4.450	0.020	71.9	0.1	944	5
160724L	Phantom Pants	80.9	0.5	4.846	0.020	81.5	0.3	1065	1
160518L	Tweedle Dee	74.0	0.1	2.020	0.020	78.1	0.1	2314	1
160518N	Tweedle Dum	82.8	0.1	1.900	0.020	81.5	0.1	2530	5
160719W	Choco Mocha	70.7	0.1	1.406	0.020	71.0	0.1	16380	10
160719Z	The Good Stuff	67.1	0.2	2.110	0.020	68.1	0.2	3605	5
Shoshone Geyser Basin									
160712C	Proudfeet	91.1	0.1	6.681	0.010	91.9	0.1	3689	30
160712E	Sarlacc Pit	70.9	0.1	7.344	0.002	70.1	0.1	2971	6
160712F	Amy Pohler	72.1	0.4	6.868	0.020	72.3	0.1	2916	4
160712G	Anton Chekhov	82.6	0.2	8.687	0.005	84.8	0.2	3919	1
160712H	Shoshone Creek	25.3	0.1	8.037	0.010	25.3	0.1	317	0
160713J	CB OF 1 (yellow mat)	68.2	0.1	8.729	0.003	69.9	0.1	3140	2
160713K	CB Source	77.9	0.1	8.584	0.004	79.8	0.1	3390	10
160713L	Gray Matter	41.6	0.2	3.856	0.004	41.9	0.1	251	0
160713M	Millenium Falcon	84.8	0.1	7.414	0.001	85.7	0.1	3573	3
160713P	Flame Leach	82.8	0.1	2.541	0.050	86.6	0.1	3410	3
160713RSMR13	Gold Digger	76.2	0.1	2.494	0.002	75.8	0.3	3208	1
160713Q	Redrum	89.4	0.5	2.479	0.004	91.1	0.2	3803	1

Table XX: Field data collected with pH and conductivity meters, continued.

Sample ID	Unofficial Sample Name	Temp. pH meter °C	Error Temp. °C	pH	Error pH	Temp. conductivity °C	Error Temp. °C	Conductivity µS/cm	Error Conductivity µS/cm
Shoshone Geyser Basin, continued									
160713N	The Cooler Strikes Back OF 1	80.3	0.5	8.080	0.004	83.0	0.2	3603	2
160713O	The Cooler Strikes Back Source	92.1	0.1	7.830	0.008	92.3	0.8	3924	8
Pocket Basin Thermal Area									
160715U	Vitamin C OF 2	42.7	0.1	9.196	0.007	42.7	0.1	2313	7
160715W	Vitamin C OF 1 (full flow)	60.4	0.1	9.237	0.005	60.6	0.1	2877	1
160715X	Vitamin C Pool (Source)	79.1	0.1	9.109	0.002	79.4	0.1	3497	2
160715Z	Cotton Candy	57.9	0.1	8.134	0.001	60.1	0.3	1929	3
Sylvan Springs Thermal Area									
160716D	Dryer	42.8	0.1	4.531	0.002	42.0	0.1	3072	5
160716F	Burning Eye	86.9	0.1	1.828	0.100	87.1	0.3	2294	5
160716G	Gunter's Fancy	71.2	3	5.291	0.200	68.8	0.5	2997	30
160716RSMR4	Peaceful Pool	32.3	0.1	2.161	0.004	32.1	0.1	4042	1
160716H	Goldilocks Source	48.7	0.1	2.363	0.002	48.8	0.1	3235	10
160716I	Sylvan Spring	76.9	0.1	5.353	0.005	78.0	0.1	5110	5
160716J	Evening Primrose	74.4	0.4	5.441	0.020	75.8	0.1	4590	10
Rabbit Creek North Thermal Area									
150919C	Hot spring near 150919B	77.1	0.5	7.700	1.000	77.1	0.1	521	5
150919E	Rabbit Creek Source	76.1	0.5	9.510	1.000	76.1	0.1	1686	5
160717N	Pink Mat	80.6	0.3	8.794	0.040	81.4	0.1	403	2
160717O	Rabbit Creek Source	83.2	0.1	8.837	0.015	83.5	0.1	406	1
160717P	Rabbit Creek Yellow	67.2	0.4	9.142	0.004	67.9	0.1	1920	2
160717Q	Rabbit Creek Orange	63.2	0.1	9.058	0.002	63.1	0.1	1943	1
160717R	Rabbit Creek Green	61.1	0.1	9.085	0.001	61.1	0.1	1960	1
160717S	Nefarious Now	78.0	0.1	3.412	0.002	77.7	0.1	580	1
160917B	Rabbit Creek Green	58.0	0.1	9.326	0.002	58.0	0.1	3208	5
160917C	Rabbit Creek Orange	60.3	0.1	9.291	0.002	60.4	0.1	3326	5
160917D	Rabbit Creek Yellow	65.8	0.1	9.242	0.002	66.2	0.1	3465	5
160917E	Rabbit Creek Source	82.0	0.1	9.069	0.002	82.3	0.1	4180	5

Table XX: Field data collected with pH and conductivity meters, continued.

Sample ID	Unofficial Sample Name	Temp. pH meter	Error Temp.	pH	Error pH	Temp. conductivity	Error Temp.	Conductivity	Error Conductivity
		°C	°C			°C	°C	µS/cm	µS/cm
Rabbit Creek South Thermal Area									
160721M	Spitting Cobra Orange	53.3	0.1	8.600	0.002	53.8	0.1	388	5
160721N	Spitting Cobra Orange-Green	57.7	0.1	8.479	0.002	58.7	0.1	433	5
160721O	Spitting Cobra Green-Black	68.4	0.1	8.526	0.002	58.7	0.1	415	5
160721P	Spitting Cobra Black	76.7	0.1	8.119	0.002	76.7	0.1	530	5
160721Q	Spitting Cobra Source	88.0	0.1	7.999	0.002	88.5	0.1	588	5
160721R	Titianina	45.4	0.1	3.760	0.002	45.9	0.1	387	5
160721S	Lil' Sebastian	66.4	0.1	3.244	0.002	64.3	0.1	537	5
160721T	Iron Fist	90.5	0.1	2.443	0.002	92.8	0.1	2187	5
Greater Obsidian Pool Thermal Area									
160517D	Figure 8 Source	63.9	0.1	3.090	0.002	65.8	0.1	4088	5
160517F	Obsidian Pool	77.8	0.1	4.460	0.002	77.1	0.1	436	5
160517G	Figure 8 OF 2	44.5	0.1	4.680	0.002	45.2	0.1	2640	5
160517H	Figure 8 OF 1	44.0	0.1	3.280	0.002	43.9	0.1	3053	5
160720F	HH/Figure 8 Mix	40.4	0.1	3.933	0.002	40.0	0.1	2813	5
160720J	Figure 8 OF 1.5	54.6	0.1	3.442	0.002	54.6	0.1	3863	5
160720C	Figure 8 Source	56.3	0.1	3.477	0.002	56.9	0.1	4012	5
160720E	Figure 8 OF 1		0.1	3.512	0.002	56.7	0.1	4016	5
160720H	Happy Harfer OF	46.1	0.1	6.576	0.002	45.1	0.1	2341	5
160720G	Figure 8 OF 2	43.7	0.1	6.742	0.002	43.6	0.1	2414	5
160720I	OB1-Heim	74.5	0.1	5.625	0.002	75.4	0.1	530	5
160720MRRS13	Spotted Grizzly	30.5	0.1	3.889	0.002	30.5	0.1	419	5
160720MRRS14	Figure 8 OF	46.0	0.1	3.437	0.002	46.1	0.1	3215	5
160724	Moose Pool	70.7	0.1	1.953	0.010	71.3	0.1	3813	3
160724I	Skippy's Bathtub	69.2	0.3	4.926	0.001	70.9	0.1	2679	1
160724K	OB1 Hime	75.9	0.1	5.516	0.006	78.1	0.3	1617	1

Table XX: Field data collected with pH and conductivity meters, continued.

Sample ID	Unofficial Sample Name	Temp. pH meter	Error Temp.	pH	Error pH	Temp. conductivity	Error Temp.	Conductivity	Error Conductivity
		°C	°C			°C	°C	µS/cm	µS/cm
Forest Springs									
160719V	Yellow Sulfur Photosynthetic Cove	52.5	<i>0.1</i>	1.952	0.020	54.7	<i>0.1</i>	5730	5
160719X	Purple Toad	48.0	<i>0.1</i>	2.035	0.010	49.7	<i>0.1</i>	5330	5
160719Y	Gunter's Dream	59.1	0.1	3.046	<i>0.002</i>	59.6	<i>0.1</i>	1130	5
Crater Hills Thermal Area									
160518K	Alice	77.5	0.1	1.890	0.002	79.1	0.1	5810	5
160518M	Jabberwocky	85.2	0.1	2.010	0.002	79.0	0.1	4470	5
160518O	Delilah	83.3	0.1	1.960	0.002	86.4	0.1	872000	5
160518P	Crater Hills Geyser	84.6	0.1	2.990	0.002	86.0	0.1	9900	5
160518MR1	Crumpet	70.4	0.1	2.900	0.002	72.1	0.1	6780	5
160724G	Crater Hills Geyser	87.1	0.3	3.356	0.010	87.5	0.5	9210	20
Boulder									
160726O	Boulder Spring OF 3	68.0	0.1	8.754	0.002	67.1	0.1	3027	1
160726P	Boulder Spring OF 2	79.4	0.2	8.645	0.002	79.4	0.1	3589	10
160726Q	Boulder Spring OF 1	88.2	<i>0.1</i>	8.200	0.005	86.9	<i>0.1</i>	3397	5
160726R	Boulder Spring Source	92.3	0.1	8.456	0.001	92.7	<i>0.1</i>	4000	2

Errors represent field fluctuations. Italicized error values were approximated because data were not collected at time of sampling. OF = outflow.

Table XXI: Major cation concentrations.

Sample ID	Unofficial Sample Name	Li ⁺	Na ⁺	K ⁺	Mg ⁺²	Ca ⁺²
		μmol/kg				
Mudpots						
160712D	Mudder's Milk	BDL	5800	220	BDL	6.5
160715T	Urinal Cake	42	840	290	290	310
160715V	Consolation Prize	13	550	450	230	190
160716E	Ol' Chunky	4.1	2600	900	55	66
150919B	Princess Peach	38	1500	160	47	72
160717M	Princess Peach	22	640	90	19	27
160517E	Pedro's Tarpit					
160724L	Phantom Pants	47	4300	1700	1100	1100
160719W	Choco Mocha	12	700	390	240	140
160719Z	The Good Stuff	BDL	130	500	65	46
160726S	Ugluk's Door	5.8	580	520	36	71
Shoshone Geyser Basin						
160712E	Sarlace Pit	110	10300	260	BDL	3.6
Pocket Basin Thermal Area						
160715Z	Cotton Candy	530	15300	400	62	27
160715X	Vitamin C (Source)	740	22200	500	25	15
Sylvan Springs Thermal Area						
160716F	Burning Eye	3.2	550	360	BDL	14
160716G	Gunter's Fancy	400	11100	430	BDL	48
160716H	Goldilocks Source	370	9500	970	BDL	110
160716I	Sylvan Spring	1400	27000	1700	BDL	97
160716D	Dryer	1900	37200	1700	33	150
Rabbit Creek North Thermal Area						
160717S	Nefarious Now	63	1500	160	BDL	10
160717O	Rabbit Creek Source	930	15500	410	BDL	1.8
Greater Obsidian Pool Thermal Area						
160517D	Fig. 8 Source	710	25900	1400	61	170
160517F	Obsidian Pool	43	2800	680	500	400
Forest Springs Thermal Area						
160719Y	Gunter's Dream	6.7	3000	1000	160	280
Boulder Spring Thermal Area						
160726R	Boulder Spring Source	590	22300	340	35	9.2

Instrumental errors are $\pm 10\%$ for all cation values. BDL = below detection limit. / =not sampled. Detection limits for Li, Na, K, Mg, Ca are 1.7, 2.7, 11.1, 9.6, 0.7 μM , respectively.

Table XXII: Major anion concentrations.

Sample ID	Unofficial Sample Name	F ⁻	Cl ⁻	Br ⁻	SO ₄ ⁻²	PO ₄ ⁻³	NO ₃ ⁻
μmol/kg							
Mudpots							
160712D	Mudder's Milk	530	3200	4.0	960	0.3	1.9
160715T	Urinal Cake	100	1700	BDL	14600	1.4	2.9
160715V	Consolation Prize	33	1300	BDL	4700	1.2	8.0
160716E	Ol' Chunky	30	1700	BDL	4500	0.7	1.1
150919B	Princess Peach	130	1000	1.4	3300	0.8	2.0
160717M	Princess Peach	54	330	BDL	1500	1.5	1.1
160517E	Pedro's Tarpit	46	1200	BDL	2000	0.3	3.1
160724L	Phantom Pants	46	8400	BDL	10000	3.8	1.2
160719W	Choco Mocha	60	120	BDL	63900	15	12
160719Z	The Good Stuff	44	140	BDL	10800	3.2	4.2
160726S	Ugluk's Door	21	1100	BDL	2700	0.7	1.4
Pocket Basin Thermal Area							
160715X	Vitamin C (Source)	1500	9500	12	450	1.6	BDL
160715Z	Cotton Candy	1300	8400	11	760	BDL	BDL
Sylvan Springs Thermal Area							
160716D	Dryer	1300	10	19	2400	BDL	BDL
160716F	Burning Eye	13	18	BDL	12500	0.5	1.0
160716G	Gunter's Fancy	530	9100	11	1600	BDL	1.7
160716H	Goldilocks Source	210	3900	6.7	9900	1.3	5.0
160716I	Sylvan Spring						
Rabbit Creek North Thermal Area							
160717O	Rabbit Creek Source	1200	8300	12	230	BDL	BDL
160717S	Nefarious Now	330	710	1.1	240	0.2	1.3
Greater Obsidian Pool Thermal Area							
160517D	Fig. 8 Source	2900	11800	12	4900	5.4	BDL
160517F	Obsidian Pool	480	810	BDL	3200	BDL	BDL
Forest Springs Thermal Area							
160719Y	Gunter's Dream	19	48	BDL	4600	3.4	0.9
Boulder Spring Thermal Area							
160726R	Boulder Spring Source	1200	6800	11	210	BDL	BDL

Instrumental errors are ± 11.9% for fluoride, ± 0.92% for chloride, ± 1.1% for bromide, ± 1.9% for sulfate, ± 10.8% for phosphate, and ± 0.87 % for nitrate.

BDL = below detection limit. Detection limits for F⁻, Cl⁻, Br⁻, SO₄⁻², PO₄⁻³, NO₃⁻, and NO₂⁻ were 0.53, 0.28, 0.13, 5.2, 0.21, 0.22, 0.16 μM, respectively. / =not sampled.

Table XXIII: Lithium, boron, aluminum, iron, and titanium concentrations.

Sample ID	Sample Location	Li	B	Al	Fe	Ti
		μmol/kg				
Mudpots						
160712D	Mudder's Milk	4.3	19	0.3	0.1	BDL
160715T	Urinal Cake	33	4.4	1300	1300	4.3
160715V	Consolation Prize	9.4	4.6	35	720	1.3
160715A	Lil' B	1.9	8.8	21	520	3.5
160716E	Ol' Chunky	3.5	120	30	270	1.7
150919B	Princess Peach	38	28	150	450	2.1
160717M	Princess Peach	20	10	70	1.3	0.5
160517C	Muddy Delight	0.9	1.3	1200	130	5.2
160517E	Pedro's Tarpit	5.5	21	170	220	49
160724K	Phantom Pants	45	33	64	270	4.3
160518L	Tweedle Dee	BDL	0.4	2200	540	4.2
160518N	Tweedle Dum	BDL	1.5	2700	420	12
160719W	Choco Mocha	BDL	19	2900	60	53
160719Z	The Good Stuff	11	1.5	890	410	4.2
160726S	Ugluk's Door	5.1	2.5	8.5	400	0.6
Shoshone Geyser Basin						
160712E	Sarlacc Pit	130	270	3.4	BDL	BDL
Pocket Basin Thermal Area						
160715X	Vitamin C Pool (Source)	370	380	4.8	BDL	BDL
160715Z	Cotton Candy	230	240	1.2	BDL	BDL
Sylvan Springs Thermal Area						
160716D	Dryer					
160716F	Burning Eye	2.0	81	640	32	4.3
160716G	Gunters Fancy	320	420	18	BDL	BDL
160716H	Goldilocks Source	210	250	790	63	3.5
Rabbit Creek North Thermal Area						
150919C	Obsidian Pool Jr.	230	120	11	3.3	BDL
150919E	Rabbit Creek Source	680	280	5.3	BDL	1.5
160717S	Nefarious Now	51	24	44	BDL	0.3

Table XXIII: Lithium, boron, aluminum, iron, and titanium concentrations, continued.

Sample ID	Sample Location	Li	B	Al	Fe	Ti
				μmol/kg		
Rabbit Creek North Thermal Area, continued						
160717N	Pink Mat	780	310	7.4	BDL	BDL
160717O	Rabbit Creek Source	740	280	6.7	BDL	BDL
160717P	Rabbit Creek Yellow	760	290	6.9	BDL	BDL
160717Q	Rabbit Creek Orange	720	330	6.9	BDL	BDL
160717R	Rabbit Creek Green	770	350	7.4	BDL	BDL
160917B	Rabbit Creek Green	780	380	7.3	BDL	BDL
160917C	Rabbit Creek Orange	820	390	7.8	BDL	BDL
160917D	Rabbit Creek Yellow	780	370	7.3	BDL	BDL
160917E	Rabbit Creek Source	800	330	7.2	BDL	BDL
Rabbit Creek South Thermal Area						
160721M	Spitting Cobra Orange	7.8	1.2	1.0	BDL	BDL
160721N	Spitting Cobra Orange-Green	8.8	1.3	1.1	BDL	BDL
160721O	Spitting Cobra Green-Black	8.2	1.4	1.2	BDL	BDL
160721P	Spitting Cobra Black	8.7	1.3	1.1	BDL	BDL
160721Q	Spitting Cobra Source	8.5	1.3	1.1	BDL	BDL
Greater Obsidian Pool Thermal Area						
160517D	Figure 8 Source	420	740	11	BDL	2.0
160517F	Obsidian Pool	31	60	21	70	2.4
160517G	Figure 8 OF 2	240	550	8.4	BDL	BDL
160517H	Figure 8 OF 1	300	660	12	BDL	1.3
Crater Hills Thermal Area						
160518K	Alice	3.9	37	320	220	7.7
160518M	Jabberwocky	6.6	27	430	220	8.2
160518O	Delilah	300	720	560	36	6.8
160518P	Crater Hills Geyser	730	1900	250	BDL	BDL
Forest Springs Thermal Area						
160719Y	Gunter's Dream	4.4	1.1	12	0.2	1.5
Boulder Spring Thermal Area						
160726R	Boulder Spring Source	380	370	10	BDL	1.0

Instrumental errors are $\pm 4.2\%$ for lithium, $\pm 4.0\%$ for boron, $\pm 5.6\%$ for aluminum, $\pm 5.2\%$ for iron, and $\pm 3.8\%$ for titanium. BDL = below detection limit. OF = outflow. Detection limits for Li, B, Al, Fe, and Ti were 0.078, 0.023, 0.019, 0.098, 0.193 μM , respectively. / =not sampled.

Table XXIV: Beryllium, vanadium, chromium, and manganese concentrations.

Sample ID	Sample Location	Be	V	Cr	Mn
		nmol/kg			
Mudpots					
160712D	Mudder's Milk	BDL	BDL	BDL	52
160715T	Urinal Cake	490	640	83	10100
160715V	Consolation Prize	150	130	BDL	7800
160715A	Lil' B	47	60	26	4400
160716E	Ol' Chunky	390	29	BDL	6000
150919B	Princess Peach	140	45	6.9	2800
160717M	Princess Peach	74	BDL	BDL	860
160517C	Muddy Delight	59	300	230	1000
160517E	Pedro's Tarpit	180	200	340	9600
160724K	Phantom Pants	290	73	BDL	39500
160518L	Tweedle Dee	570	1000	530	4200
160518N	Tweedle Dum	BDL	1300	370	2000
160719W	Choco Mocha	BDL	1100	2200	1400
160719Z	The Good Stuff	BDL	230	BDL	5200
160726S	Ugluk's Door	190	26	BDL	4100
Shoshone Geyser Basin					
160712E	Sarlacc Pit	130	BDL	BDL	BDL
Pocket Basin Thermal Area					
160715X	Vitamin C Pool Source	BDL	BDL	BDL	BDL
160715Z	Cotton Candy	BDL	BDL	BDL	306
Sylvan Springs Thermal Area					
160716D	Dryer				
160716F	Burning Eye	69	17	BDL	410
160716G	Gunters Fancy	0.3	BDL	BDL	330
160716H	Goldilocks Source	150	23	BDL	3100
Rabbit Creek North Thermal Area					
150919C	Obsidian Pool Jr.	BDL	14	5.0	49
150919E	Rabbit Creek Source	BDL	BDL	BDL	BDL
160717S	Nefarious Now	78	BDL	BDL	350

Table XXIV: Beryllium, vanadium, chromium, and manganese concentrations, continued.

Sample ID	Sample Location	Be	V	Cr	Mn
		nmol/kg			
Rabbit Creek North Thermal Area, continued					
160717N	Pink Mat	BDL	BDL	BDL	BDL
160717O	Rabbit Creek Source	BDL	BDL	BDL	BDL
160717P	Rabbit Creek Yellow	BDL	BDL	BDL	BDL
160717Q	Rabbit Creek Orange	BDL	BDL	BDL	BDL
160717R	Rabbit Creek Green	BDL	BDL	BDL	BDL
160917B	Rabbit Creek Green	BDL	BDL	BDL	BDL
160917C	Rabbit Creek Orange	BDL	BDL	BDL	BDL
160917D	Rabbit Creek Yellow	BDL	BDL	BDL	BDL
160917E	Rabbit Creek Source	BDL	BDL	BDL	BDL
Rabbit Creek South Thermal Area					
160721M	Spitting Cobra Orange	BDL	BDL	BDL	BDL
160721N	Spitting Cobra Orange-Green	78	BDL	BDL	79
160721O	Spitting Cobra Green-Black	76	BDL	BDL	98
160721P	Spitting Cobra Black	39	BDL	BDL	68
160721Q	Spitting Cobra Source	77	BDL	BDL	140
Greater Obsidian Pool Thermal Area					
160517D	Figure 8 Source	BDL	BDL	BDL	520
160517F	Obsidian Pool	230	BDL	BDL	9200
160517G	Figure 8 OF 2	BDL	BDL	BDL	470
160517H	Figure 8 OF 1	120	BDL	BDL	480
Crater Hills Thermal Area					
160518K	Alice	BDL	110	56	9000
160518M	Jabberwocky	BDL	82	81	5900
160518O	Delilah	510	130	61	4100
160518P	Crater Hills Geyser	960	BDL	BDL	2900
Forest Springs Thermal Area					
160719Y	Gunter's Dream	69	26	BDL	4200
Boulder Spring Thermal Area					
160726R	Boulder Spring Source	BDL	BDL	BDL	BDL

Instrumental errors are $\pm 3.7\%$ for beryllium, $\pm 3.8\%$ for vanadium, $\pm 4.4\%$ for chromium, and $\pm 5.0\%$ for manganese. BDL = below detection limit. OF = outflow. Detection limits for Be, V, Cr, and Mn are 22.2, 9.8, 4.6, 36.4 nM, respectively. / =not sampled.

Table XXV: Arsenic, rubidium, molybdenum, and cesium concentrations.

Sample ID	Sample Location	As	Rb	Mo	Cs
		nmol/kg			
Mudpots					
160712D	Mudder's Milk	730	62	260	5.7
160715T	Urinal Cake	4500	2000	130	480
160715V	Consolation Prize	1600	1300	94	96
160715A	Lil' B	850	350	69	48
160716E	Ol' Chunky	92	2000	45	15
150919B	Princess Peach	570	350	BDL	280
160717M	Princess Peach	32	230	62	220
160517C	Muddy Delight	65	680	BDL	13
160517E	Pedro's Tarpit	73	1400	110	16
160724K	Phantom Pants	1100	3200	BDL	16
160518L	Tweedle Dee	130	560	94	BDL
160518N	Tweedle Dum	580	510	BDL	15
160719W	Choco Mocha	280	680	BDL	BDL
160719Z	The Good Stuff	580	1800	BDL	100
160726S	Ugluk's Door	620	1200	28	120
Shoshone Geyser Basin					
160712E	Sarlace Pit	11200	1600	600	510
Pocket Basin Thermal Area					
160715X	Vitamin C Source	15500	2300	2300	2400
160715Z	Cotton Candy	12600	2200	700	2400
Sylvan Springs Thermal Area					
160716D	Dryer				
160716F	Burning Eye	94	660	BDL	9.5
160716G	Gunters Fancy	14100	1300	2100	780
160716H	Goldilocks Source	3700	1700	BDL	430
Rabbit Creek North Thermal Area					
150919C	Obsidian Pool Jr.	9600	630	580	240
150919E	Rabbit Creek Source	17900	1600	1400	3500
160717S	Nefarious Now	370	430	BDL	370

Table XXV: Arsenic, rubidium, molybdenum, and cesium concentrations, continued.

Sample ID	Sample Location	As	Rb	Mo	Cs
		nmol/kg			
Rabbit Creek North Thermal Area, continued					
160717N	Pink Mat	19000	1700	1300	3200
160717O	Rabbit Creek Source	18400	1600	1100	3000
160717P	Rabbit Creek Yellow	18700	1600	1100	3200
160717Q	Rabbit Creek Orange	20200	1800	1100	3200
160717R	Rabbit Creek Green	20800	1900	1300	3400
160917B	Rabbit Creek Green	20600	1900	1100	3300
160917C	Rabbit Creek Orange	20400	1800	1100	3400
160917D	Rabbit Creek Yellow	19800	1800	1200	3300
160917E	Rabbit Creek Source	18800	1600	1000	3200
Rabbit Creek South Thermal Area					
160721M	Spitting Cobra Orange	92	1100	310	120
160721N	Spitting Cobra Orange-Green	87	1100	290	120
160721O	Spitting Cobra Green-Black	83	1100	310	100
160721P	Spitting Cobra Black	86	1100	320	110
160721Q	Spitting Cobra Source	85	1100	300	110
Greater Obsidian Pool Thermal Area					
160517D	Figure 8 Source	8600	4000	1900	460
160517F	Obsidian Pool	27	1800	BDL	BDL
160517G	Fig. 8 OF 2	5000	2900	1500	330
160517H	Fig. 8 OF 1	6400	3500	1900	430
Crater Hills Thermal Area					
160518K	Alice	170	1700	BDL	69
160518M	Jabberwocky	84	1400	BDL	50
160518O	Delilah	24400	5800	BDL	1800
160518P	Crater Hills Geyser	50300	13100	BDL	4500
Forest Springs Thermal Area					
160719Y	Gunter's Dream	39	2100	BDL	95
Boulder Spring Thermal Area					
160726R	Boulder Spring Source	16300	1300	1400	1900

Instrumental errors are $\pm 3.8\%$ for arsenic, $\pm 3.0\%$ for rubidium, $\pm 3.9\%$ for molybdenum, and $\pm 3.0\%$ for cesium. BDL = below detection limit. OF = outflow. Detection limits for As, Rb, Mo, and Cs are 2.7, 8.1, 21.4, 3.8 nmol/kg, respectively. / =not sampled.

Table XXVI: Cobalt, nickel, copper, and zinc concentrations.

Sample ID	Sample Location	Co	Ni	Cu	Zn
		nmol/kg			
Mudpots					
160712D	Mudder's Milk	BDL	BDL	BDL	BDL
160715T	Urinal Cake	110	1000	BDL	9100
160715V	Consolation Prize	45	1300	BDL	5200
160715A	Lil' B	BDL	380	24	12200
160716E	Ol' Chunky	BDL	42	BDL	9400
150919B	Princess Peach	31	290	210	25700
160717M	Princess Peach	12	95	220	2700
160517C	Muddy Delight	BDL	440	BDL	5800
160517E	Pedro's Tarpit	140	1700	520	8600
160724K	Phantom Pants	BDL	580	57	3300
160518L	Tweedle Dee	180	3100	BDL	21500
160518N	Tweedle Dum	260	3200	78	10200
160719W	Choco Mocha	BDL	BDL	BDL	7500
160719Z	The Good Stuff	130	1700	BDL	7000
160726S	Ugluk's Door	BDL	300	BDL	3400
Shoshone Geyser Basin					
160712E	Sarlacc Pit	BDL	BDL	BDL	BDL
Pocket Basin Thermal Area					
160715X	Vitamin C Pool (Source)	BDL	BDL	BDL	BDL
160715Z	Cotton Candy	BDL	BDL	BDL	BDL
Sylvan Springs Thermal Area					
160716D	Dryer				
160716F	Burning Eye	BDL	BDL	BDL	2900
160716G	Gunters Fancy	BDL	BDL	BDL	300
160716H	Goldilocks Source	BDL	BDL	BDL	2000
Rabbit Creek North Thermal Area					
150919C	Obsidian Pool Jr.	BDL	BDL	BDL	740
150919E	Rabbit Creek Source	BDL	BDL	BDL	BDL
160717S	Nefarious Now	BDL	BDL	BDL	940

Table XXVI: Cobalt, nickel, copper, and zinc concentrations, continued.

Sample ID	Sample Location	Co	Ni	Cu	Zn
		nmolal			
Rabbit Creek North Thermal Area, continued					
160717N	Pink Mat	BDL	BDL	BDL	BDL
160717O	Rabbit Creek Source	BDL	BDL	BDL	BDL
160717P	Rabbit Creek Yellow	BDL	BDL	BDL	BDL
160717Q	Rabbit Creek Orange	BDL	BDL	BDL	BDL
160717R	Rabbit Creek Green	BDL	BDL	BDL	BDL
160917B	Rabbit Creek Green	BDL	BDL	BDL	BDL
160917C	Rabbit Creek Orange	BDL	BDL	BDL	BDL
160917D	Rabbit Creek Yellow	BDL	BDL	BDL	BDL
160917E	Rabbit Creek Source	BDL	BDL	BDL	BDL
Rabbit Creek South Thermal Area					
160721M	Spitting Cobra Orange	BDL	BDL	BDL	BDL
160721N	Spitting Cobra Orange-Green	BDL	BDL	BDL	99
160721O	Spitting Cobra Green-Black	BDL	BDL	BDL	BDL
160721P	Spitting Cobra Black	BDL	BDL	BDL	BDL
160721Q	Spitting Cobra Source	BDL	BDL	BDL	BDL
Greater Obsidian Pool Thermal Area					
160517D	Figure 8 Source	BDL	BDL	BDL	BDL
160517F	Obsidian Pool	BDL	BDL	BDL	560
160517G	Figure 8 OF 2	BDL	BDL	BDL	930
160517H	Figure 8 OF 1	BDL	BDL	BDL	1000
Crater Hills Thermal Area					
160518K	Alice	BDL	1400	BDL	3100
160518M	Jabberwocky	BDL	700	BDL	2500
160518O	Delilah	BDL	BDL	BDL	1500
160518P	Crater Hills Geyser	BDL	BDL	BDL	BDL
Forest Springs Thermal Area					
160719Y	Gunter's Dream	BDL	BDL	BDL	370
Boulder Spring Thermal Area					
160726R	Boulder Spring Source	BDL	BDL	BDL	BDL

Instrumental errors are $\pm 4.1\%$ for cobalt, $\pm 4.1\%$ for nickel, $\pm 4.3\%$ for copper, and $\pm 4.8\%$ for zinc. BDL = below detection limit. OF = outflow. Detection limits for Co, Ni, Cu, and Zn are 8.5, 32.5, 22.8, 55.2 nmol/kg, respectively. / =not sampled.

Table XXVII: Gallium, selenium, strontium, and zirconium concentrations

Sample ID	Sample Location	Ga	Se	Sr	Zr
		nmol/kg			
Mudpots					
160712D	Mudder's Milk	BDL	BDL	BDL	BDL
160715T	Urinal Cake	BDL	160	BDL	BDL
160715V	Consolation Prize	BDL	BDL	120	67
160715A	Lil' B	61	BDL	110	85
160716E	Ol' Chunky	200	BDL	300	BDL
150919B	Princess Peach	83	140	170	BDL
160717M	Princess Peach	92	BDL	67	BDL
160517C	Muddy Delight	BDL	BDL	1700	BDL
160517E	Pedro's Tarpit	370	100	3000	127
160724K	Phantom Pants	580	BDL	11200	28
160518L	Tweedle Dee	BDL	BDL	1000	BDL
160518N	Tweedle Dum	100	BDL	1600	BDL
160719W	Choco Mocha	1300	BDL	5800	BDL
160719Z	The Good Stuff	0.2	BDL	400	BDL
160726S	Ugluk's Door	360	BDL	210	BDL
Shoshone Geyser Basin					
160712E	Sarlacc Pit	200	BDL	BDL	BDL
Pocket Basin Thermal Area					
160715X	Vitamin C Pool (Source)	370	BDL	100	BDL
160715Z	Cotton Candy	160	220	74	BDL
Sylvan Springs Thermal Area					
160716D	Dryer				
160716F	Burning Eye	170	BDL	150	BDL
160716G	Gunters Fancy	BDL	BDL	120	BDL
160716H	Goldilocks Source	190	BDL	270	BDL
Rabbit Creek North Thermal Area					
150919C	Obsidian Pool Jr.	130	BDL	24	BDL
150919E	Rabbit Creek Source	590	1000	BDL	BDL
160717S	Nefarious Now	37	BDL	38	BDL

Table XXVII: Gallium, selenium, strontium, and zirconium concentrations, continued.

Sample ID	Sample Location	Ga	Se	Sr	Zr
		nmol/kg			
Rabbit Creek North Thermal Area, continued					
160717N	Pink Mat	680	BDL	BDL	BDL
160717O	Rabbit Creek Source	670	BDL	BDL	BDL
160717P	Rabbit Creek Yellow	670	BDL	BDL	BDL
160717Q	Rabbit Creek Orange	700	BDL	BDL	BDL
160717R	Rabbit Creek Green	750	BDL	BDL	BDL
160917B	Rabbit Creek Green	BDL	BDL	BDL	BDL
160917C	Rabbit Creek Orange	750	BDL	BDL	BDL
160917D	Rabbit Creek Yellow	BDL	BDL	BDL	BDL
160917E	Rabbit Creek Source	600	BDL	BDL	BDL
Rabbit Creek South Thermal Area					
160721M	Spitting Cobra Orange	BDL	BDL	BDL	BDL
160721N	Spitting Cobra Orange-Green	BDL	BDL	BDL	BDL
160721O	Spitting Cobra Green-Black	BDL	BDL	BDL	BDL
160721P	Spitting Cobra Black	BDL	BDL	BDL	BDL
160721Q	Spitting Cobra Source	BDL	BDL	BDL	BDL
Greater Obsidian Pool Thermal Area					
160517D	Figure 8 Source	BDL	BDL	300	BDL
160517F	Obsidian Pool	BDL	BDL	2100	BDL
160517G	Figure 8 OF 2	BDL	BDL	300	BDL
160517H	Figure 8 OF 1	BDL	BDL	290	BDL
Crater Hills Thermal Area					
160518K	Alice	BDL	BDL	670	BDL
160518M	Jabberwocky	BDL	BDL	390	BDL
160518O	Delilah	BDL	BDL	1800	BDL
160518P	Crater Hills Geyser	BDL	BDL	1200	BDL
Forest Springs Thermal Area					
160719Y	Gunter's Dream	83	BDL	4000	BDL
Boulder Spring Thermal Area					
160726R	Boulder Spring Source	860	BDL	BDL	BDL

Instrumental errors are $\pm 4.2\%$ for gallium, $\pm 4.6\%$ for selenium, $\pm 3.8\%$ for strontium, and $\pm 3.9\%$ for zirconium. BDL = below detection limit. OF = outflow. Detection limits for Ga, Se, Sr, and Zr are 18.0, 29.0, 13.8, 10.7 nmol/kg, respectively. / =not sampled.

Table XXVIII: Antimony, barium, lanthanum, and cerium concentrations.

Sample ID	Sample Location	Sb	Ba	La	Ce
		nmol/kg			
Mudpots					
160712D	Mudder's Milk	BDL	82	BDL	BDL
160715T	Urinal Cake	9.0	430	10	33
160715V	Consolation Prize	BDL	670	BDL	BDL
160715A	Lil' B	35	1500	14	34
160716E	Ol' Chunky	BDL	8700	85	230
150919B	Princess Peach	12	1300	7.0	25
160717M	Princess Peach	4.4	2800	4.7	15
160517C	Muddy Delight	BDL	1400	32	87
160517E	Pedro's Tarpit	BDL	14600	490	1300
160724K	Phantom Pants	BDL	27700	59	130
160518L	Tweedle Dee	BDL	1200	140	460
160518N	Tweedle Dum	BDL	2100	83	270
160719W	Choco Mocha	BDL	4700	580	1000
160719Z	The Good Stuff	BDL	BDL	59	170
160726S	Ugluk's Door	8.2	16200	3.3	13
Shoshone Geyser Basin					
160712E	Sarlacc Pit	360	75	BDL	BDL
Pocket Basin Thermal Area					
160715X	Vitamin C Pool (Source)	890	BDL	BDL	BDL
160715Z	Cotton Candy	270	400	BDL	BDL
Sylvan Springs Thermal Area					
160716D	Dryer				
160716F	Burning Eye	BDL	3600	140	310
160716G	Gunters Fancy	540	1500	BDL	BDL
160716H	Goldilocks Source	0.039	2100	120	250
Rabbit Creek North Thermal Area					
150919C	Obsidian Pool Jr.	230	250	BDL	1.8
150919E	Rabbit Creek Source	1200	BDL	BDL	BDL
160717S	Nefarious Now	31	1600	BDL	1.6

Table XXVIII: Antimony, barium, lanthanum, and cerium concentrations, continued.

Sample ID	Sample Location	Sb	Ba	La	Ce
		nmol/kg			
Rabbit Creek North Thermal Area, continued					
160717N	Pink Mat	1200	BDL	BDL	BDL
160717O	Rabbit Creek Source	1100	BDL	BDL	BDL
160717P	Rabbit Creek Yellow	1100	BDL	BDL	BDL
160717Q	Rabbit Creek Orange	1100	BDL	BDL	BDL
160717R	Rabbit Creek Green	1200	BDL	BDL	BDL
160917B	Rabbit Creek Green	1200	BDL	BDL	BDL
160917C	Rabbit Creek Orange	1200	BDL	BDL	BDL
160917D	Rabbit Creek Yellow	1200	BDL	BDL	BDL
160917E	Rabbit Creek Source	1000	BDL	BDL	BDL
Rabbit Creek South Thermal Area					
160721M	Spitting Cobra Orange	10	BDL	BDL	BDL
160721N	Spitting Cobra Orange-Green	9.7	210	BDL	BDL
160721O	Spitting Cobra Green-Black	9.4	130	BDL	BDL
160721P	Spitting Cobra Black	8.0	79	BDL	BDL
160721Q	Spitting Cobra Source	8.7	71	BDL	BDL
Greater Obsidian Pool Thermal Area					
160517D	Figure 8 Source	360	800	BDL	BDL
160517F	Obsidian Pool	BDL	6100	BDL	BDL
160517G	Figure 8 OF 2	320	840	BDL	BDL
160517H	Figure 8 OF 1	320	800	BDL	BDL
Crater Hills Thermal Area					
160518K	Alice	BDL	2600	38	160
160518M	Jabberwocky	BDL	2900	22	110
160518O	Delilah	270	1500	110	250
160518P	Crater Hills Geyser	2700	4300	24	57
Forest Springs Thermal Area					
160719Y	Gunter's Dream	BDL	3700	BDL	6.6
Boulder Spring Thermal Area					
160726R	Boulder Spring Source	860	BDL	BDL	BDL

Instrumental errors are $\pm 3.5\%$ for antimony, $\pm 3.8\%$ for barium, $\pm 4.0\%$ for lanthanum, and $\pm 4.1\%$ for cerium. BDL = below detection limit. OF = outflow. Detection limits for Sb, Ba, La, and Ce are 2.9, 64.8, 1.4, 1.6 nmol/kg, respectively. / =not sampled.

Table XXIX: Praseodymium, neodymium, tungsten, thallium, and uranium concentrations.

Sample ID	Sample Location	Pr	Nd	W	Tl	U
		nmol/kg				
Mudpots						
160712D	Mudder's Milk	BDL	BDL	79	BDL	BDL
160715T	Urinal Cake	6.2	250	BDL	4.5	51
160715V	Consolation Prize	BDL	24	BDL	3.4	14
160715A	Lil' B	3.3	72	30	BDL	5.2
160716E	Ol' Chunky	34	830	17	BDL	2.5
150919B	Princess Peach	4.1	150	BDL	1.1	8.7
160717M	Princess Peach	2.3	79	14	BDL	7.2
160517C	Muddy Delight	10	240	17	BDL	BDL
160517E	Pedro's Tarpit	130	2700	33	2.5	16
160724K	Phantom Pants	13	310	BDL	BDL	4.6
160518L	Tweedle Dee	58	1500	BDL	BDL	7.0
160518N	Tweedle Dum	31	680	BDL	BDL	13.9
160719W	Choco Mocha	91	1600	220	BDL	BDL
160719Z	The Good Stuff	23	550	BDL	BDL	4.5
160726S	Ugluk's Door	1.9	60	4.8	5.0	8.1
Shoshone Geyser Basin						
160712E	Sarlacc Pit	BDL	BDL	4800	BDL	BDL
Pocket Basin Thermal Area						
160715X	Vitamin C Pool (Source)	BDL	BDL	7700	BDL	BDL
160715Z	Cotton Candy	BDL	BDL	7000	BDL	BDL
Sylvan Springs Thermal Area						
160716D	Dryer					
160716F	Burning Eye	29	600	12	BDL	2.4
160716G	Gunters Fancy	BDL	BDL	1700	BDL	BDL
160716H	Goldilocks Source	25	540	41	BDL	BDL
Rabbit Creek North Thermal Area						
150919C	Obsidian Pool Jr.	BDL	BDL	2700	BDL	BDL
150919E	Rabbit Creek Source	BDL	BDL	8500	BDL	BDL
160717S	Nefarious Now	BDL	BDL	11	1.1	1.7

Table XXIX: Praseodymium, neodymium, tungsten, thallium, and uranium concentrations, continued.

Sample ID	Sample Location	Pr	Nd	W	Tl	U
		nmol/kg				
Rabbit Creek North Thermal Area, continued						
160717N	Pink Mat	BDL	BDL	7800	BDL	BDL
160717O	Rabbit Creek Source	BDL	BDL	7700	BDL	BDL
160717P	Rabbit Creek Yellow	BDL	BDL	8000	BDL	BDL
160717Q	Rabbit Creek Orange	BDL	BDL	8000	BDL	BDL
160717R	Rabbit Creek Green	BDL	BDL	8700	BDL	BDL
160917B	Rabbit Creek Green	BDL	BDL	8200	BDL	BDL
160917C	Rabbit Creek Orange	BDL	BDL	8600	BDL	BDL
160917D	Rabbit Creek Yellow	BDL	BDL	8500	BDL	BDL
160917E	Rabbit Creek Source	BDL	BDL	7800	BDL	BDL
Rabbit Creek South Thermal Area						
160721M	Spitting Cobra Orange	BDL	BDL	51	1.3	BDL
160721N	Spitting Cobra Orange-Green	BDL	BDL	43	1.3	BDL
160721O	Spitting Cobra Green-Black	BDL	BDL	47	1.4	BDL
160721P	Spitting Cobra Black	BDL	BDL	48	1.1	BDL
160721Q	Spitting Cobra Source	BDL	BDL	44	1.2	BDL
Greater Obsidian Pool Thermal Area						
160517D	Figure 8	BDL	BDL	2700	BDL	BDL
160517F	Obsidian Pool	BDL	BDL	60	BDL	BDL
160517G	Figure 8 OF 2	BDL	BDL	2200	BDL	BDL
160517H	Figure 8 OF 1	BDL	BDL	2200	BDL	BDL
Crater Hills Thermal Area						
160518K	Alice	26	720	BDL	BDL	BDL
160518M	Jabberwocky	22	650	BDL	BDL	BDL
160518O	Delilah	25	530	BDL	BDL	BDL
160518P	Crater Hills Geyser	BDL	130	2600	42	BDL
Forest Springs Thermal Area						
160719Y	Gunter's Dream	BDL	24	28	BDL	BDL
Boulder Spring Thermal Area						
160726R	Boulder Spring Source	BDL	BDL	8500	BDL	BDL

Instrumental errors are $\pm 4.2\%$ for praseodymium, $\pm 4.1\%$ for neodymium, $\pm 3.2\%$ for tungsten, $\pm 3.5\%$ for thallium, and $\pm 4.1\%$ for uranium. BDL = below detection limit. OF = outflow. Detection limits for Pr, Nd, W, Tl, and U are 1.4, 8.1, 4.1, 1.0, 0.9 nmol/kg, respectively. / =not sampled.

Table XXX: Palladium, silver, cadmium, lead, and thorium concentrations.

Sample ID	Sample Location	Pd	Ag	Cd	Pb	Th
		nmol/kg				
Mudpots						
160712D	Mudder's Milk	BDL	BDL	BDL	BDL	BDL
160715T	Urinal Cake	77	BDL	53	BDL	BDL
160715V	Consolation Prize	BDL	BDL	BDL	BDL	BDL
160715A	Lil' B	BDL	BDL	BDL	6.0	2.2
160716E	Ol' Chunky	38	BDL	BDL	1.6	1.3
150919B	Princess Peach	54	BDL	BDL	BDL	0.9
160717M	Princess Peach	38	BDL	BDL	BDL	BDL
160517C	Muddy Delight	BDL	BDL	BDL	BDL	BDL
160517E	Pedro's Tarpit	68	BDL	25	88	72
160724K	Phantom Pants aka Better Late Than Never Mudpot	BDL	BDL	BDL	3.8	BDL
160518L	Tweedle Dee	BDL	BDL	BDL	BDL	3.2
160518N	Tweedle Dum	BDL	BDL	BDL	BDL	2.7
160719W	Choco Mocha	BDL	BDL	BDL	230	160
160719Z	The Good Stuff	BDL	BDL	BDL	BDL	BDL
160726S	Ugluk's Door	BDL	BDL	32	5.0	BDL
Shoshone Geyser Basin						
160712E	Sarlacc Pit	BDL	24	BDL	BDL	BDL
Pocket Basin Thermal Area						
160715X	Vitamin C Pool (Source)	BDL	BDL	BDL	BDL	BDL
160715Z	Cotton Candy	BDL	BDL	BDL	BDL	BDL
Sylvan Springs Thermal Area						
160716D	Dryer					
160716F	Burning Eye	BDL	BDL	BDL	7.5	11
160716G	Gunters Fancy	BDL	BDL	BDL	BDL	BDL
160716H	Goldilocks Source	BDL	BDL	BDL	8.3	13
Rabbit Creek North Thermal Area						
150919C	Obsidian Pool Jr.	BDL	BDL	BDL	BDL	BDL
150919E	Rabbit Creek Source	BDL	BDL	BDL	BDL	BDL
160717S	Nefarious Now	BDL	BDL	BDL	2.2	BDL

Table XXX: Palladium, silver, cadmium, lead, and thorium concentrations, continued.

Sample ID	Sample Location	Pd	Ag	Cd	Pb	Th
		nmol/kg				

Rabbit Creek North Thermal Area, continued						
160717N	Pink Mat	BDL	BDL	BDL	BDL	BDL
160717O	Rabbit Creek Source	BDL	BDL	BDL	BDL	BDL
160717P	Rabbit Creek Yellow	BDL	BDL	BDL	BDL	BDL
160717Q	Rabbit Creek Orange	BDL	BDL	BDL	BDL	BDL
160717R	Rabbit Creek Green	BDL	BDL	BDL	BDL	BDL
160917B	Rabbit Creek Green	BDL	BDL	BDL	BDL	BDL
160917C	Rabbit Creek Orange	BDL	BDL	BDL	BDL	BDL
160917D	Rabbit Creek Yellow	BDL	BDL	BDL	BDL	BDL
160917E	Rabbit Creek Source	BDL	BDL	BDL	BDL	BDL
Rabbit Creek South Thermal Area						
160721M	Spitting Cobra Orange	BDL	BDL	BDL	BDL	BDL
160721N	Spitting Cobra Orange-Green	BDL	BDL	BDL	BDL	BDL
160721O	Spitting Cobra Green-Black	BDL	BDL	BDL	BDL	BDL
160721P	Spitting Cobra Black	BDL	BDL	BDL	BDL	BDL
160721Q	Spitting Cobra Source	BDL	BDL	BDL	BDL	BDL
Greater Obsidian Pool Thermal Area						
160517D	Figure 8 Source	BDL	BDL	BDL	BDL	BDL
160517F	Obsidian Pool	BDL	BDL	BDL	BDL	BDL
160517G	Figure 8 OF 2	BDL	BDL	BDL	BDL	BDL
160517H	Figure 8 OF 1	BDL	BDL	BDL	BDL	BDL
Crater Hills Thermal Area						
160518K	Alice	BDL	BDL	BDL	BDL	BDL
160518M	Jabberwocky	BDL	BDL	BDL	BDL	BDL
160518O	Delilah	BDL	BDL	BDL	BDL	12
160518P	Crater Hills Geyser	BDL	BDL	BDL	BDL	BDL
Forest Springs Thermal Area						
160719Y	Gunter's Dream	BDL	BDL	BDL	BDL	BDL
Boulder Spring Thermal Area						
160726R	Boulder Spring Source	BDL	BDL	BDL	BDL	BDL

Instrumental errors are $\pm 4.5\%$ for palladium, $\pm 0.9\%$ for silver, $\pm 4.0\%$ for cadmium, $\pm 4.0\%$ for lead, and $\pm 4.3\%$ for thorium. BDL = below detection limit. OF = outflow. Detection limits for Pd, Ag, Cd, Pb, Th, Nb, and Sn are 21.0, 3.6, 13.9, 1.0, 0.9, 5.4, 4.2 nmol/kg, respectively. Niobium and tin concentrations have been omitted because they are below detection limit for all sampled hot springs and mudpots. / =not sampled.

Table XXXI: Relative abundances of trace elements in sampled mudpots.

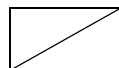
Sample ID	Sample Name	Li	B	Al	Ti	Mn	Fe	Zn	As	Rb	Sr	Mo	Ba	Nd	% Al and fingerp
-----------	-------------	----	---	----	----	----	----	----	----	----	----	----	----	----	---------------------

Table XXXII: $\delta^{18}\text{O}$ and δD vs VSMOW values for mudpots and hot springs.

Sample ID	Unofficial Sample Name	$\delta^{18}\text{O}$ vs VSMOW	Error	δD vs VSMOW	Error
		‰	‰	‰	‰
Mudpots					
160712D	Mudder's Milk	-11.5	0.1	-127	1
160715T	Urinal Cake				
160715V	Consolation Prize				
160715A	Lil' B				
160716E	Ol' Chunky	-6.2	0.1	-102	1
150919B	Princess Peach	-9.9	0.1	-129	1
160717M	Princess Peach	-8.8	0.1	-121	1
160517C	Muddy Delight	-7.0	0.1	-111	1
160517E	Pedro's Tarpit				
160724L	Phantom Pants	-13.2	0.1	-133	1
160518L	Tweedle Dee				
160518N	Tweedle Dum				
160719W	Choco Mocha	-12.5	0.1	-114	1
160719Z	The Good Stuff	-7.3	0.1	-106	1
160726S	Ugluk's Door	-11.0	0.1	-126	1
Shoshone Geyser Basin					
160712C	Proudfeet	-15.2	0.1	-134	1
160712E	Sarlacc Pit	-15.1	0.1	-133	1
160712F	Amy Pohler	-15.1	0.1	-136	1
160712G	Anton Chekhov	-16.0	0.1	-139	1
160712H	Shoshone Creek	-17.0	0.1	-135	1
160713J	CB Outflow 1 (yellow mat)	-15.9	0.1	-137	1
160713K	CB Source	-16.4	0.1	-139	1
160713L	Gray Matter	-9.0	0.1	-114	1
160713M	Millenium Falcon	-16.4	0.1	-138	1
160713N	The Cooler Strikes Back OF 1	-15.5	0.1	-135	1
160713O	The Cooler Strikes Back Source	-16.5	0.1	-138	1
160713P	Flame Leach	-15.6	0.1	-132	1
160713RSMR13	Gold Digger	-14.7	0.1	-130	1
Pocket Basin Thermal Area					
160715U	Vitamin C OF 2	-15.6	0.1	-142	1
160715W	Vitamin C OF 1	-14.9	0.1	-141	1
160715X	Vitamin C Source	-15.2	0.1	-142	1
160715Z	Cotton Candy	-14.4	0.1	-139	1
Sylvan Springs Thermal Area					
160716D	Dryer	-11.4	0.1	-125	1
160716F	Burning Eye	-10.2	0.1	-118	1
160716G	Gunters Fancy	-9.5	0.1	-121	1
160716H	Goldilocks Source	-13.4	0.1	-129	1
160716I	Sylvan Spring	-9.0	0.1	-122	1
160716J	Evening Primrose	-8.4	0.1	-121	1
Rabbit Creek North Thermal Area					
150919C	Obsidian Pool Jr.	-13.9	0.1	-139	1
150919E	Rabbit Creek Source	-15.4	0.1	-143	1
160717N	Pink Mat	-16.1	0.1	-143	1
160717O	Rabbit Creek Source	-16.2	0.1	-144	1

Table XXXII: $\delta^{18}\text{O}$ and δD vs VSMOW values for mudpots and hot springs in YNP, continued.

Sample ID	Unofficial Sample Name	$\delta^{18}\text{O}$ vs VSMOW	Error	δD vs VSMOW	Error
		‰	‰	‰	‰
Rabbit Creek North Thermal Area, continued					
160717P	Rabbit Creek Yellow	-16.0	0.1	-143	1
160717Q	Rabbit Creek Orange	-15.9	0.1	-143	1
160717R	Rabbit Creek Green	-16.0	0.1	-143	1
160717S	Nefarious Now	-9.2	0.1	-131	1
Rabbit Creek South Thermal Area					
160721M	Spitting Cobra Orange	-18.4	0.1	-143	1
160721N	Spitting Cobra Orange-Green	-19.3	0.1	-145	1
160721O	Spitting Cobra Green-Black	-19.4	0.1	-146	1
160721P	Spitting Cobra Black	-19.5	0.1	-146	1
160721Q	Spitting Cobra Source	-19.6	0.1	-146	1
160721R	Titianina	-10.4	0.1	-123	1
160721S	Lil' Sebastian	-12.1	0.1	-134	1
160721T	Iron Fist	-14.0	0.1	-129	1
Greater Obsidian Pool Thermal Area					
160517D	Figure 8 Source	-13.1	0.1	-133	1
160517F	Obsidian Pool	-12.8	0.1	-130	1
160517G	Fig. 8 OF 2	-12.1	0.1	-131	1
160517H	Fig. 8 Outflow 1	-12.0	0.1	-131	1
160720C	Figure 8 Source	-13.5	0.1	-134	1
160720E	Fig. 8 OF 1	-13.4	0.1	-134	1
160720F	HH/Fig. 8 Mix	-13.1	0.1	-132	1
160720G	Fig. 8 OF 2	-12.8	0.1	-131	1
160720H	Happy Harfer OF	-13.0	0.1	-132	1
160720I	OB1-Heim	-12.8	0.1	-130	1
160720J	Fig. 8 OF 1.5	-13.0	0.1	-132	1
160724I	Skippy's Bathtub	-7.1	0.1	-108	1
160724K	OB1 Hime	-7.7	0.1	-108	1
Crater Hills Thermal Area					
160518K	Alice	-10.9	0.1	-125	1
160518M	Jabberwocky	-11.6	0.1	-127	1
160518O	Delilah	-12.0	0.1	-126	1
160518P	Crater Hills Geyser	-11.6	0.1	-125	1
Forest Springs Thermal Area					
160719V	Yellow Sulfur Photosynthetic Cove	-16.5	0.1	-139	1
160719X	Purple Toad	-18.7	0.1	-143	1
160719Y	Gunter's Dream	-9.9	0.1	-118	1
Boulder Spring Thermal Area					
160726O	Boulder Spring OF 3	-16.1	0.1	-143	1
160726P	Boulder Spring OF 2	-16.4	0.1	-143	1
160726Q	Boulder Spring OF 1	-16.2	0.1	-143	1
160726R	Boulder Spring Source	-16.9	0.1	-144	1



Sample Not Collected. Errors are instrumental in nature. BDL = below detection limit

9. Appendix C: Sampling Blanks and Detection Limits

Table XXXIII: Lithium, boron, aluminum, iron, and titanium concentrations for blanks.

Sample ID	Sample Location	Li	B	Al	Fe	Ti
		$\mu\text{mol/kg}$				
160712B	Filter	BDL	0.66	0.28	0.20	BDL
160715S	Filter	BDL	0.30	0.02	BDL	BDL
160716C	Filter	BDL	0.29	0.24	BDL	BDL
150919D	Filter	BDL	0.34	0.36	0.56	0.25
160717L	Filter	BDL	0.33	0.17	BDL	BDL
160517B	Filter	BDL	BDL	0.13	BDL	BDL
160518J	Filter	BDL	0.15	0.10	BDL	BDL
160719U	Filter	BDL	0.21	0.08	BDL	BDL
160726N	Filter	0.34	0.65	1.23	BDL	BDL
160715R	Field	BDL	0.13	0.08	BDL	BDL
160716B	Field	BDL	0.21	0.84	BDL	BDL
150919A	Field	BDL	0.10	0.12	0.19	BDL
160717K	Field	BDL	0.34	0.08	BDL	BDL
160917A	Field	BDL	1.02	0.09	BDL	BDL
160518I	Field	BDL	0.06	0.05	BDL	BDL
160719T	Field	BDL	0.18	0.03	BDL	BDL
160721L	Field	BDL	0.27	0.07	BDL	BDL
160517A	Field	BDL	BDL	0.04	BDL	BDL
160726M	Field	BDL	0.11	0.07	BDL	BDL

Filter blanks were associated with the mudpot filtration apparatus and field blanks with the hot spring syringe filtration. Instrumental errors for Li, B, Al, Fe, and Ti are $\pm 4.2\%$, $\pm 4.0\%$, $\pm 5.6\%$, $\pm 5.2\%$, and $\pm 3.8\%$, respectively. BDL = below detection limit.

Table XXXIV: Lithium, boron, aluminum, iron, and titanium detection limits.

Element	Detection Limit	Error
	$(\mu\text{mol/kg})$	
Li	0.078	0.003
B	0.023	0.001
Al	0.019	0.001
Fe	0.098	0.008
Ti	0.193	0.007

Table XXXV: Manganese, arsenic, rubidium, molybdenum, and cesium concentrations for blanks.

Sample ID	Sample Location	Mn	As	Rb	Mo	Cs
		nmol/kg				
160712B	Filter	BDL	3.0	BDL	BDL	BDL
160715S	Filter	BDL	BDL	BDL	BDL	BDL
160716C	Filter	51	BDL	BDL	BDL	BDL
150919D	Filter	BDL	5.6	BDL	BDL	3.8
160717L	Filter	BDL	BDL	BDL	BDL	BDL
160517B	Filter	BDL	BDL	BDL	BDL	BDL
160518J	Filter	BDL	BDL	BDL	BDL	BDL
160719U	Filter	BDL	BDL	BDL	BDL	BDL
160726N	Filter	53	BDL	10	BDL	BDL
160715R	Field	BDL	BDL	BDL	BDL	BDL
160716B	Field	BDL	4.7	BDL	BDL	BDL
150919A	Field	BDL	BDL	BDL	22	BDL
160717K	Field	BDL	4.1	BDL	BDL	BDL
160917A	Field	BDL	BDL	BDL	BDL	BDL
160518I	Field	BDL	BDL	BDL	BDL	BDL
160719T	Field	BDL	BDL	BDL	BDL	BDL
160721L	Field	36	BDL	BDL	BDL	BDL
160517A	Field	BDL	BDL	BDL	BDL	BDL
160726M	Field	BDL	BDL	BDL	BDL	BDL

Beryllium, vanadium, chromium, cobalt, nickel, and copper were omitted from this table because measured concentrations were below the detection limit. Filter blanks were associated with the mudpot filtration apparatus and field blanks with the hot spring syringe filtration. Instrumental errors are $\pm 3.7\%$ for beryllium, $\pm 3.8\%$ for vanadium, $\pm 4.4\%$ for chromium, $\pm 5.0\%$ for manganese, $\pm 3.8\%$ for arsenic, $\pm 3.0\%$ for rubidium, $\pm 3.9\%$ for molybdenum, $\pm 4.1\%$ for cobalt, $\pm 4.1\%$ for nickel, $\pm 4.3\%$ for copper, and $\pm 3.0\%$ for cesium. BDL = below detection limit.

Table XXXVI: Beryllium, vanadium, chromium, manganese, arsenic, rubidium, molybdenum, cesium, cobalt, nickel, and copper detection limits.

Element	Detection Limit	Error
	(nmol/kg)	
Be	22.19	0.82
V	9.84	0.35
Cr	4.59	0.14
Mn	36.40	1.02
As	2.67	0.06
Rb	8.11	0.07
Mo	21.37	0.68
Cs	3.76	0.04
Co	8.48	0.24
Ni	32.49	1.04
Cu	22.75	0.59

Errors are instrumental in nature.

Table XXXVII: Zinc, selenium, strontium, barium, and tungsten concentrations for blanks.

Sample ID	Sample Location	Zn	Se	Sr	Ba	W
		nmol/kg				
160712B	Filter	1200	BDL	BDL	BDL	BDL
160715S	Filter	270	BDL	BDL	BDL	BDL
160716C	Filter	550	BDL	BDL	BDL	5.5
150919D	Filter	1400	60	BDL	110	15
160717L	Filter	610	BDL	BDL	BDL	BDL
160517B	Filter	610	BDL	BDL	BDL	8.4
160518J	Filter	720	BDL	15	BDL	BDL
160719U	Filter	360	BDL	BDL	BDL	4.8
160726N	Filter	1300	BDL	BDL	BDL	BDL
160715R	Field	250	BDL	BDL	BDL	BDL
160716B	Field	860	BDL	BDL	BDL	6.5
150919A	Field	BDL	BDL	BDL	BDL	4.9
160717K	Field	250	BDL	BDL	BDL	BDL
160917A	Field	680	BDL	BDL	BDL	BDL
160518I	Field	280	BDL	BDL	BDL	BDL
160719T	Field	380	BDL	BDL	BDL	4.6
160721L	Field	860	BDL	BDL	BDL	BDL
160517A	Field	260	BDL	BDL	BDL	15
160726M	Field	610	BDL	BDL	BDL	BDL

Gallium, zirconium, niobium, palladium, silver, cadmium, tin, antimony, lanthanum, cerium, praseodymium, neodymium, thallium, lead, thorium, and uranium were omitted from this table because measured concentrations were below the detection limit. Detection limits for Zn, Se, Sr, Ba, W, Ga, Zr, Nb, Pd, Ag, Cd, Sn, Sb, La, Ce, Pr, Nd, Tl, Pb, Th, U are 55.2, 29.0, 13.8, 64.8, 4.1, 18.0, 10.7, 5.4, 21.0, 13.9, 4.2, 2.9, 1.4, 1.6, 1.4, 8.1, 1.0, 1.0, 0.9, 0.9 $\mu\text{mol/kg}$, respectively. Filter blanks were associated with the mudpot filtration apparatus and field blanks with the hot spring syringe filtration. Instrumental errors are $\pm 4.8\%$ for zinc, $\pm 4.6\%$ for selenium, $\pm 3.8\%$ for strontium, $\pm 3.8\%$ for barium, $\pm 3.2\%$ for tungsten. BDL = below detection limit.

Table XXXVIII: Sodium and calcium concentrations for blanks.

Sample ID	Type of Blank	Na	Ca
		$\mu\text{mol/kg}$	
160715S	Filter	BDL	BDL
160717L	Filter	BDL	BDL
160719U	Filter	BDL	BDL
160726N	Filter	40	6.3
160720B	Filter	11	1.5
160715R	Field	BDL	BDL
160717K	Field	BDL	BDL
160917A	Field	BDL	BDL
160719T	Field	BDL	BDL
160721L	Field	BDL	BDL
160517A	Field	BDL	BDL
160726M	Field	BDL	BDL
160720A	Field	BDL	BDL

Lithium, Potassium, and Magnesium were omitted from this table because measured concentrations were below the detection limit. Instrumental errors are $\pm 10\%$ for all cation values. BDL = below detection limit. Detection limits for Li, Na, K, Mg, Ca are 1.7, 2.7, 11.1, 9.6, 0.7 μM , respectively.

Table XXXIX: Zinc, gallium, selenium, strontium, zirconium, niobium, palladium, silver, cadmium, tin, antimony, barium, tungsten, lanthanum, cerium, praseodymium, neodymium, thallium, lead, thorium, and uranium detection limits.

Element	Detection Limit	Error
	(nmol/kg)	
Zn	55.16	1.76
Ga	17.98	0.20
Se	29.01	0.81
Sr	13.82	0.36
Zr	10.65	0.15
Nb	5.38	0.08
Pd	21.04	0.32
Ag	3.58	0.07
Cd	13.90	0.46
Sn	4.21	0.07
Sb	2.87	0.09
Ba	64.83	2.01
W	4.11	0.06
La	1.44	0.02
Ce	1.61	0.02
Pr	1.42	0.02
Nd	8.07	0.15
Tl	0.98	0.03
Pb	0.97	0.02
Th	0.86	0.01
U	0.85	0.01

Errors are instrumental in nature.

Table XL: Lithium, sodium, potassium, magnesium, and calcium detection limits.

Cation	Detection Limit	Error
	μmol/kg	
Lithium	1.7	28.8
Sodium	2.7	8.7
Potassium	11.1	1.3
Magnesium	9.6	2.1
Calcium	0.7	0.7

Errors are instrumental in nature.

Table XLI: Fluoride, sulfate, and nitrate concentrations for blanks.

Sample ID	Type of Blank	F ⁻	Cl ⁻	SO ₄ ⁻²	NO ₃ ⁻
		μmol/kg			
160715S	Filter	BDL	420	BDL	BDL
160716C	Filter	8.2	150	11	BDL
160717L	Filter	13	46	120	1.1
160517B	Filter	4.3	330	7.3	BDL
160518J	Filter	5.4	210	12	0.9
160719U	Filter	2.5	11	13	0.8
160726N	Filter	29	860	68	1.9
160715R	Field	BDL	8.0	9.7	1.1
160716B	Field	BDL	7.9	BDL	0.9
160717K	Field	BDL	7.8	11	BDL
160917A	Field	BDL	10	BDL	1.0
160518I	Field	1.4	5.8	5.2	BDL
160719T	Field	2.5	9.2	13	1.2
160721L	Field	BDL	11	14	0.9
160517A	Field	BDL	5.2	BDL	BDL
160726M	Field	BDL	11	14	BDL

Bromide, phosphate, and nitrite were omitted from this table because measured concentrations were below the detection limit. Instrumental errors are $\pm 11.9\%$ for fluoride, $\pm 0.92\%$ for chloride, $\pm 1.1\%$ for bromide, $\pm 1.9\%$ for sulfate, $\pm 10.8\%$ for phosphate, and $\pm 0.87\%$ for nitrate. BDL = below detection limit. Detection limits for F⁻, Cl⁻, Br⁻, SO₄⁻², PO₄⁻³, NO₃⁻, and NO₂⁻ were 0.53, 0.28, 0.13, 5.2, 0.21, 0.22, 0.16 μM, respectively.

Table XLII: Fluoride, chloride, bromide, sulfate, phosphate, nitrite, and nitrate detection limits.

Anion	Detection limit	Error
	μmol/kg	
Fluoride	0.526	0.063
Chloride	0.282	0.003
Bromide	0.125	0.001
Sulfate	5.205	0.099
Phosphate	0.211	0.023
Nitrite	0.220	0.007
Nitrate	0.161	0.001

Errors are instrumental in nature.

10. Appendix D: Field Guide to Sampling Locations

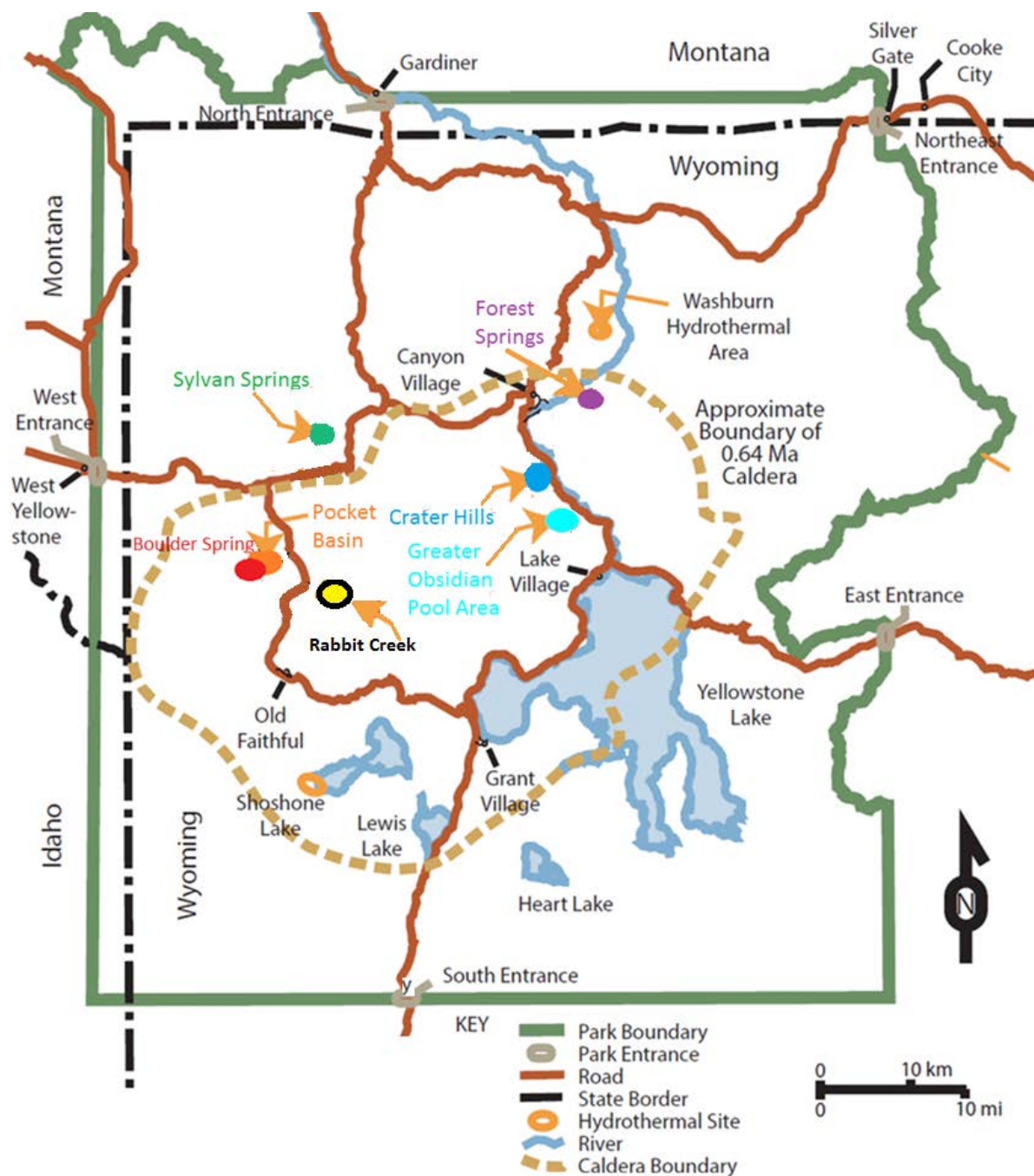


Figure 41: Same as Figure 2.

10.1. Eastern Sites

10.1.1. Geology

The eastern sampling sites are hosted in younger rock, whereas the western sampling sites may be in more eroded areas, with older outcrops nearby (Table D - 1). The three eastern sampling sites are underlain by the Hayden Valley Flow. The Hayden Valley and Solfatara Flows of the Pleistocene Central Plateau Member have variation in trace element bulk compositions in relation to the other flows in the Central Plateau Member (Girard *et al.*, 2010).

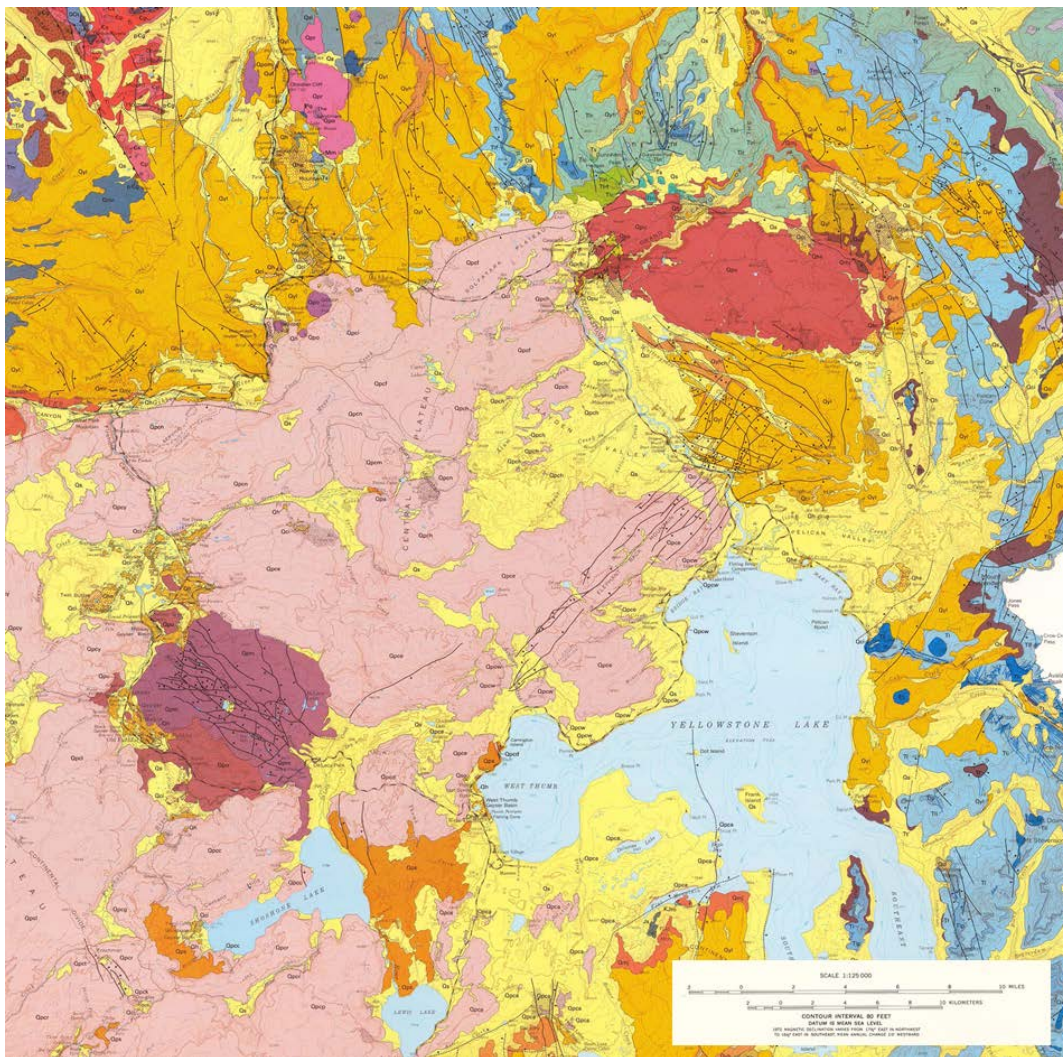


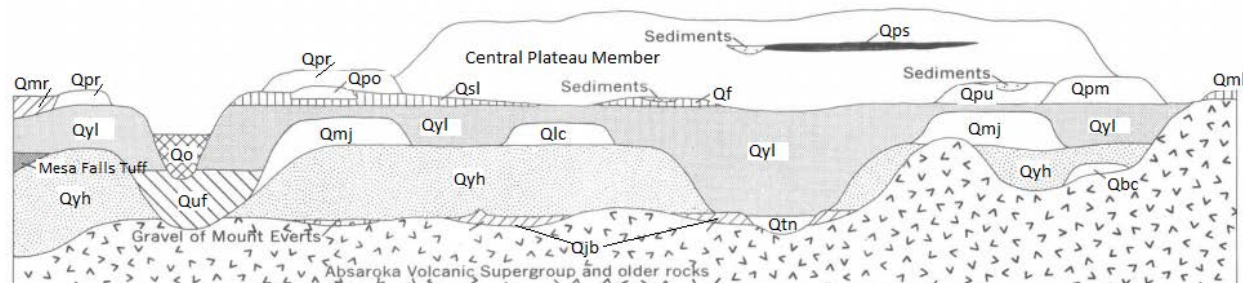
Figure 42: Same as Figure 3.

Table XLIII: Same as Table I.

<u>Thermal Area</u>	<u>Surficial Geology*</u>	<u>Probable Next Rock Unit**</u>
Forest Springs	Qpu, Upper Basin Member (260 ka) Qs, detrital deposits (Holocene)	Qpu, Upper Basin Member (260 ka) Qpch, Hayden Valley Flow (102 ka)
Crater Hills	Qs, detrital deposits (Holocene) Qci, cemented ice contact deposits localized by hot springs (Holocene)	Qpch, Hayden Valley Flow (102 ka) Qpcf, Solfatara Plateau Flow (102.8 ka)
GOPA	Qs, detrital deposits (Holocene) Qci, cemented ice contact deposits localized by hot springs (Holocene)	Qpch, Hayden Valley Flow (102 ka) Qpce, Elephant Back Flow (150 ka) Qpcw, West Thumb Flow (144 ka)
Sylvan Springs	Qyl, Lava Creek Tuff (620 ka)	Qpo, Obsidian Creek Member (500 ka) Qpci, Gibbon River Flow (80 ka)
Pocket Basin	Qh, hot spring deposits (Holocene) Qhe, hydrothermal explosion deposits (Holocene)	Qpu, Upper Basin Member (260 ka) Qpcy, West Yellowstone Flow (114 ka) Qpce, Elephant Back Flow (150 ka)
Rabbit Creek	Qh, hot spring deposits (Holocene)	Qpu, Upper Basin Member (260 ka) Qpm, Mallard Lake Member (164 ka)
Shoshone	Qh, hot spring deposits (Holocene) Qci, cemented ice contact deposits localized by hot springs (Holocene)	Qps, Shoshone Lake Tuff Member (180 ka) Qpcc, Spring Creek Flow (160 ka)

Numbers parentheses indicate age in ka (kilo-annum) (Richmond, 1972; Christiansen and Blank, 1972; Beus, 1986; Girard, 2010). Standard abbreviations for rock units precede the rock unit name and age. *Read from USGS map (Richmond, 1972). **Inferred from nearby outcrops from USGS map (Richmond, 1972).

The general stratigraphic diagram of Yellowstone National Park shows volcanic rock units layering in a psudeo-sedimentary fashion, however the older eruptive cycles have caused subregional rock units. These subregional rock units, such as the Shoshone Lake Tuff Member, outcrop in a relatively small area of the park and may be dissimilar in composition from larger and more widespread flows and tuffs, such as most flows in the Central Plateau Member Rhyolite or the Lava Creek Tuff. The localized rock units may have a dramatic effect on the chemistry of surficial thermal features.



Rock Unit	Code	Relative Age
Roaring Mountain Member*	Qpr	Third Volcanic Cycle
Shoshone Lake Tuff Member*	Qps	
Obsidian Creek Member*	Qpo	
Upper Basin Member*	Qpu	
Mallard Lake Member*	Qpm	
Osprey Basalt	Qo	
Madison River Basalt	Qmr	
Swan Lake Flat Basalt	Qsl	
Falls River Basalt	Qf	
Basalt of Mariposa Lake	Qml	
Lava Creek Tuff	Qyl	
Undine Falls Basalt	Quf	
Mount Jackson Rhyolite	Qmj	
Mesa Falls Tuff		Second Volcanic Cycle
Sediments and Basalts of The Narrows	Qtn	
Lewis Canyon Rhyolite	Qlc	First Volcanic Cycle
Huckleberry Ridge Tuff	Qyh	
Rhyolite of Broad Creek	Qbc	
Junction Butte Basalt	Qjb	

Figure 43: Same as Figure 4.

10.1.2. Eastern Thermal Areas

The eastern sampling sites are Forest Springs, Crater Hills, and the Greater Obsidian Pool Area (GOPA) (Fig. D - 1). The eastern half of YNP is underlain by the Hayden Valley and Solfatara Plateau Flows. Thermal waters in this area have noticeably higher rare earth elements (REE) compared to the western half of the park. This increase in REE may be linked to hydrothermal interaction with the underlying rock units at depth, resulting in higher REE concentrations measured in thermal features at the surface. The Hayden Valley and Solfatara

Plateau Flows have been described as chemically different in composition from all other older Central Plateau Member rhyolite flows. This chemical difference is derived from four factors: a gradual disappearance of plagioclase with an increase of sanidine and quartz, indicating lower crystallization temperatures; clinopyroxene becoming less magnesium rich; decreasing titanium content in quartz crystals; and an increase in incompatible elements (Nb, Y, Tb, Dy, Ho, Er, Tm, Yb, Lu) (Girard, 2010).

Forest Springs is subdivided into two regions; one with low to mid-range viscosity mudpots and the other with photosynthetic hot springs. One sampled mudpot had a high measured non-temperature-corrected conductivity (> 16 mS/cm) and precipitated salt sludge during filtration, whereas the other was a more typical mud sample taken from a large mudpot with visually identified heterogeneous viscosity. The other mudpots in the area (not sampled) had low viscosities not suitable for successful filtration for the current setup.

Mudpots with low viscosities likely have particulate much less than $100\text{ }\mu\text{m}$, but much greater than $1.2\text{ }\mu\text{m}$, making gravity pre-filtration ineffective and syringe filtration tedious and resource intensive. Mudpots with extremely high viscosities clog $100\text{ }\mu\text{m}$ bag filters quickly and lack enough fluid per sampling aliquot to syringe filter a full suite of vials for geochemical sampling.



Figure 44: Forest Springs thermal area mudpot unofficially named The Good Stuff.



Figure 45: Forest Springs thermal area mudpot unofficially named, Choco Mocha.

Crater Hills contained noticeably more sulfur than the other sites and elemental sulfur precipitated from many of the thermal features, including the Crater Hills Geyser. The area features turbid and clear hot springs, and a cluster of deep coned homogenous viscosity mudpots. The Crater Hills mudpots had small openings with the actual mud roughly six to ten feet below ground surface. Each Crater Hills mudpot did not have discernable areas of different viscosities, as seen in Forest Springs and GOPA mudpots. The two samples collected in different subregions of the Crater Hills are geochemically similar.



Figure 46: Crater Hills thermal area mudpot cluster, one of which is unofficially named, Tweedledee.

The Greater Obsidian Pool Area (GOPA) has two distinct subregions, with mudpots in each. GOPA is also closer to the Sour Creek resurgent dome than Forest Springs or Crater Hills, which may make the thermal area more dynamic. During the last two weeks of July 2016, a mudpot formed and is geochemically and microbially distinct from the preexisting mudpots.

Before the thermal feature became a mudpot, it was a frying pan. As it accumulated more fluid and suspended sediment, the viscosity was highly variable. Within the span of an hour, the mudpot's viscosity increased dramatically, such that the mudpot become not viable for sampling, however roughly a week later, the mudpot's viscosity stabilized, allowing it to be sampled. The suspended sediment contained larger fragments and the collected sample was sandy when oven dried, rather than a powdered clay material. In contrast, sample collected from the preexisting mudpot is mostly clay with more sandy material than kaolinite-based mudpots in western YNP. Mudpots in this thermal area form dark brown films of unknown composition on the surface. The films may be organic or mineral in nature. Branched alkanes have been extracted from the suspended sediment of a GOPA mudpot (Bradley, 2005). There has been no further published work on branched alkanes in mudpots.



Figure 47: GOPA mudpot unofficially named, The Gomper.



Figure 48: GOPA mudpot unofficially named, Muddy Delight.



Figure 49: GOPA mudpot unofficially named, Phantom Pants, formed during July 2016.

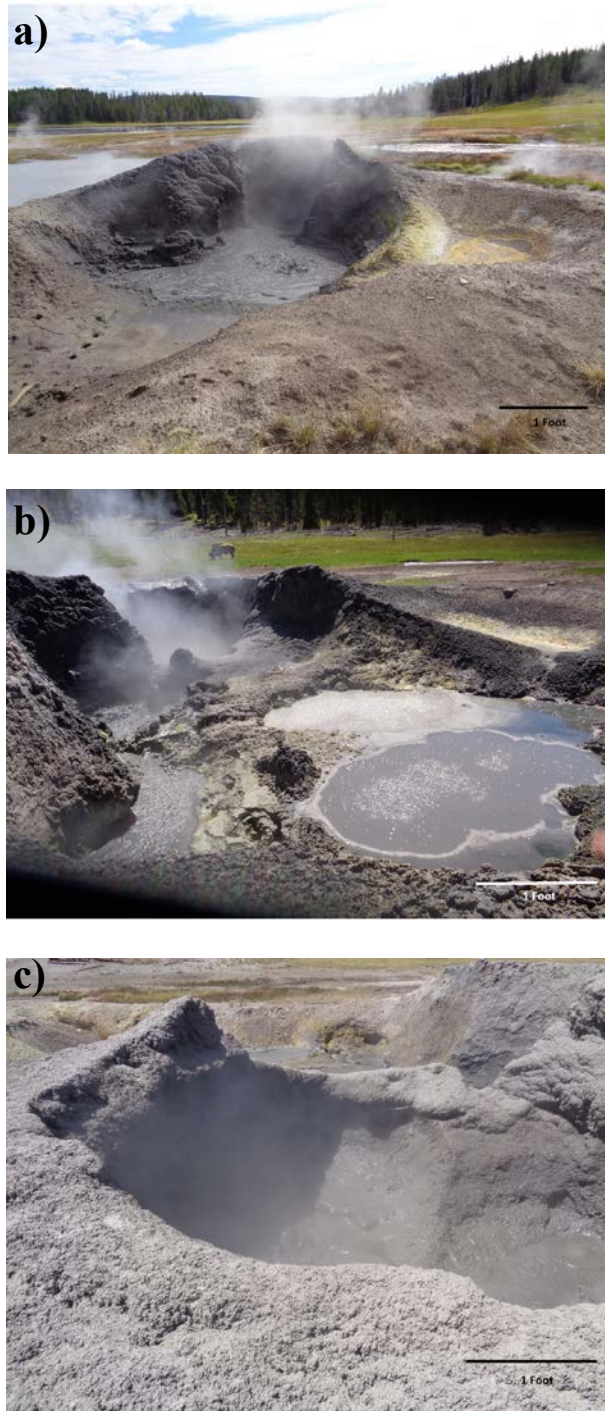


Figure 50: GOPA mudpot unofficially named, Pedro's Tar Pit. This mudpot is dynamic and changes shape and viscosity, as shown in a) July 2015, b) May 2016, and c) July 2016.

10.2. Western Sites

10.2.1. Geology

The western sites may be divided into three groups based on underlying geology: Sylvan Springs, the Midway Geyser Basin (Pocket Basin, Boulder Spring, and Rabbit Creek), and Shoshone Geyser Basin. Sylvan is underlain by the Gibbon River Flow, Shoshone by the Spring Creek Flow and Shoshone Lake Tuff member, and the Midway Geyser Basin by the Upper Basin Member. The far western edge of the park has columnar basalt outcrops and many older, more mafic volcanics (Table D - 1, Fig. D - 3).

10.2.2. Thermal Areas

Thermal areas in the western half of the park were sampled in the summer and fall. From north to south, the thermal areas are Amphitheater, Sylvan Springs, Pocket Basin, Boulder Spring, Rabbit Creek, and Shoshone Basin. Complex volcanic stratigraphy and abundant faulting in western YNP lead to fewer broad conclusions linking surficial trace element chemistry to underlying rock units than in eastern YNP.

During July 2016, dried muddy features at Amphitheater prevented feasible mudpot sampling. In previous years, the Amphitheater thermal area was more active and turbid and clear hot springs were sampled. Lack of mudpots prevented comparison with hot spring geochemistry in this thesis.

Descriptions of Sylvan Spring from Allen and Day are of a clear, possibly alkaline chloride hot spring, rather than the turbid, acidic hot spring cloudy with sulfurous particulate that was witnessed in 2016. One mudpot, a former fumarole, and turbid acidic features were sampled in the Sylvan Springs area. These opposing descriptions suggest a dramatic change in the underground source water behavior, leading to more steam fractionation.



Figure 51: Sylvan Spring thermal area mudpot unofficially named, Ol' Chunky.

Pocket Basin had many mudpots with fairly similar chemistries and a range of viscosities. The mudpot, Urinal Cake (Fig. D - 13), has a strong ammonia-based odor. The mudpots are separate from the photosynthetic hot springs that have outflows into the Firehole River.



Figure 52: Pocket Basin thermal area mudpot unofficially named, Consolation Prize.



Figure 53: Pocket Basin thermal area mudpot unofficially named, Urinal Cake.

The nearby Boulder Spring thermal area is small and features a single mudpot, in addition to the alkaline Boulder Spring and its photosynthetic outflow. This area is slightly northwest of the Pocket Basin, but does not have direct outflow into the Firehole River. The Boulder Spring thermal area lies west of the Sentinel Meadows thermal area, containing historically named superheated alkaline hot springs; Steep Cone, Flat Cone, and Mound Spring.



Figure 54: Boulder Spring thermal area mudpot unofficially named, Ugluk's Door.

Rabbit Creek is divided into Rabbit Creek North, which includes the source, its photosynthetic outflow, a suite of mudpots, and a meteoric mixing region and Rabbit Creek South, which mostly has meteoric mixed thermal fluids. East of the Rabbit Creek thermal area is

the Tomato Soup subregion which has a cluster of colloidal iron hot springs. The Rabbit Creek thermal area is on the edge of the Mallard Lake resurgent dome.

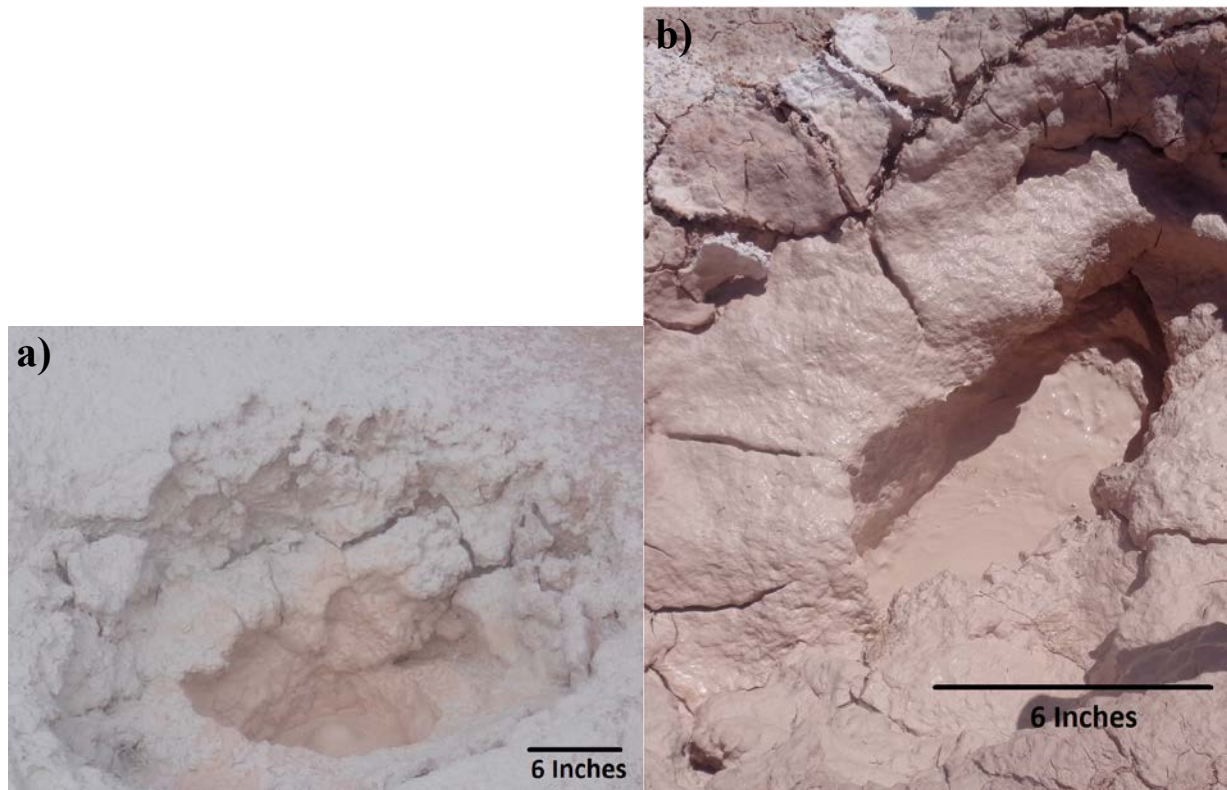


Figure 55: Rabbit Creek North thermal area mudpot unofficially named, Princess Peach. Chemical differences between the two samples a) September 2015 and b) July 2016 have been attributed to dilution.

The Shoshone Geyser Basin is a backcountry site accessible by boat or hike. The thermal area has acidic hot springs directly on the shore of the lake, circumneutral hot springs slightly more inland, and some turbid, slightly viscous pH 6 thermal features that look like mudpots, but are chemically dissimilar to other mudpots in YNP. The thermal area is underlain by the Shoshone Lake Tuff, which is localized and may partially account for the odd mudpot-like thermal features.



Figure 56: Shoshone Geyser Basin mudpot unofficially named, Mudder's Milk. This mudpot exhibits low viscosity and is chemically unlike other YNP mudpots sampled. This mudpot is circumneutral (pH 6.08, 74.6 °C).

11. Appendix E: CHNOSZ Input File

CHNOSZ input file

```
data(thermo)
basis(c("Al+3", "Na+", "K+", "SO4-2", "SiO2", "H+", "H2O", "e-"))
species(c("kaolinite", "halloysite", "dickite", "alunite", "muscovite", "k-feldspar"))
a <- affinity(pH=c(0, 14, 400), Eh=c(-1.5, 1.5, 400), T=92.5)
diagram(a, fill=NULL)
water.lines(T=365.65, col="red")
title(main=paste("Clay, 92.5 °C, 1 bar, a=10^-3", sep="\n"))
```

```
data(thermo)
basis(c("B(OH)3", "H2O", "H+", "e-"))
species(c("B(OH)3", "BO2-"))
a <- affinity(pH=c(0, 14, 400), Eh=c(-1.5, 1.5, 400), T=92.5)
diagram(a, fill=NULL)
water.lines(T=365.65, col="red")
title(main=paste("Boron Speciation 92.5 °C, 1 bar, a=10^-3", sep="\n"))
```

SIGNATURE PAGE

This is to certify that the thesis prepared by Georgia Dahlquist entitled "Foundations for a Geobiochemical Characterization of Mudpots in Yellowstone National Park" has been examined and approved for acceptance by the Department of Chemistry and Geochemistry, Montana Tech of The University of Montana, on this 1st day of July, 2017.



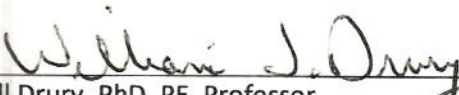
Alysia Cox, PhD, Assistant Professor
Department of Chemistry and Geochemistry
Chair, Examination Committee



Chris Gammons, PhD, Professor and Department Head
Department of Geological Engineering
Member, Examination Committee



Katie Hailer, PhD, Associate Professor
Department of Chemistry and Geochemistry
Member, Examination Committee



Bill Drury, PhD, PE, Professor
Department of Environmental Engineering
Member, Examination Committee