



Mineralogical and geochemical characterization of two Sulfolobales harboring hot spring systems :
Rabbit Creek and Ragged Hills, Yellowstone National Park, WY, USA
by Braden Thomas Hanna

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in
Earth Sciences
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Abstract:

The fine scale mineralogy of two Sulfolobus and Acidianus harboring acid sulfate geothermal areas in Yellowstone National Park was investigated to determine the interrelationships between these microbial populations and the ambient mineralogy. The two geothermal areas are a highly altered solfatara in Rabbit Creek, Midway Geyser Basin and a less mature solfatara in Ragged Hills, Norris Geyser Basin. The fine scale mineralogy was determined using X-ray diffraction (XRD) in combination with scanning electron microscopy (SEM) equipped with energy dispersive spectroscopy (EDS). Cryogenic SEM was used to investigate in situ potential interactions between the mineralogy and microbial populations using both natural and cultured samples. Water chemistry of the natural springs was monitored over a one and a half year time span, and thermodynamic modeling was used to predict the equilibrium mineral assemblages.

The mineralogy of the two sites was found to be significantly different. Abundant sulfide and sulfate minerals were present at the Ragged Hills site, while no sulfate minerals and only trace amounts of sulfides were present at Rabbit Creek. Both sites contained kaolinite, quartz, a variety of paracrystalline silica phases, amorphous aluminum hydroxides, and a various residual volcanic phases. Rabbit Creek also contained hematite and halloysite. Elemental sulfur was only observed at one unique spring at the Ragged Hills site.

The mineralogy observed at these sites was found to influence the populations of sulfur metabolizing, acidophilic, hyperthermophiles in several ways. The microbes from high temperature springs at these sites were found to attach, both in situ and in culture, to a variety of mineral surfaces, including quartz, alunite, sulfur and kaolinite. Microbes from these springs were also found to consume elemental sulfur. It was determined that the kaolinite and amorphous aluminum hydroxide present at both sites are capable of buffering the pH in the range acceptable to sustain Sulfolobus and Acidianus. Observations indicate that elemental sulfur is potentially consumed by metabolic oxidation as rapidly as it forms.

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APPROVAL

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Braden Thomas Hanna

This thesis has been read by each member of the thesis committee and has been found to be satisfactory regarding content, English usage, format, citations, bibliographic style, and consistency, and is ready for submission to the College of Graduate Studies.

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ABSTRACT

The fine scale mineralogy of two *Sulfolobus* and *Acidianus* harboring acid sulfate geothermal areas in Yellowstone National Park was investigated to determine the interrelationships between these microbial populations and the ambient mineralogy. The two geothermal areas are a highly altered solfatara in Rabbit Creek, Midway Geyser Basin and a less mature solfatara in Ragged Hills, Norris Geyser Basin. The fine scale mineralogy was determined using X-ray diffraction (XRD) in combination with scanning electron microscopy (SEM) equipped with energy dispersive spectroscopy (EDS). Cryogenic SEM was used to investigate *in situ* potential interactions between the mineralogy and microbial populations using both natural and cultured samples. Water chemistry of the natural springs was monitored over a one and a half year time span, and thermodynamic modeling was used to predict the equilibrium mineral assemblages.

The mineralogy of the two sites was found to be significantly different. Abundant sulfide and sulfate minerals were present at the Ragged Hills site, while no sulfate minerals and only trace amounts of sulfides were present at Rabbit Creek. Both sites contained kaolinite, quartz, a variety of paracrystalline silica phases, amorphous aluminum hydroxides, and a various residual volcanic phases. Rabbit Creek also contained hematite and halloysite. Elemental sulfur was only observed at one unique spring at the Ragged Hills site.

The mineralogy observed at these sites was found to influence the populations of sulfur metabolizing, acidophilic, hyperthermophiles in several ways. The microbes from high temperature springs at these sites were found to attach, both *in situ* and *in culture*, to a variety of mineral surfaces, including quartz, alunite, sulfur and kaolinite. Microbes from these springs were also found to consume elemental sulfur. It was determined that the kaolinite and amorphous aluminum hydroxide present at both sites are capable of buffering the pH in the range acceptable to sustain *Sulfolobus* and *Acidianus*. Observations indicate that elemental sulfur is potentially consumed by metabolic oxidation as rapidly as it forms.

CHAPTER 1

PURPOSE OF STUDY

The primary purpose of this study was to determine the mineralogy of two geothermal systems in the Rabbit Creek (Midway Geyser Basin) and Ragged Hills (Norris Geyser Basin) areas of Yellowstone National Park, and to compare and contrast the older, more mature system (Rabbit Creek) with the younger, less mature system (Ragged Hills). Comparing and contrasting the older and younger systems provides the opportunity to determine the potential influences of the mineralogical evolution of the system on the microbiological populations in the system. This study also investigated the extent to which the mineralogy of each system influences and is influenced by the thermophilic microbial populations present in these springs) The potential relationships investigated include the attachment of these organisms to mineral surfaces, the use of certain minerals for energy or nutrients, and the implications of the buffering capacity of specific mineral precipitation reactions.

CHAPTER 2

BACKGROUND

Microorganisms Can Influence Their Environment

Microorganisms, such as bacteria and archaea, comprise the majority of the diversity of life, and perhaps the majority of Earth's biomass is composed of subterranean lithotrophs (Pace, 1997). They are found in nearly all environments, from underneath the surface of rocks and ice, to the depths of the oceans, to up to 3.5 kilometers beneath the surface of the Earth's crust (Amend and Shock, 2001). In fact, they are capable of living in the most extreme environments of any known life forms. They are capable of altering their local environment, thereby influencing physical parameters such as pH, Eh, concentration of various aqueous elements and complexes, and precipitation and dissolution of minerals. Some of these microorganisms are chemotrophic, deriving their metabolic energy directly from the free energy associated with changes in the chemical state of their environment. These chemotrophic organisms allow ecosystems to develop where the energy source is from geologic processes rather than sunlight (Nealson and Stahl, 1997). In fact, all known hyperthermophiles and many thermophiles are chemosynthetic, using oxidation reduction reactions of inorganic or organic compounds (Amend and Shock, 2001).

Microbially induced chemical transformations can occur in aqueous, solid, or gaseous phases. Solid phase alteration has been well investigated with respect to the microbially induced oxidation of pyrite (FeS_2) and subsequent acidification in acid mine

drainages (Bigham and Nordstrom, 2000). In this process the sulfur and iron in pyrite are microbially oxidized, leading to the dissolution of pyrite and the acidification of surface waters. Microbes can also indirectly mediate mineral dissolution or precipitation by depleting or increasing concentrations of various dissolved chemical species, driving dissolution and precipitation through equilibrium reactions (Fortin *et al.*, 1997).

Microbial mediation occurs when these reactions are “thermodynamically favored but kinetically inhibited” (Amend and Shock, 2001), as in the oxidation of pyrite at surface conditions. Microorganisms are capable of directly mediating the precipitation of minerals such as iron and manganese oxides, various other metals, opaline silica, carbonates, on and or inside their cell perimeters (Tebo *et al.*, 1997, de Vrind-de Jong and de Vrind, 1997). Microbes have also been shown to selectively mediate mineral dissolution to gain access to limiting nutrients such as the phosphate contained in the mineral apatite ($\text{Ca}_5(\text{PO}_4)_3(\text{F}, \text{Cl}, \text{OH})$) (Rogers *et al.*, 1999). Microbial populations have been shown to accelerate weathering rates of quartz and feldspar even when aqueous concentrations are at saturation (Heibert and Bennett, 1992). The coupling of high temperatures and microbial activity provides an opportunity for greatly accelerated weathering processes and microbially mediated mineral transformations.

The microbial alteration of physical conditions can have profound implications for the system. The oxidation state of metals such as iron and arsenic influence characteristics such as mobility (Xu *et al.*, 1991), toxicity (Stauffer *et al.*, 1980), and bioavailability (Banfield and Hamers, 1997). In order to understand the ramifications of microbially mediated physical environmental change, it is necessary to investigate the

role that microorganisms play in influencing their environment, as well as the details of the physical environments they inhabit.

Mineralogy Can Influence Microbial Populations

Minerals can influence microbial populations by controlling physical and chemical processes in a variety of ways. In addition to providing a source of energy through potential oxidation-reduction pathways and a serving as a source of nutrients such as phosphorous, calcium, sodium, potassium, iron, etc, minerals can also influence microbial populations in other ways. The presence of certain minerals can buffer concentrations of chemical species. For example, the presence of calcite will buffer the pH of surface waters with which it is in contact at a pH of 8.3. The precipitation of minerals that contain certain metals will prevent the respective metals from exceeding a given aqueous concentration. Minerals such as clays are capable of retaining large amounts of water, as well as inhibiting the flow of groundwater. These properties directly influence the spatial distribution of organisms. Clays can also serve as a temporary reservoir for elements such as calcium, potassium, and other biological elements, thus keeping them available to organisms after their liberation from the host rock. Surface properties of minerals vary widely, and can provide conditions that enable microorganisms to attach to mineral surfaces.

Sulfur oxidizing organisms from hydrothermal environments have been shown to attach to a variety of surfaces (Guezennec *et al.*, 1998). Dynamics of surficial attachment of microbes to minerals are diverse and complex. Attachment can range from

electrostatic and transient to the permanent attachment of entire colonies via exopolymers produced by the organisms. Attachment and biofilm production can provide refuge in particularly dynamic environments by inhibiting transport across thermoclines and chemoclines (Little *et al.*, 1997). Attachment also enables colony forming organisms to generate localized physical conditions between mineral and biofilm surfaces. Rocks and soils with different mineralogies also have porosities and permeabilities that vary of several orders of magnitude. The mineralogy of an environment can drastically influence and even control the chemical composition of water, the processes of attachment and transport, and the availability of water, nutrients, and energy.

Site Selection

Two different geothermal areas within the Yellowstone caldera were chosen for this study. These two sites are referred to here as Rabbit Creek and Ragged Hills (see Figure 1). High resolution maps were created for each of these sites and are provided in the cd-rom insert.

The Rabbit Creek study site is located along the otherwise alkaline Rabbit Creek drainage in Midway Geyser Basin (UTM zone 12, 0515034E, 4929794N). A photograph of the Rabbit Creek thermal site is shown in Figure 2. This site is a relatively mature geothermal area and appears to be highly altered. No dead trees are remaining from the emergence of the thermal area, although a few stunted pines do continue to live in the thermal area. The presence of this acidic sulfate rich thermal site was recorded as early as 1935 (Allen and Day).

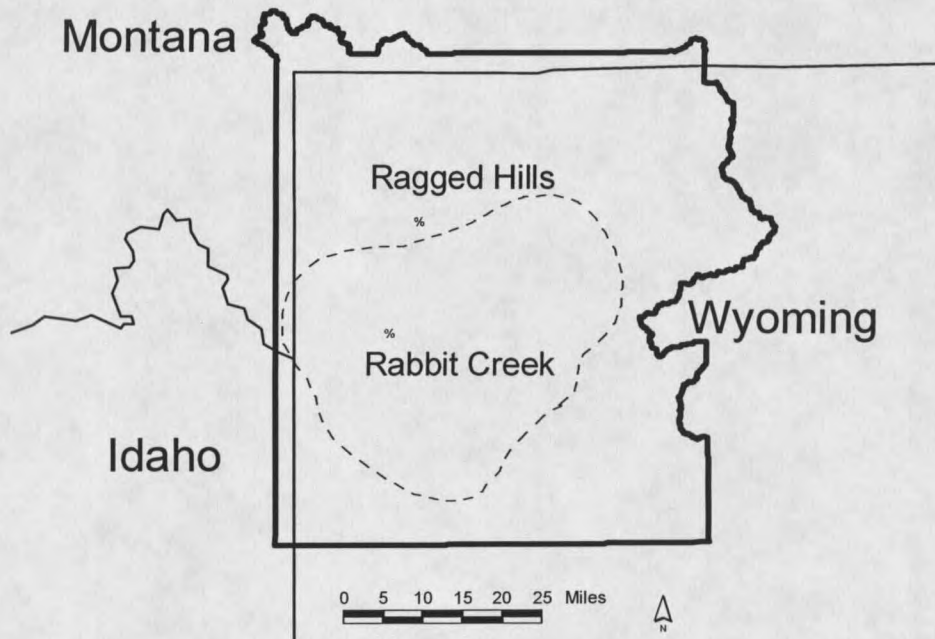


Figure 1: Map of study sites. Rabbit Creek and Ragged Hills study sites are shown as black circles. Boundary of Yellowstone National Park is marked with the thick black line, state borders are shown in thin black (from Spatial Analysis Center, Yellowstone National Park, 1995), and the most recent caldera limits (from Hildreth *et al.*, 1991) are marked with the dashed line.

The second study site, Ragged Hills, is located in the Ragged Hills area of Norris Geyser Basin (UTM zone 12, 521000 E, 4952543 N). A photograph of the Ragged Hills site is shown in Figure 3. Ragged Hills is composed of silica cemented glacial gravels and as of 1935 there was no evidence of any past thermal activity (Allen and Day, 1935). This site is a much less mature geothermal area than Rabbit Creek. There are abundant dead trees, killed off from the recent emergence of the thermal area, and the emergence of new springs at Ragged Hills over the 3 year interval from 1999-2001 has been recently noted (Nordstrom *et al.*, 2001). Both sites have a variety of geothermal features including

mud pots, thermal springs, outflow channels, and fumaroles. Both sites contain springs which maintain pH in the range of 2-4, and temperatures in the range of 65 °C to 90 °C. In addition, they both have alteration mineral assemblages derived from similar underlying rhyolite flows.

The geomicrobiological aspect of this study focuses on the highly acidic (pH 2-4), high temperature (65 °C to 90 °C) springs. The organisms that inhabit this environment are referred to as acidophilic hyperthermophiles (Barns and Nierzwicki-Bauer, 1997). Although numerous types of organisms do inhabit this niche, it has much less diversity than other less extreme habitats. While this study focuses on the sulfur oxidizing organisms *Sulfolobus* and *Acidianus*, it is important to note that other acidophilic thermophiles are undoubtedly present at these sites. However, the microbial diversity and potential role of other microbial organisms at these sites was beyond the scope of this study.

Extremophiles

Extremophiles, organisms that grow in environments that are inhospitable to most living things, have recently become an important topic for research. Categories of extremophiles include thermophiles and hyperthermophiles (which grow optimally at >80C), psychrophiles (which grow optimally at <15C) halophiles (which grow in high salinity), endoliths (which grow inside rocks), barophiles (which grow at high hydrostatic pressure), acidophiles (which grow at low pH), as well as many others. Extremophiles

can also demonstrate the ability to grow multiple extreme environments such as thermophilic acidiphiles.

The construction of a universal phylogenetic tree using evolutionary distances between different organisms based on their 18S and 16S rDNA sequences (Figure 4) has indicated that there are three major domains of life, or urkingdoms (Woese, 1987). The hypothetical last common ancestor is represented by the root of the tree. Theoretically, the closer an organism is to the root of the tree, the closer it is to the last common ancestor (Woese, 1987). The fact that extremophiles plot close to the base of the tree is evidence that they may serve as analogues of extraterrestrial life and of early life on Earth as well.

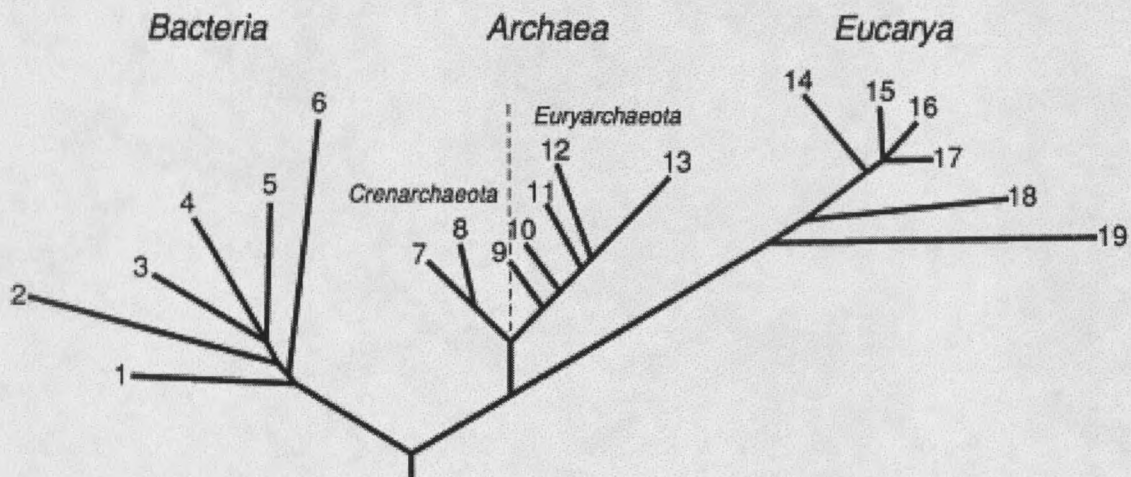


Figure 4: Phylogenetic tree of life. *Sulfolobus* lies in the group labeled 8 on this tree. Branching order and length are based on rRNA sequences. Length of branches and distance from the base of the tree are proportional to evolutionary distance from the hypothetical common ancestor. Numbers on the tree represent the following groups: 1, the Thermotogales; 2, the flavobacteria; 3, the cyanobacteria; 4, the purple bacteria; 5, the Gram-positive bacteria; 6, the green nonsulur bacteria; 7, *Pyrodictum*; 8, *Thermoproteus*; 9, the Thermococcales; 10, the Methanococcales; 11, the Methanobacteriales; 12, the Methanomicrobiales; 13, the extreme halophiles; 14, the animals; 15, the ciliates; 16, the green plants; 17, the fungi; 18, the flagellates; and 19, the microsporidia. (From Woese *et al.*, 1990).

Research focus on these organisms has grown dramatically in recent years. They are important for a number of reasons. Their environments serve as analogues for potential extraterrestrial ecosystems. In fact, hot springs are a primary potential site for the origin of life on Earth and are currently a primary target for the search for life and fossils of past life outside of Earth (Farmer, 2000). They are also very important for industrial and biomedical applications because of the novel proteins they use to catalyze reactions under such unique conditions (Madigan and Mairs, 1997). The extremophiles of direct interest in this study are acidophilic hyperthermophiles.

Hyperthermophilic Sulfur Metabolizing Organisms

The domain archaea consists of two phyla, the Crenarchaeota and the Euryarchaeota. The phylum Crenarchaeota is composed of acidophilic, obligately thermophilic (Garrity, G.M., 2001), sulfur-dependent organisms (Howland, 2000). Most cultured Crenarchaeotes are hyperthermophilic (Snyder and Young, in press). Within the phylum Crenarchaeota, the sole class, *Thermoprotei*, has three orders: *Thermoproteales*, *Desulfurococcales*, and *Sulfolobales* (Garrity, G.M., 2001). Among these, the sulfur reducers, *Thermoproteales*, and the sulfur oxidizers, *Sulfolobales* are the most widely studied (Snyder and Young, in press). Two genus from the family *Sulfolobaceae* order *Sulfolobales*, *Sulfolobus* and *Acidianus*, have been isolated from the high temperature areas of the Rabbit Creek and Ragged Hills sites (although a true *Acidianus* type-member has not yet been isolated from Rabbit Creek) (Rice *et al.*, 2001).

The first acidophilic hyperthermophile to be characterized was the Crenarchaeote *Sulfolobus* (Brock *et al.*, 1972), found in Yellowstone National Park. Since then, a variety of acidophilic hyperthermophiles have been identified in solfatara environments around the world. There are currently six recognized species of the genus *Sulfolobus*: *S. acidocaldarius*, *S. solfataricus*, *S. hakonensis*, *S. metallicus*, *S. shibatae*, and *S. yangmingensis* (as well as two other proposed species, *S. islandicus* and *S. thuringiensis*) (Garrity, G.M., 2001). The *Sulfolobus* species isolated from Rabbit Creek and Ragged Hills most closely resemble *S. acidocaldarius* and *S. solfataricus*, based on 16S rRNA sequencing (Rice *et al.*, 2001).

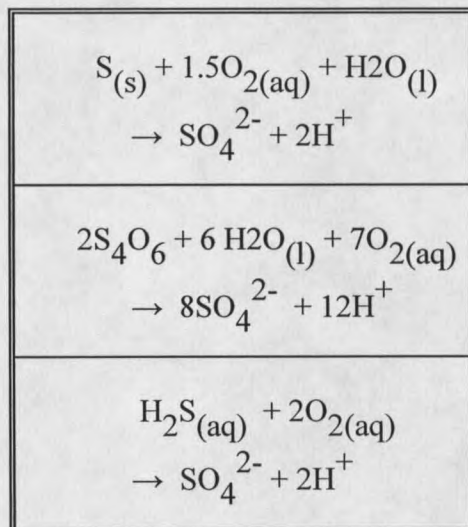
Sulfolobus cells are coccoid in shape and can be highly lobed or spherical (Garrity, G.M., 2001). They have a diameter ranging from 0.7 to 2 microns, can be either motile or immotile, and flagella, pillus-like, and pseudopodium-like structures have all been observed. They live in a pH range from 1- 5.5 and a temperature range from 65 to 85°C. *Sulfolobus* is a facultative chemolithoautotroph, meaning it can use inorganic or organic carbon sources and attains energy from oxidation reduction reactions of organic molecules or inorganic sulfur and iron compounds. All species are obligate aerobes, requiring oxygen (although some species can use Fe^{3+} , or MoO_4^{2-}) as electron acceptors. Lithotrophic growth can occur using sulfide ores, sulfide, elemental sulfur, tetrathionate, or Fe^{2+} ions as electron donors. Organotrophic growth occurs using complex organic materials, sugars, or amino acids. (Garrity, G.M., 2001).

Although *Sulfolobus* is one of the most widely studied archaebacteria, its identification and characterization is often confounding. In fact, widely available

Sulfolobus cultures have been found to be impure and misidentified (Grogan, 1989), even after repeated RNA polymerase, DNA binding protein and ribosomal protein analyses.

Sulfolobus acidocaldarius was found to have narrower temperature ranges, more sensitivity to organic carbon sources, and less tolerance to phosphate than other wild types investigated (Grogan, 1989). Some known metabolic reactions of *Sulfolobus acidocaldarius* are given by Amend and Shock (2001) and have been listed in Table 1.

Table 1: Metabolic reactions of *Sulfolobus acidocaldarius*. (Reactions from Amend and Shock, 2001).



The sensitivity of *Sulfolobus* to salts, heavy metals, and organics has been shown to be similar for the *acidocaldarius* and *solfataricus* strains investigated by Grogan (1989). The sensitivity to several anions, cations, and metals are listed in Table 2.

Table 2: Sensitivity of *Sulfolobus acidocaldarius* and *Sulfolobus solfataricus* to important cations, anions, and metals. (From Grogan, 1989)

Ion	Growth Inhibiting Concentration (Molarity)
SO ₄ ²⁻	>0.64
Cl ⁻	0.64
NO ₃ ²⁻	0.08 – 0.16
PO ₄ ²⁻	<0.06
K ⁺	0.32
Na ⁺	0.32
Li ⁺	0.32
Mg ²⁺	0.64
Ag ⁺	8 * 10 ⁻⁶
Cr ₂ O ₇ ²⁻	65 * 10 ⁻⁶
Cu ²⁺	5 * 10 ⁻³
Hg ²⁺	2 * 10 ⁻⁶
Ni ²⁺	6 * 10 ⁻⁴
Pb ²⁺	1.6 * 10 ⁻³
Zn ²⁺	5 * 10 ⁻²

In addition to *Sulfolobus*, an *Acidianus*-like organism has also been isolated from Rabbit Creek and Ragged Hills (Rice, personal communication). The genus *Acidianus* has three species, *A. infernus*, *A. ambivalens*, and *A. brierleyi*. *Acidianus* cells are coccoid in shape and highly irregular. They range from 0.5 to 2 microns in diameter, and occur almost exclusively as single cells. (Garrity, G.M., 2001). They grow in a pH range from 1 to 6, and a temperature range from 45 to 96 °C (Segerer *et al.*, 1986). *Acidianus* is capable of using CO₂ (autotrophic) or organic carbon (mixotrophic) as a carbon source. *A. brierleyi* and *A. ambivalens* are obligate chemotrophs, capable of reducing elemental sulfur with H₂ to H₂S under anaerobic conditions or oxidizing elemental sulfur to sulfate under aerobic conditions (Segerer *et al.*, 1986) (or under anaerobic conditions in the

presence of MoO_4^{2-} (Garrity, G.M., 2001)). *A. brierleyi* can also use oxidation of ferrous iron and can grow heterotrophically on complex organics like yeast extract and peptone in the presence of oxygen (Seegerer *et al.*, 1986). Some species have been reported to directly mobilize metals from oxidation of sulfide ores (Garrity, G.M., 2001).

Acidophilic hyperthermophiles are known to inhabit acid sulfate springs from Yellowstone National Park (Fliermans and Brock, 1972). Oily films on the surface of mud pots have been found to have very high *Sulfolobus* populations (Garrity, G.M., 2001). *Sulfolobus* and *Acidianus* have been isolated from springs at Rabbit Creek and Ragged Hills. These organisms are known to be capable of obtaining energy by catalyzing reactions involving a variety of gasses, minerals, and dissolved ions in their environment. The physical, chemical, and mineralogical properties of their environment strongly influence the ability of these organisms to survive.

Acid Sulfate Springs

Not all geothermal springs provide conditions suitable to acidophilic hyperthermophiles. White *et al.* (1971) characterized two end-member types of geothermal systems, vapor-dominated and hot-water systems. Each type produces hot spring environments with specific physical and chemical properties.

Water-dominated systems tend to have high outflow in the range of several hundred to several thousand liters per minute, low sulfate to chloride ratios, and a near neutral pH. Occasionally these systems have dominantly sub-surface outflow. These water-dominated systems with primarily subsurface outflow have significantly different

properties. In these cases Fe^{3+} , Al^{3+} , Ca^{2+} , and Mg^{2+} are dominant relative to Na^+ and K^+ , the pH ranges from 2.5-5, the ground is usually bleached white and contains kaolinite ($\text{Al}_2\text{Si}_2\text{O}_5(\text{OH})_4$), elemental sulfur, and orange, yellow, and white sulfate minerals, and chloride concentrations below 20 mg/L. The water-dominated systems which have primarily subsurface flow have some characteristics, such as a pH of 2.5-5, that could provide a suitable environment for acidophilic hyperthermophiles.

Vapor-dominated systems tend to have smaller pools with little (less than 100 liters per minute) to no outflow, chloride concentrations below 2 mg/L, sulfate concentrations on the order of thousands of mg/L, little to no sinter, and are strongly acidic pH (2-3). According to White *et al.*, the vapor-dominated systems are "uncommon and poorly understood compared with hot-water systems" and have received very little research drilling attention. These characteristics associated with vapor-dominated springs are consistent with the type of environment where acidophilic hyperthermophiles such as *Sulfolobus* and *Acidianus* are commonly found.

The model vapor-dominated system (White *et al.*, 1971) evolves from an originally water-dominated system. With a high heat supply or a lack of sufficient recharge the water-dominated systems begin to transform. When recharge does not keep pace with outflow and boiling, the system begins to become vapor-dominated. The surface expression of a well developed vapor-dominated system is then characterized by fumaroles at or near boiling temperature, a lack of high chloride springs (>2 mg/L), the presence of acidic springs and mud pots (occasionally they have nearly neutral pH from abundant NH_3 oxidation and limited H_2S oxidation) with very low surface flow (<100

lpm), high sulfate concentrations (>1000 mg/L), and little or no silica sinter. They also commonly exhibit bleached ground and little or no vegetation. The temperature in this type of system increases rapidly below the surface before tapering off and following the hydrostatic boiling curve. This results in sediment with temperatures in the optimal range for hyperthermophiles (80-90°C) within a meter from the ground surface. Heat transfer in this near surface region occurs from both convective and conductive processes. Within this near surface region there are isolated areas of up-flowing steam and condensate as well as areas of down-flowing condensate and meteoric water. This model for a vapor-dominated system explains many of the characteristics observed in the acid sulfate springs found in Yellowstone National Park. Apparently vapor-dominated springs, mud pots, and fumaroles are found locally within the major geyser basins in areas of high relief and abundant H₂S (White *et al.*, 1971). In this study the Rabbit Creek and Ragged Hills systems are investigated within the framework of this model.

The mineralogy and water chemistry associated with thermal features in Yellowstone National Park have been studied extensively (Allen and Day, 1935, Fournier, 1989, Stauffer *et al.*, 1980, Walter, 1976, White *et al.*, 1988). The majority of those studies have focused on the alkaline silica and travertine depositing systems. Lithofacies, biofacies, and taphofacies models have been developed for siliceous sinters in those alkaline systems (Walter, 1976). Acid sulfate springs have been studied less extensively than SiO₂ and CaCO₃ depositing springs (White *et al.*, 1971, Foos, 2000), although some characterization of the mineralogy of acid sulfate springs has been done (White *et al.*, 1971, Foos, 2000, Kokaly *et al.*, 1998). Alunite

$((K,Na,H_3O)Al_3(SO_4)_2(OH)_6)$ and kaolinite deposits have been observed as being associated with acidic sulfate systems, while dominant silica and montmorillonite $((Na,Ca)_{0,3}(Al,Mg)_2Si_4O_{10}(OH)_2 \cdot n(H_2O))$ are interpreted as indicative of neutral to alkaline conditions (White *et al.*, 1988). The model of White *et al.* (1971) proposed that the interaction of the upwelling CO_2 rich vapor with the surrounding silicate minerals resulted in the formation of kaolinite and montmorillonite.

Mineralogical mapping of acid sulfate areas in Yellowstone has been done using AVIRIS remote spectroscopy (Kokaly *et al.*, 1998), however, the spatial resolution of this technique is 20 meter by 20 meter pixels. AVIRIS data was used to produce mineral maps for the acid sulfate system in the Roaring Mountain hydrothermal area, as well as for the primarily neutral to alkaline systems of Mammoth Hot Springs, Norris Geyser and Upper and Lower Geyser Basins. The mineral maps of Roaring Mountain show abundant alunite, an aluminum sulfate mineral, in the center of the area. The alunite region is surrounded by the clay minerals kaolinite and halloysite $(Al_2Si_2O_5(OH)_4)$. The perimeter of the area has some minor outcrops of montmorillonite and siliceous sinter. The maps indicate that sulfide oxidation products dominate the mineralogy at the center of the thermal area and silicate alteration products dominate the perimeter.

Acid sulfate springs have a large flux of H_2S . Brock and Mosser (1975) determined the flux of H_2S derived sulfate through Moose Pool, an acid sulfate spring, to be in excess of 44 kilograms per day. In addition to H_2S , a variety of other gases are dissolved in the spring water, including H_2 , CO_2 , and other various sulfur gasses (SO_x) (Xu *et al.*, 1998, Sheppard *et al.*, 1992). These constituents are difficult to measure and

results are highly dependent on location of sample collection and on collection technique (Sheppard *et al.*, 1992). These gasses are involved in a variety of chemotrophic reactions, such as CO₂ fixation, H₂ oxidation, and sulfide oxidation (Amend and Shock, 2001). This supply of reduced sulfur, along with other reduced metals and gases, to an oxidizing environment results in a system that is energetically out of equilibrium. These reduced phases are metastable under oxidizing conditions. Transformation to their oxidized states is thermodynamically favored, but is often inhibited by kinetics. This thermodynamic disequilibrium is used advantageously by microbes which catalyze the oxidation of these and other reduced species as an energy source to sustain life.

A detailed study of the mineralogy of the specific acid sulfate systems is necessary to facilitate a better understanding of the microbe mineral interactions occurring in a particular acid sulfate system. Two of the areas recently identified as being significant gaps in the understanding of thermophiles and hyperthermophiles are the impact of solid phases and the quantification of the energetics of reactions under the physical and chemical conditions of the natural systems (Amend and Shock, 2001). The research described below is directed at addressing some of these gaps.

Research Approach

This study examines in detail the mineralogy, water chemistry, and physico-chemical characteristics in and around the high temperature acidic springs at the Rabbit Creek and Ragged Hills sites. The mineralogy was determined using a combination of XRD and SEM with EDS. The use of these two methods allowed for the determination of

the major and minor mineral phases present, as well as the morphology of the minerals. A combination of *in situ* field experiments and experiments with organisms cultured from the field sites were used to determine some of the potential influences of the microbiology on the mineralogy and vice versus. Detailed field mapping was performed for each site. These maps show the spatial aspect of variability and provide a reference for the location, distribution, and relative size of geothermal features studied. The water chemistry, Eh, pH, and temperature of a variety of spring types were measured. Several springs were also monitored periodically over time to gain some insight into their temporal variation. Geochemical modeling was performed to determine whether or not the mineral assemblages observed represented equilibrium assemblages, and if not to determine how they differed from equilibrium assemblages. This information was used to further analyze the potential influences between the microbial population and the mineralogy.

CHAPTER 3

STUDY SITES

Each of the sites studied had a very heterogeneous nature. At each location, a comprehensive suite of samples was collected from pools, mud pots, fumaroles, soils, and outflow channels. In addition to this general survey of the mineralogy of the site, several features representative of the diversity of each site were studied in more detail. These springs only represent a portion of the thermal features present at the site. Most, but not all, of the representative features chosen were in the temperature and pH range of *Sulfolobus* and *Acidiamus*, the organisms of interest. They serve as representative examples, at least with respect to appearance, of all of the features at the site with appropriate temperature and pH ranges. The characteristics of the representative springs in Rabbit Creek are given in Table 3, and those for Ragged Hills are given in Table 4. One spring at each of the two study areas, Rabbit Creek spring 1 and Ragged Hills spring 1, was selected for a more comprehensive chemical and mineralogical investigation.

Rabbit Creek

The Rabbit Creek study site, as previously mentioned, contained a wide variety of types of thermal features such as springs, mud pots, and fumaroles. These features were quite diverse in appearance, having a wide range of sizes, colors, turbidity, clarity, and consistency. Pools ranged in clarity and color from completely transparent to very murky browns, grays, and reds. Mud pots ranged in color from red to white to brown and also

Table 3: Characteristics of representative detailed study springs from Rabbit Creek.

Spring	Diameter	Color	Clarity/Viscosity	Outflow Channel	Notes
Rabbit Creek spring 1	30 cm	Brown	Murky	No	Has repeatedly produced cultures of <i>Acidianus</i> and <i>Sulfolobus</i> over the past 3-4 years
Rabbit Creek spring 2	75 cm	Red to Brown	Mud Pot to Murky	No	Located on small topographic mound on south end of site, spring is recessed below surrounding ground
Rabbit Creek spring 3	20 cm	Brown	Murky	Small, Ephemeral	Fed outflow channel on west edge of the site
Rabbit Creek spring 4, 7	5-10 cm	White to Lt. Gray	Mud Pot	No	Pools were recessed 5-10 cm below surrounding ground, pools bridged when water was high
Rabbit Creek spring 5	35 cm	Red	Murky	Small, Ephemeral	Tributary to larger westward flowing marshy outflow channel that fed main outflow channel
Rabbit Creek spring 6	<10 cm	Red	Mud Pot	No	Thin black oily film on surface/ Pool dried up by 09/02
Rabbit Creek spring 8	25 cm x 50 cm	None	Clear	Yes, Perennial	Located within the outflow channel of other springs, small gas bubbles constantly rose from circular features on pool floor, circular features had distinct clear and black grains
Rabbit Creek spring 9	10 cm x 20 cm	Brown	Murky	No	Pool was recessed about 5 cm below surrounding ground, bubbled vigorously
Rabbit Creek spring 10	1 m	Red	Clear	Yes, Perennial	Heart shaped pool, sinter deposit around perimeter, fine grained sediment on pool bottom, tranquil, alkaline, source of main outflow channel

Table 4: Characteristics of representative detailed study springs from Ragged Hills.

Spring	Diameter	Color	Clarity/ Viscosity	Outflow Channel	Notes
Ragged Hills spring 1	15 m	Brown	Murky	Yes, perennial	Repeatedly produce cultures of <i>Acidianus</i> and <i>Sulfolobus</i> over past 2-3 years, nicknamed the Plaza, recessed below ground surface 30 cm, bridged to Ragged Hills spring 2 by small isthmus, unclear if it contributes to channel draining Ragged Hills spring 2 and 3
Ragged Hills spring 4	4 m	Blue	Hazy	No	Higher up on the hill than other pools at this site, walls around pool had unique blue and red color, intensity of blue diminished significantly over 3 years since emergence
Ragged Hills spring 6	2 m	Grey to Brown	Murky	No	Had frothy scum floating on pool surface, smooth crust on surface of water logged soil surrounding the pool
Ragged Hills spring 8	5 m x 2 m	Blue	Hazy	Yes, perennial	This pool has a steel gray center, surrounded by a 20 cm wide reddish orange annular ring, surrounded by another ring of black to dark gray spicular geyselite at the air water interface, surrounded by sandy sediment
Ragged Hills spring 12	1 m	Dark Grey	Clear	Yes, perennial	This pool had 2 terraces around its perimeter, upper terrace was red, lower was dark grey
Ragged Hills spring 15	2m	Dark Grey	Murky	No	Had frothy scum on pool surface, had some waterlogged soil around perimeter
Ragged Hills spring 19	10 cm	Yellow	Murky	Yes, ephemeral	This pool had fine grained yellow sediment on pool bottom and along the outflow channel, only occurrence of elemental sulfur
Ragged Hills spring 20	50 cm	Brown	Murky	No	This pool was only present on one occasion, but produced a strong environmental culture

had a wide range of viscosity. Fumaroles ranged from vapor escape cracks to sediment cones a foot tall. The individual springs and mud pots ranged in size from very small pools, less than 10 cm in diameter, to medium sized pools as much as 5 meters in diameter.

There was also a temporal aspect to the variability of these features. New springs, mud pots, and outflow channels appeared occasionally throughout the study period. Some of the features dried up or disappeared, failing to persist throughout the period of study. The Rabbit Creek site was approximately 40 meters by 30 meters (see cd-rom: Rabbit Creek site map). Photographs of the representative springs from the Rabbit Creek site are shown in Figure 5 (cd-rom), and their locations are marked on the map in (cd-rom).

Ragged Hills

The Ragged Hills site was several times larger than the Rabbit Creek site, approximately 500 meters by 100 meters (see cd-rom: Ragged Hills site map). The site is located across the northwest striking ridge of a small saddle connecting the Ragged Hills area of Norris Geyser Basin to another small hill to the north. Most of the features at the Ragged Hills site drain to the northeast, into the One Hundred Springs Plain area of Norris Geyser Basin and eventually north into the Gibbon River. The southwestern half of the site drains to the southwest, away from Norris Geyser Basin and directly into the Gibbon River.

Photographs of the representative thermal features from the Ragged Hills site are shown in Figure 6, and their locations are marked on the map (cd-rom). Many of the

springs at the Ragged Hills site are significantly larger than those of the Rabbit Creek site, ranging from about 10 centimeters in diameter to as much as 13 meters in diameter.

Many of the outflow channels at the Ragged Hills site were perennial and appeared to have substantially more discharge than the Rabbit Creek outflow channels, although there were springs in Ragged Hills with ephemeral outflow as well as with no apparent outflow. The flowing springs had a diverse range of colors including clear, hazy blue, murky grey, murky brown, and murky white. The non flowing pools also had a variety of colors, but were often murky and yellow to brown in color. The outflow channels in the area ranged from <1 centimeter wide to greater than 20 meters wide. The larger channels were perennial, but smaller ephemeral channels were also present. There was also a wide array of colors found in the outflow channels, often changing downstream as well as through time. The outflow channels were often brown, yellow, red, orange and white. Some, if not most, of their color was apparently from the microbial mats which occurred as thin films as well as thick streaming mats. The outflow channels were all shallow, ranging from well under a centimeter to about 20 cm deep in places along the main drainage to the north. The shallow outflows did not maintain the exceptionally high temperatures found in some of the springs and were consequently capable of supporting a more diverse ecological community than the springs.

There were also some fumaroles and mud pots in Ragged Hills, but they were not as abundant or pronounced as those in Rabbit Creek. None of the mud pots at Ragged Hills were as viscous as those in Rabbit Creek, and there were not any well developed sediment cones around any of the fumaroles in Ragged Hills.

CHAPTER 4

WATER CHEMISTRY

Chemical Composition

The presence of specific mineral phases and the ability of microorganisms to survive and metabolize is, at least in part, governed by the temperature and chemical composition of the springs. In order to gain some understanding of the chemical character of the springs, the chemistry from a variety of features was determined, and the chemistry of select springs was monitored over time. Table 5 and 6 show the results of chemical analyses of select representative springs in Rabbit Creek and Ragged Hills. Results show that the chemistry of the springs is highly variable both over space and through time, and that the two sites have diverse yet distinct chemistries. The chemical composition of the springs shifts in and out of stability fields of various minerals over short periods of time.

Temperature and pH

The pH and temperature were recorded periodically for Ragged Hills spring 1 and Rabbit Creek spring 1 using a field calibrated, temperature compensated, Ag/AgCl electrode, Orion pH meter. The temperature and pH of Ragged Hills spring 1 was measured periodically over a period of 21 months and is shown in Figure 7. The range is about 13° C for temperature and about 1 pH unit. The temperature and pH of Rabbit Creek spring 1 was measured periodically over a 2 year period and is shown in Figure 8. There is a temperature range of about 15° C and a pH range of more than 2 pH units.

Table 6: Chemistry from Rabbit Creek and Ragged Hills, 6/17/2001, 10/2001, and 9/2002. 6/17/2001 samples were analyzed at Montana State University Soil Testing Laboratory. All other samples were analyzed at Energy Laboratories in Billings, MT. "na" indicates sample was not analyzed for that component. "nd" indicates concentration was below detection. All concentrations in mg/L.

Spring	Date	Temp °C	pH	SO ₄	Si	K	Al	Ca	Fe	Cl	P	Na
RC6	6/17/2001	85	3.3	57.5	23.8	2.7	0.24	0.7	0.1	3	na	4.7
RC7	6/17/2001	80.7	2.98	58.4	10.4	1.1	8.13	0.3	0.1	0.9	na	1.2
RC8	6/17/2001	77	5.05	18.0	1.9	2.3	0.71	0.1	0.04	72.8	na	56.2
RC1	6/17/2001	78.5	3.96	38.3	31.8	5.8	0.7	0.3	0.37	25.3	na	21.2
RC5	6/17/2001	74	3.4	67.1	40.4	4.6	2.71	0.7	0.37	49.1	na	36.4
RC9	6/17/2001	78.3	3.6	118.3	23.4	2.6	7.23	1.2	2.42	9.8	na	17.9
RC10	6/17/2001	77.7	9.02	16.2	121	10.5	0.57	0.5	0.02	267.8	na	312
	Spring	Date	Cu	Mg	Mn	As	Zn	Cr				
	RC6	cont.	0.01	0.5	0.09	nd	0.17	nd				
	RC7	cont.	nd	0.1	0.02	nd	0.02	nd				
	RC8	cont.	0.02	0.1	nd	0.09	0.02	nd				
	RC1	cont.	nd	0.1	0.03	nd	0.05	nd				
	RC5	cont.	0.01	0.2	0.07	nd	0.09	nd				
	RC9	cont.	nd	0.3	0.05	nd	0.03	nd				
	RC10	cont.	nd	nd	nd	1.83	0.01	nd				
Spring	Date	Temp °C	pH	SO ₄	Si	K	Al	Ca	Fe	Cl	P	
RC2	10/17/01	87	2.6	83	126	3	1.6	<1	0.04	na	0.03	
RC1	10/24/01	73	3.7	77	35.7	1	8.1	1	0.09	na	0.03	
RC2	10/24/01	83.1	2.8	61	123	3	1.8	1	0.05	na	0.03	
RH1	9/16/2002	71.5	2.65	164	393	49	2.1	6	4.59	583	0.09	
RH4	9/16/2002	78.9	3.42	92	385	41	2.5	6	5.66	687	0.1	
RH6	9/16/2002	61.7	3.18	115	301	42	4.3	8	7.08	651	0.27	
RH8	9/16/2002	86		88	336	44	2.1	5	8.93	606	0.15	
RH12	9/16/2002	86.1	3.1	84	289	38	1.8	4	4.85	521	0.17	
RH19	9/16/2002	57.1	3	42	196	7	0.4	nd	0.26	18	0.58	
RC1	9/18/2002	67.5	3.6	318	49.4	13	26.2	8	24.9	44	0.09	
RC2	9/18/2002	81.9	2.7	116	140	6	3.4	1	2.73	28	0.04	

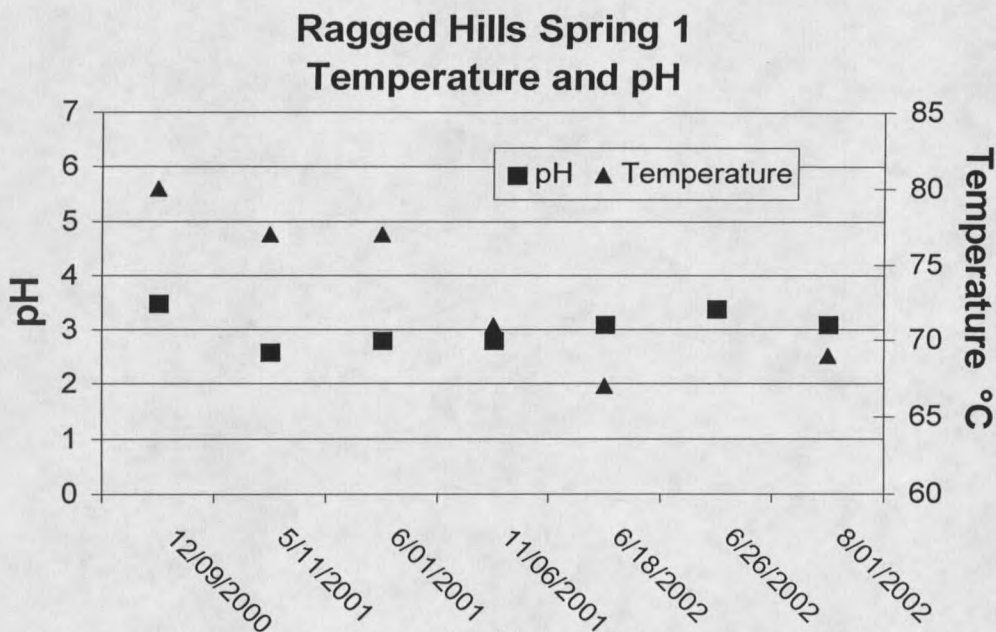


Figure 7: Temperature and pH variation in Ragged Hills spring 1. Values of field measured pH and temperature were recorded periodically throughout the study period. Temperature is shown as solid triangles and pH is shown as solid squares.

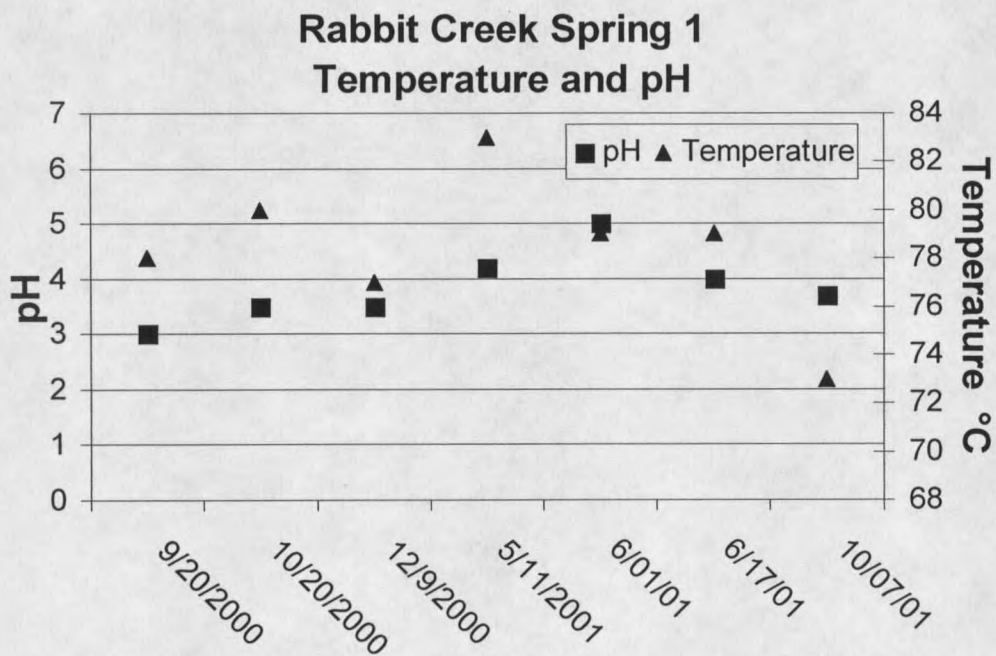


Figure 8: Temperature and pH variation in Rabbit Creek spring 1. Values of field measured pH and temperature were recorded periodically throughout the study period. Temperature is shown as solid triangles and pH is shown as solid squares.

The temperature of Rabbit Creek spring 1 and Ragged Hills spring 1 was also monitored using an automated data logger. Figure 9 and Figure 10 show the short-term temperature variation within Rabbit Creek spring 1 and Ragged Hills spring 1 respectively.

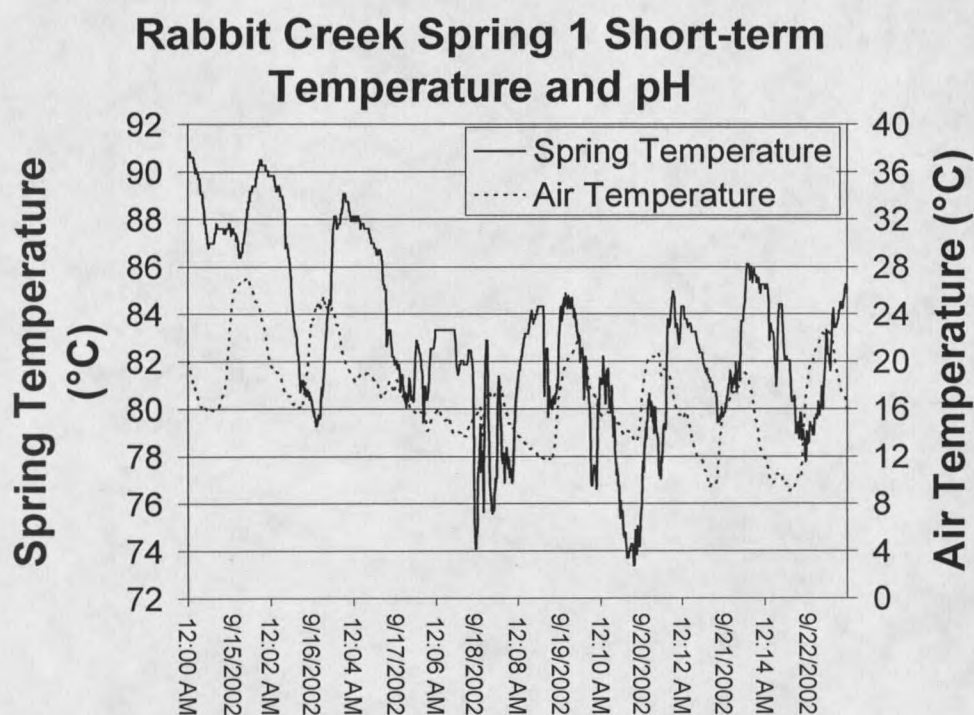


Figure 9: Short term temperature variation in Rabbit Creek spring 1. The graph shows the temperature of the spring approximately 10 cm below the water surface and the air temperature for the 8 day period including the 9/16/02 and 9/18/02 sample collection dates. Temperatures were recorded every 7 minutes with an automated data logger. The spring temperature is shown as a solid black line and the air temperature as a dashed black line.

Over an 8 day period there is a temperature range of about 20 °C in Rabbit Creek spring 1, with a strong diurnal component accounting for about 10°C of the temperature fluctuation. In Ragged Hills spring 1 there is an overall fluctuation of about 8 °C, and the

diurnal variation accounts for up to 7 °C of the variation. The spring temperature is 7 hours out of phase with the air temperature in Rabbit Creek spring 1 and 12 hours out of phase with the air temperature in Ragged Hills spring 1, with respective correlation coefficients of 0.59 and 0.53. The significantly larger volume of water in Ragged Hills spring 1 probably accounts for the slower response time.

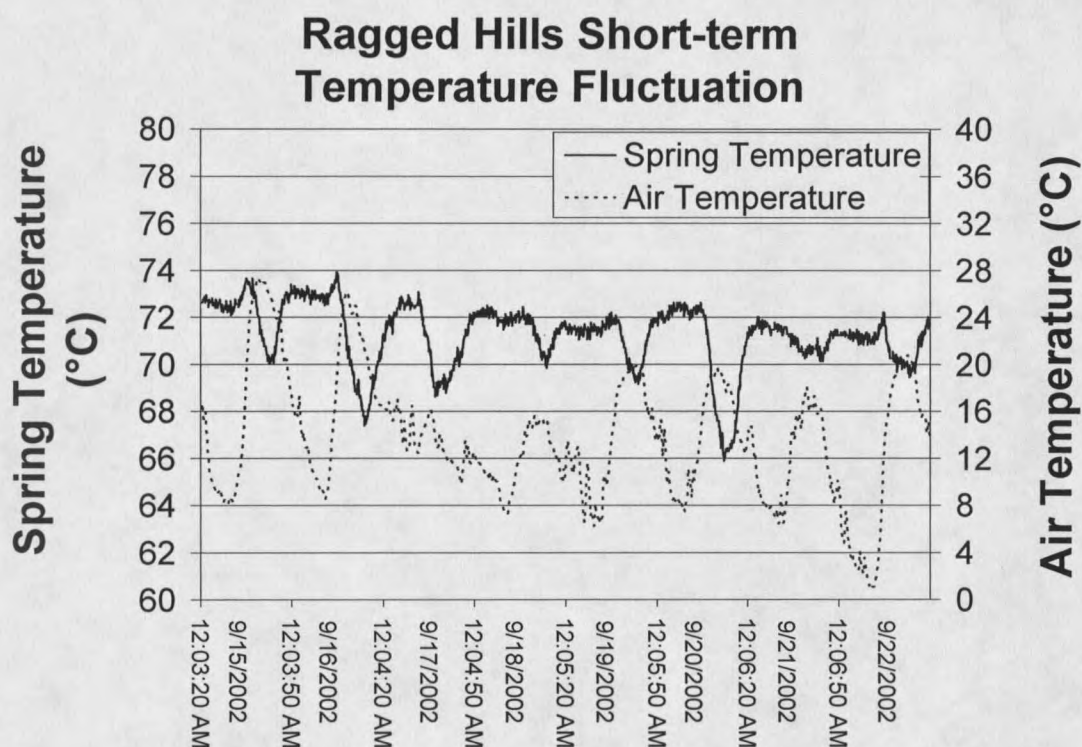


Figure 10: Short term temperature variation in Ragged Hills spring 1. The graph shows the temperature of the spring approximately 10 cm below the water surface and the air temperature for the 8 day period including the 9/16/02 and 9/18/02 sample collection dates. Temperatures were recorded every 7 minutes with an automated data logger. The spring temperature is shown as a solid black line and the air temperature as a dashed black line.

Dissolved Ionic Concentrations

The aqueous concentrations of dissolved cations, anions, and metals were measured in Rabbit Creek spring 1 and Ragged Hills spring 1 in February of 2000 (see maps on the cd-rom for spring locations). The results are shown in Figure 11 and Figure 12 respectively. Both springs have sodium as the dominant cation and chloride as the dominant anion. They also both have strong silica and sulfate components. Rabbit Creek spring 1 had a total measured dissolved solids value of 180 mg/L and Ragged Hills spring 1 had a total dissolved solids value of 1281 mg/L. Neither spring fits the vapor-dominated system model of White et. al (1971), where sulfate is the dominant anion and chloride values are less than about 10 mg/L.

Rabbit Creek Spring 1 Relative Abundance of Dissolved Ions 2/2001

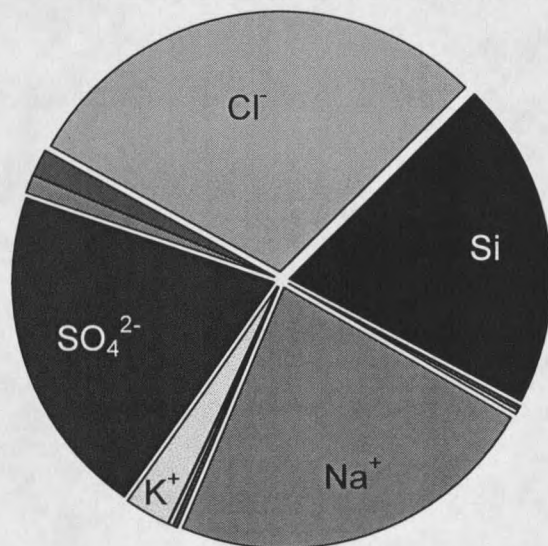


Figure 11: Composition of Rabbit Creek spring 1. The composition is normalized and plotted as a percentage of the measured dissolved ions. The dissolved solids measured totaled 180 mg/L.

Ragged Hills Spring 1 Relative Abundance of Dissolved Ions 2/2001

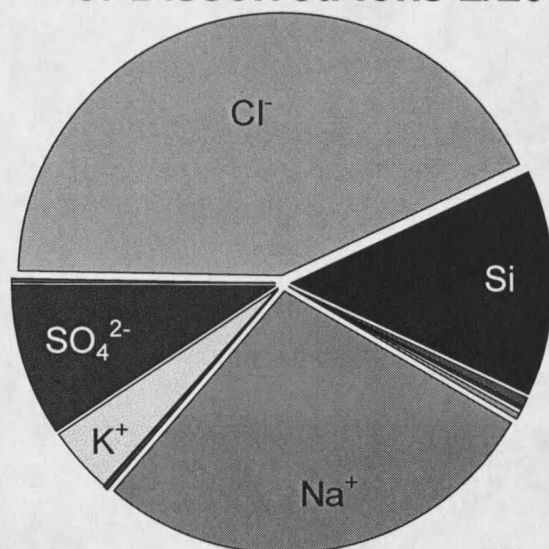


Figure 12: Composition of Ragged Hills spring 1. The composition is normalized and plotted as a percentage of the measured dissolved ions. The dissolved solids measured totaled 1281 mg/L.

A reduced suite of dissolved species was chosen for further analysis of a variety of spring types in both Rabbit Creek and Ragged Hills. Preliminary mineralogic and chemical analyses were used to determine the dominant ions in solution and the elements which corresponded to the major observed mineral phases. These parameters were also measured at several points in time for Rabbit Creek spring 1 and Ragged Hills spring 1 in order to constrain the temporal variability of the springs.

Figure 13 shows the variation of these dissolved species in Rabbit Creek spring 1 over time. The concentration of all measured ions was highest in the September 2002

sample. The concentration of sulfur (sulfate sulfur reported as mg/L of sulfur) is much higher during the fall than during the winter or early summer, increasing by as much as a factor of 10. The dissolved aluminum follows a similar pattern to the sulfate, except that it increases by more than a factor of 25. The increase in concentration was particularly dramatic for iron, which increased by a factor of approximately 75. However, this increase only occurred in the September 2002 sample and not in the October 2001 sample.

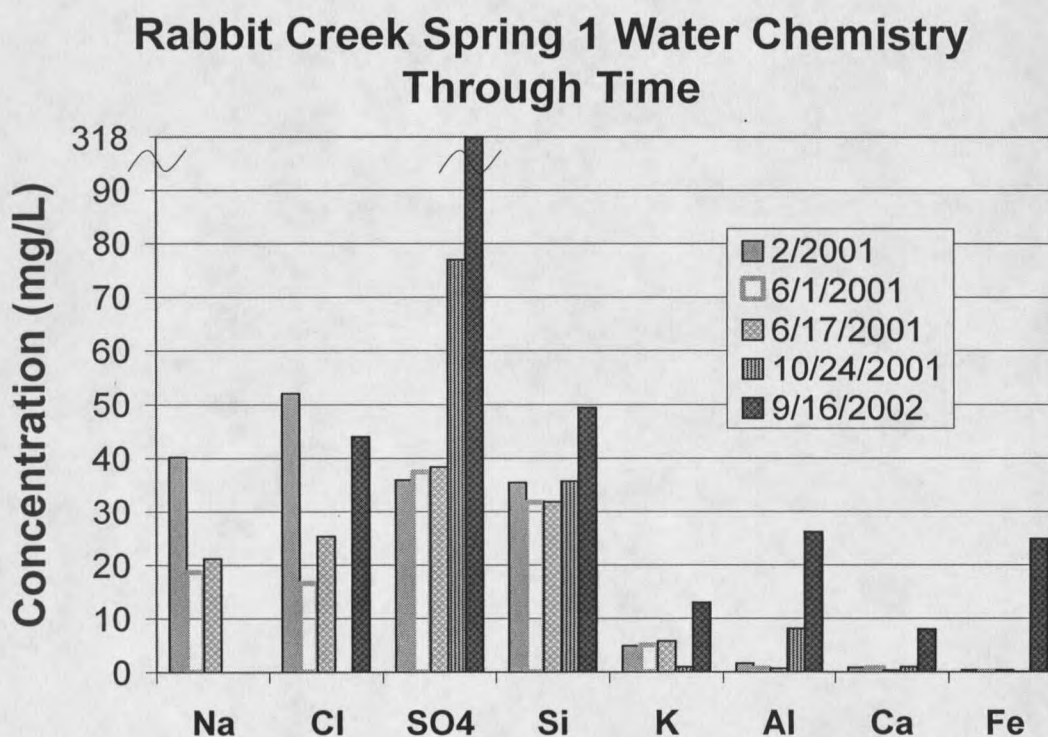


Figure 13: Rabbit Creek spring 1 chemistry over time. Concentrations of select ions dissolved in Rabbit Creek spring 1 are shown from periodic analyses of water chemistry during the course of the study. The fluctuations in concentration are as large as one order of magnitude and concentrations of various ions do not vary proportionally.

Figure 14 shows the variation of the dissolved species in Ragged Hills spring 1 over time. The concentration of dissolved species did not change seasonally as much as it did in Rabbit Creek spring 1. The chloride, potassium, iron, aluminum, and calcium remained relatively constant. There was a five fold increase in sulfate, but the highest sulfate concentrations occurred in the mid summer, not in the fall. The silica concentration in Ragged Hills spring 1 doubled in the fall 2002 sample.

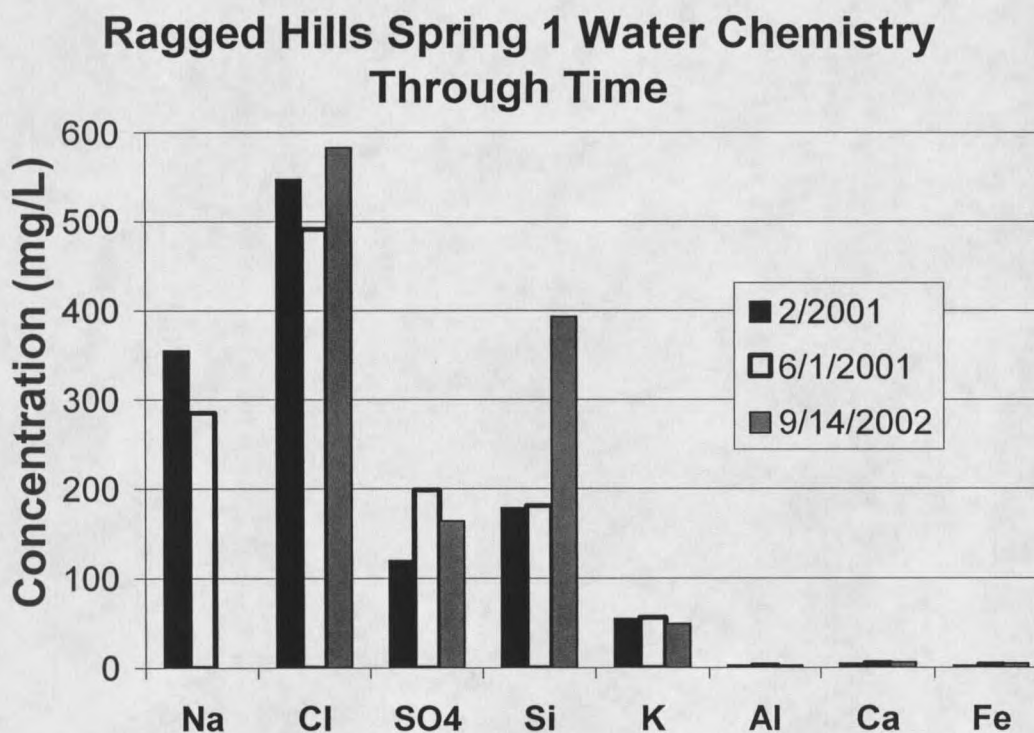


Figure 14: Ragged Hills spring 1 chemistry over time. Concentrations of select ions dissolved in Rabbit Creek spring 1 are shown from periodic analyses of water chemistry during the course of the study. The fluctuations in concentration are as high as a factor of four and concentrations of various ions do not vary proportionally.

The chloride concentrations in Rabbit Creek vary widely over time and space.

They range from <1 mg/L to >250 mg/L. This range is not consistent with the value of

<10 mg/L appropriate for vapor-dominated systems or with the value of <20 mg/L for water-dominated systems with primarily subsurface outflow (White *et al.*, 1971). Nor is it consistent with the higher chloride levels expected for a system with dominantly water phase hydrothermal input.

The chloride concentrations in Ragged Hills are generally in the 500 to 670 mg/L range. This range is nowhere near that suggested for vapor-dominated or subsurface outflow water-dominated systems. In fact, these concentrations well exceed the chloride concentration of 310 mg/L in the deep thermal water of the Yellowstone hydrothermal system (Truesdell, *et al.*, 1977). Concentration from boiling as the geothermal fluids rise, as well as evaporative effects at the surface can account for these elevated levels of chloride (Truesdell, *et al.*, 1977). Since the partitioning of chloride into the vapor phase is minimal, these high chloride concentrations clearly indicate that there is a significant liquid water hydrothermal input to the surface hydrology at the Ragged Hills site.

The water chemistry analyses from all of the samples analyzed were used to construct frequency distribution plots for several key chemical components. The frequency distribution plots for chloride, silica, aluminum, potassium, calcium, sulfate, and iron are shown in Figure 15. The distribution of chloride, silica, potassium, and calcium are all clearly bimodal, with modalities corresponding with the two sites.

The Ragged Hills concentrations are consistently in the higher grouping, while the Rabbit Creek concentrations are consistently in the lower grouping. There are a couple of exceptions to this grouping of distributions. Rabbit Creek spring 1 had exceptionally high concentrations of iron, aluminum, and calcium in the September of 2002 sample. As a

result, it appears as the outlier to the Rabbit Creek group in the aluminum, iron, and calcium frequency distribution plots.

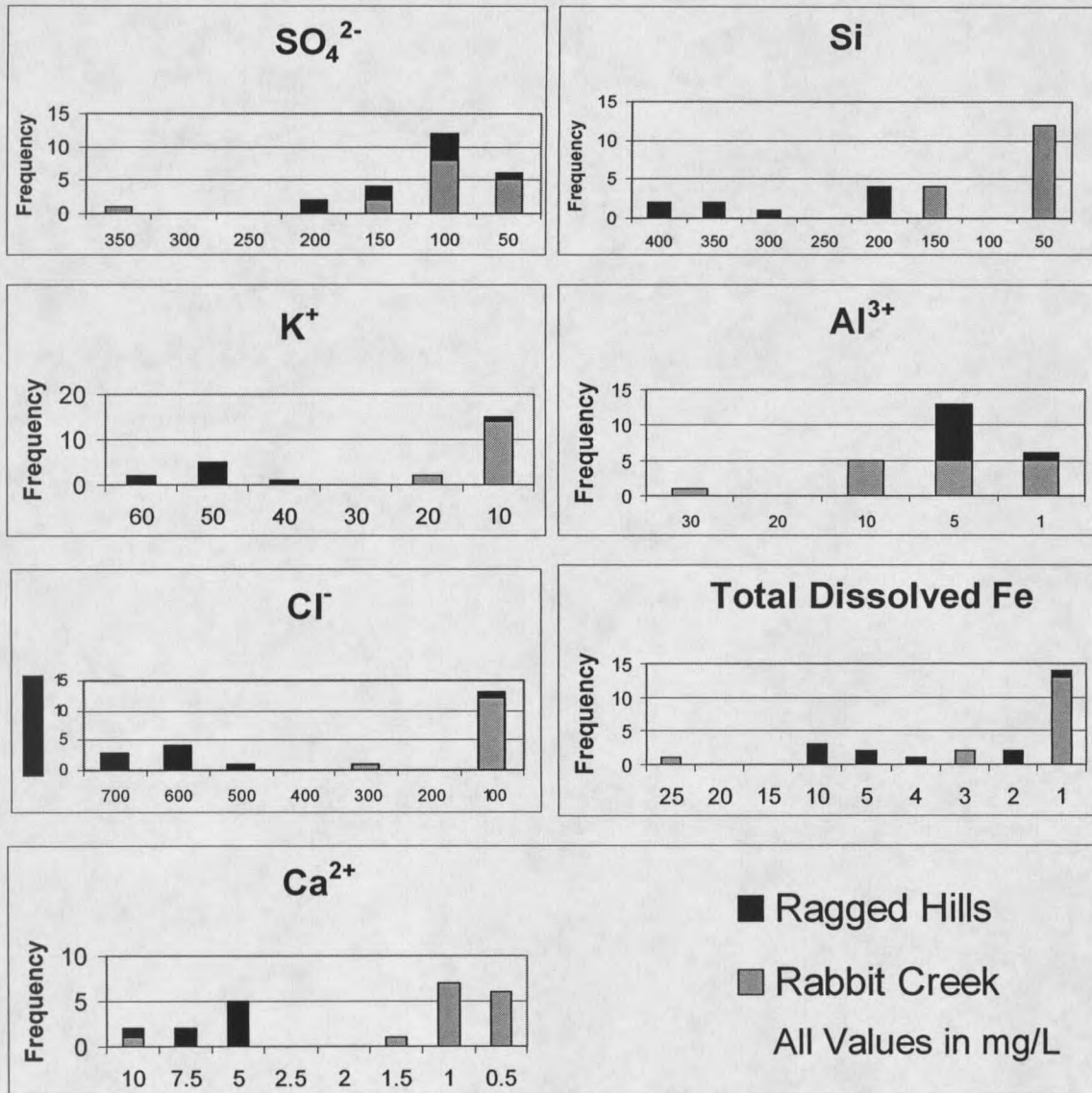


Figure 15: Frequency distribution plots for select ions in Rabbit Creek and Ragged Hills. Plots for a) SO_4^{2-} , b) Si, c) K^+ , d) Al^{3+} , e) Cl^- , f) Fe, and g) Ca^{2+} show the frequency of measured concentrations within a given range. The values on the x-axis indicate the maximum value of the range plotted immediately above.

The most likely explanation for the anomalous chemical character of the Rabbit Creek spring 1, September 2002 sample is desorption and dissolution of solid phases induced by a drop in pH. While the pH on this date was the lowest of all of the sampling dates, it was only 0.1 pH units lower than the next lowest pH. Although this observed change in pH is minimal, it is quite possible that the pH had dropped significantly lower during the interim.

Ragged Hills spring 19 (see Ragged Hills map on the cd-rom for spring location) had exceptionally low values for potassium and chloride relative to all of the other springs in Ragged Hills. Ragged Hills spring 19 is apparently not controlled by the same processes as the other study springs in the Ragged Hills site. These data clearly indicate that the Rabbit Creek and Ragged Hills compositions are distinctly different.

Trilinear diagrams showing the relative concentrations of the major cations and anions in solution are called Piper diagrams. They are useful for indicating the dominant chemical signature of a solution, as well as for indicating simple mixing of two solutions. Major cations are plotted in the ternary region on the left, major anions in the ternary region on the right and then both are projected into the upper domain of the diagram. Where the data plots in the upper portion indicates the dominant chemistry, and simple mixing of two waters is indicated by data that plots in a straight line in the upper portion of the diagram. The composition of Rabbit Creek spring 1 and Ragged Hills spring 1 are plotted on a Piper diagram shown in Figure 16. Both springs plot in the sodium chloride dominant region. The HCO_3^- is assumed to be negligible because H_2CO_3^* (which was not analyzed) is the dominant carbonate species at a pH below about 6.

A modified piper diagram with potassium equivalence substituted for magnesium, and silica equivalence (as a monovalent species) substituted for HCO_3 is shown in Figure 17 and Figure 18. The scatter in Figure 17 shows high variability in the water chemistry of features in the small (approximately 40m by 30m) Rabbit Creek geothermal site. Mixing of two water types would have simply plotted as a straight line in the upper portion of the Piper diagram. The scatter of the samples in Rabbit Creek indicates that simple mixing can not account for the distribution of chemical composition of springs in Rabbit Creek. Rabbit Creek spring 8 and Rabbit Creek spring 10 both plot as sodium and chloride dominant and sulfate depleted relative to the other springs in Rabbit Creek.

Figure 18 shows that the samples from Ragged Hills cluster closely together with the one exception of Ragged Hills spring 19, which has much lower chloride and sodium concentrations. Rabbit Creek spring 10, the only alkaline pool from the Rabbit Creek site, plots in the cluster of Ragged Hills springs. It is the only spring from Rabbit Creek in that cluster. The winter sample from Rabbit Creek spring 1 plots nearby, but has proportionately higher sulfate and lower chloride. Rabbit Creek spring 8, the other outlier from Figure 17 has similar sulfate, and much lower silica and chloride concentrations than Rabbit Creek spring 10.

The Rabbit Creek and Ragged Hills Piper plots further illustrate the difference in the two sites, and indicate that simple mixing of two waters does not account for the chemical variation seen at either site. The variation appears to be due to temporal changes in the relative input of geothermal liquid and geothermal vapor, which have distinctly

different chemical signatures. The occasional rapid dissolution of fine grained particulates such as amorphous gibbsite and iron oxides also causes some of the variation.

2/2001 Piper Diagram

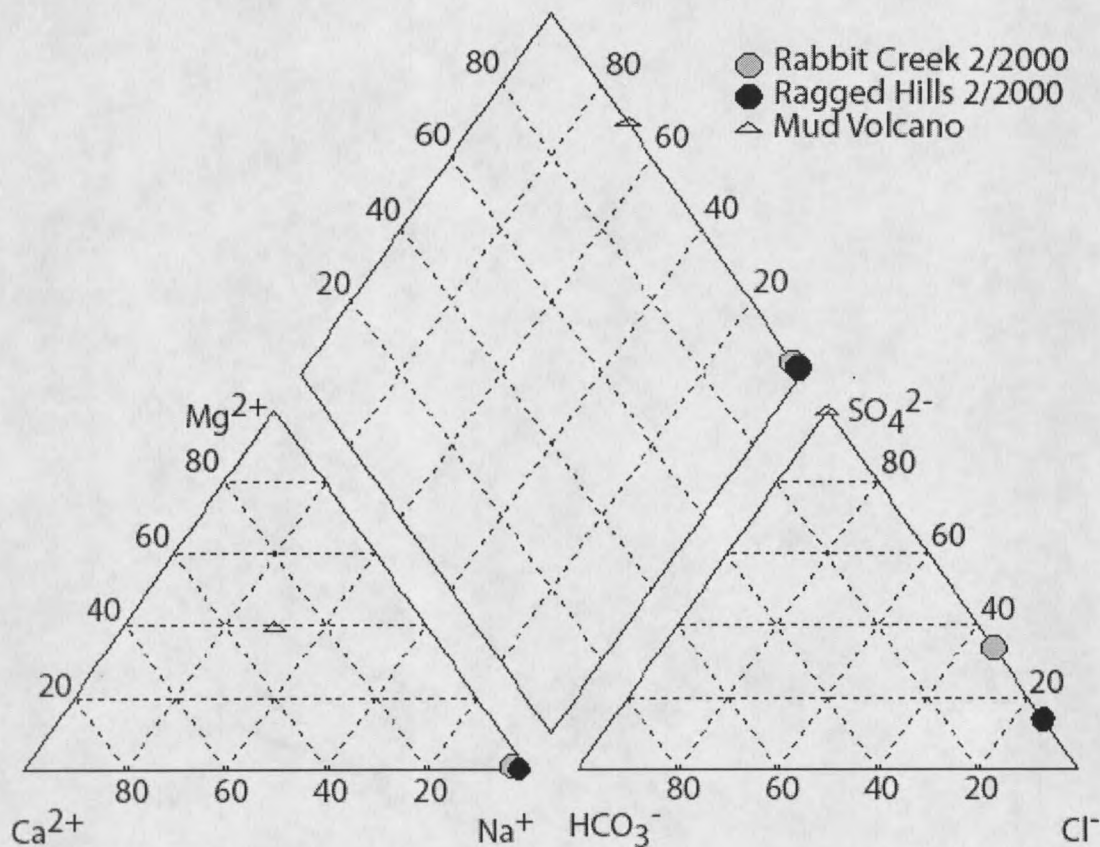


Figure 16: Piper diagram of Ragged Hills spring 1 and Rabbit Creek spring 1. The 2/2001 chemical analyses from Rabbit Creek and Ragged Hills are plotted on a conventional Piper diagram. Components were plotted as charge equivalents and normalized to 100% for each ternary diagram. Compositions of individual samples are plotted in the quadrilateral region by projecting their composition from the ternary regions parallel to the dashed lines, and then plotting the composite value at the intersection of 2 lines of projection. The concentration of HCO_3^- was assumed to be zero because the stable dissolved carbonate phase under the acidic conditions observed in these springs is H_2CO_3 , and not HCO_3^- .

**Modified Piper Diagram of Rabbit Creek
Water Chemistry from 6/01/2001 and 6/17/2001**

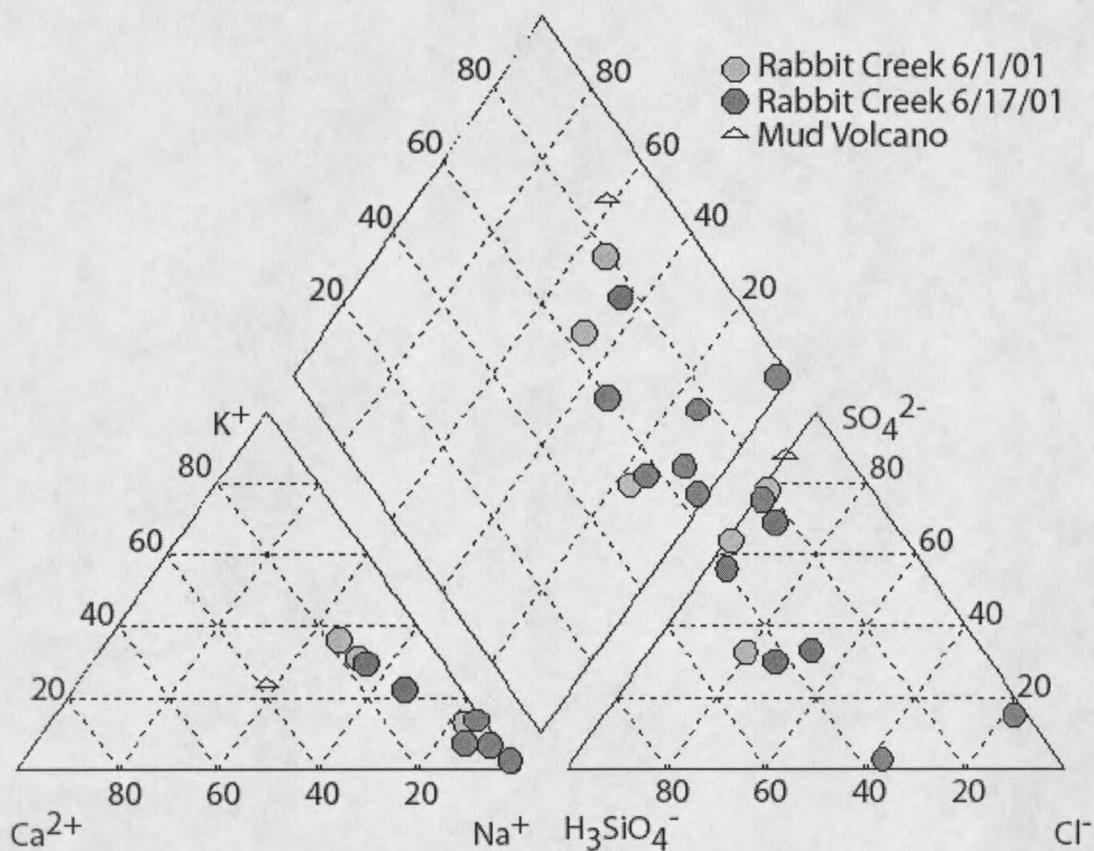


Figure 17: Modified Piper plot of Rabbit Creek samples from 6/1/2001 and 6/17/2001. The Piper diagram was modified by replacing Mg^+ with K^+ and HCO_3^- with H_2SiO_3^- . Although the stable phase under the observed conditions is actually H_4SiO_4 , the monovalent Si equivalent H_2SiO_3^- was chosen for consistency with the convention of plotting cations on the left ternary diagram and anions on the right ternary diagram. Samples from 6/01/01 are shown as black circles and samples from 6/17/2001 are shown as black rectangles. Individual springs are labeled in the composite portion of the diagram.

Modified Piper Diagram of Rabbit Creek and Ragged Hills Water Chemistry

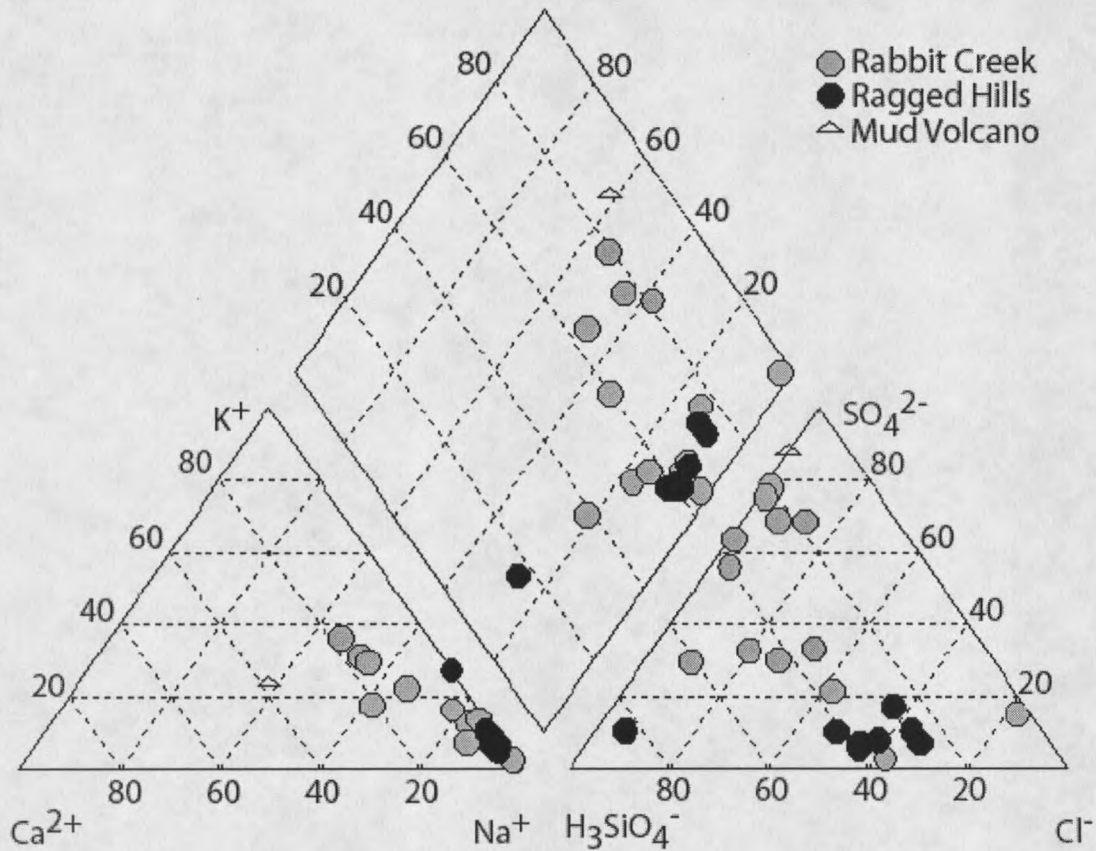


Figure 18: Modified Piper plot of Ragged Hills and Rabbit Creek samples from 2/2001, 6/2001, and 9/2002. The Piper diagram was modified as in Figure 16. The Rabbit Creek samples are all shown as solid black circles and The Ragged Hills samples are all shown as white circles with a black outline. Select springs are labeled in the SO_4^{2-} , Cl^- , H_3SiO_3^- region of the plot.

Oxidation State

The redox state of the system was measured using an Orion Ag/AgCl combination electrode, an Fe²⁺/Fe³⁺ Hach colorimetric Ferrozine test kit, and a Hach S²⁻ colorimetric test kit. The test kits did not provide acceptable results, primarily due to the suspended sediment and coloration of the water samples. The Orion probe produced highly variable readings for any given spring. The readings for Rabbit Creek spring 1 on 9/18/02 wavered between 110 mV and 250 mV. The maximum reading obtained for any spring was 290 mV and the lowest was 90 mV. This gives a range of measured Eh of 0.3 to 0.5 V. The failure of Eh measurements to stabilize is characteristic of solutions which are not in a state of redox equilibrium (Langmuir, 1997). These measured values represent a weighted value of all redox pairs in the system and may not be representative of any one redox pair or of the redox state of the system as a whole.

Predicted Mineralogy (Thermodynamic Modeling)

The water chemistry was used to predict the stable mineralogy of several spring types using the geochemical thermodynamic model PHREEQCI (Parkhurst *et al.*, 1997). The model was used to calculate the mineral phases at and above saturation with respect to the observed water chemistry. All modeling calculations were performed setting kaolinite and amorphous silica as equilibrium phases. Due to the unreliable nature of the Eh measurements, the default value of 4 (standard log units) was used for pe, which

corresponds to the value characteristic of transitional acidic groundwater environments (Langmuir, 1997).

The saturation index for the minerals predicted by the model to be at saturation with respect to the water chemistry in Rabbit Creek spring 1 are shown in Figure 19. The dynamic nature of the springs is evident in the fluctuations of the saturation indices of the minerals in Rabbit Creek 1 through time. The saturation index for quartz and chalcedony vary slightly because of temperature effects, but are essentially fixed because amorphous silica and kaolinite were set as equilibrium phases. The mineral phases predicted to be at or above saturation for various springs in Rabbit Creek and Ragged Hills are given in Table 7 and Table 8.

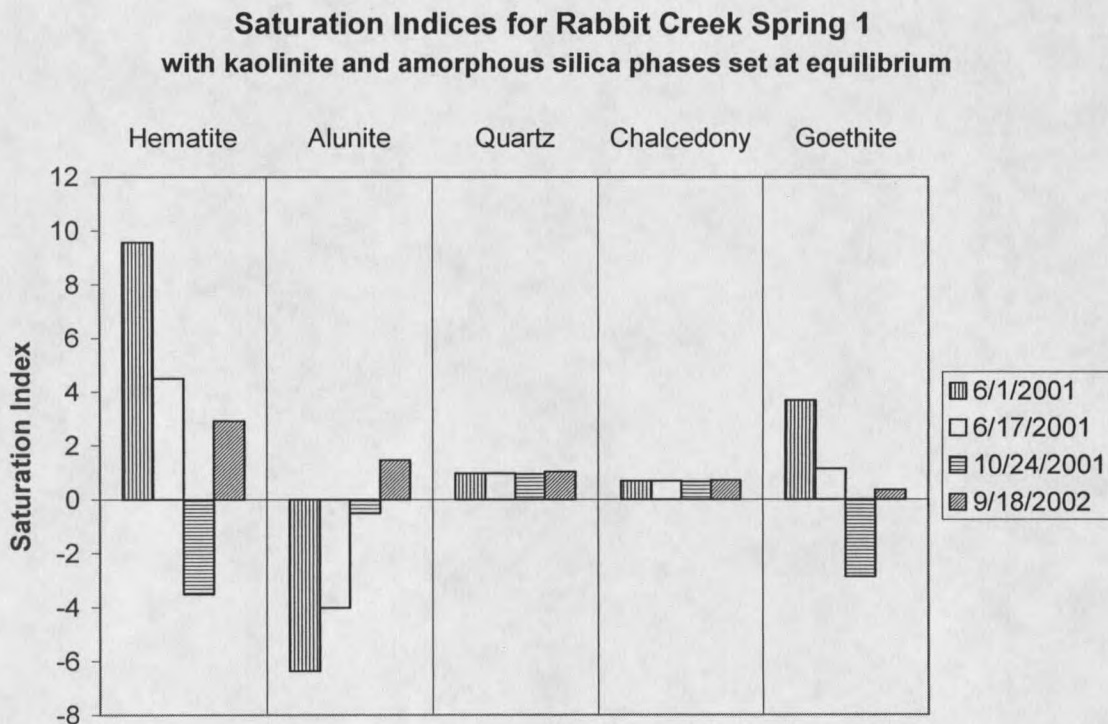


Figure 19: Saturation indices for Rabbit Creek spring 1. Values were calculated using the geochemical model PHREEQCI v.2.6. Positive values indicate that the spring is saturated and negative values indicate that the spring is undersaturated. Saturation indices were calculated with the solution set in equilibrium with amorphous silica and kaolinite.

Table 7: Thermodynamically stable mineral phases based on chemical analyses from 6/01 and 10/01. Kaolinite and amorphous silica were set at equilibrium. As, Cr, and Ti were not included in the database (Parkhurst *et al.*, 1997) used for geochemical calculations. Estimated value of 4 was used for pE (≈ 250 mV). For each sample, saturated phases are listed in descending order of degree of supersaturation.

Date	6/1/2001	6/1/2001	6/1/2001	6/1/2001
Sample ID	6121	6115	6113	6111
Spring	RH1	RC7	RC3	RC1
Saturated Mineral Phases	Quartz Chalcedony Alunite	Quartz Chalcedony	Quartz Chalcedony Hematite	Hematite Goethite Quartz Chalcedony
Date	6/17/2001	6/17/2001	6/17/2001	6/17/2001
Sample ID	61713	61722	61742	61752
Spring	RC1	RC5	RC6	RC7
Saturated Mineral Phases	Hematite Goethite Quartz Chalcedony	Quartz Chalcedony	Quartz Chalcedony Hematite	Quartz Chalcedony
Date	6/17/2001	6/17/2001	6/17/2001	
Sample ID	61761	61772	61782	
Spring	RC8	RC9	RC10	
Saturated Mineral Phases	Hematite Goethite Quartz Chalcedony	Hematite Quartz Chalcedony	Hematite Goethite Willemite K-mica K-feldspar Quartz Albite Chalcedony	
Date	10/17/2001	10/24/2001	10/24/2001	
Sample ID	1017-1	1024-1	1024-2	
Spring	RC2	RC2	RC1	
Saturated Mineral Phases	Quartz Chalcedony	Quartz Chalcedony	Quartz Chalcedony	

Table 8: Thermodynamically stable mineral phases based on chemical analyses from 9/02. Kaolinite and amorphous silica were set at equilibrium. As, Cr, and Ti were not included in the database (Parkhurst *et al.*, 1997) used for geochemical calculations. Estimated value of 4 was used for pE (≈ 250 mV). For each sample, saturated phases are listed in descending order of degree of supersaturation.

Date	9/16/2002	9/16/2002	9/16/2002
Sample ID	RH1	RH4	RH6
Spring	RH1	RH4	RH6
Saturated Mineral Phases	Alunite Quartz Chalcedony	Hematite Quartz Chalcedony Goethite	Quartz Alunite Chalcedony Hematite
Date	9/16/2002	9/16/2002	
Sample ID	RH8	RH12	
Spring	RH8	RH12	
Saturated Mineral Phases	Hematite Goethite K-mica Quartz Fe(OH) ₃ (a) Chalcedony K-feldspar	Hematite Quartz Chalcedony Alunite	
Date	9/18/2002	9/18/2002	
Sample ID	RC1	RC2	
Spring	RC1	RC2	
Saturated Mineral Phases	Hematite Alunite Quartz Chalcedony Goethite	Quartz Chalcedony Alunite	

Eh/pH Diagram

The oxidation state of components known to be involved in metabolic redox reactions of *Sulfolobus*, sulfur and iron, are of particular interest. In order to investigate the state of these components, an Eh/pH diagram was constructed for the Fe, S, and H₂O system at 85° C (Figure 20) using the concentrations from Rabbit Creek spring 1 for Fe and SO₄ from 2/2000. There is a stable region for elemental sulfur between the H₂S and the Fe²⁺ + SO₄²⁻ regions. This stable elemental sulfur field shrinks as the temperature

increases, and is no longer discernable at a temperature of 85° C. The conditions observed in Rabbit Creek spring 1 (in the simplified Fe, S, H₂O system) plot on the border of the Fe²⁺ + SO₄²⁻ ions in solution and the hematite + SO₄²⁻ fields. At the higher pH and the higher oxidation states, the solid iron oxide and SO₄²⁻ ions in solution become the stable phases. At the lower limits the dissolved Fe²⁺ and SO₄²⁻ ions are stable.

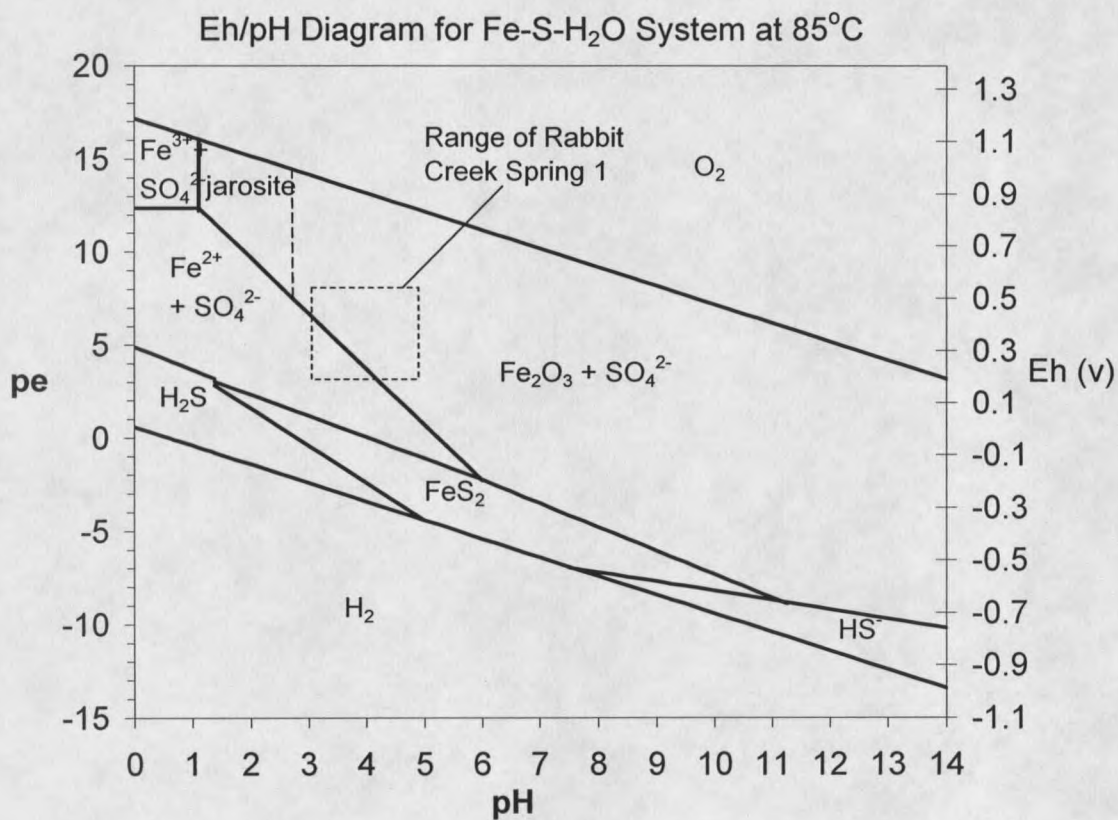


Figure 20: Eh/pH diagram for the Fe-S-H₂O system at 85° C. Total dissolved Fe was set at 0.37 mg/L and total S was set at 12 mg/L based on conditions observed in Rabbit Creek. Conditions typical of Rabbit Creek spring 1 are marked with a box labeled RC 1. Jarosite field is estimated. (Fields created using data from Amend and Shock, 2001)

Under equilibrium conditions the dissolution and precipitation of minerals is a reversible process governed by the free energy equation $\log K = \Delta G_r^0 / (RT)$. In this

equation K is the equilibrium constant and is the product of the activities of the products divided by that of the reactants. Assuming ideal behavior, the equilibria are a function of the temperature and the dissolved concentrations of the reaction components. When the temperature is known, the value of any single component can be viewed as a dependant variable, controlled by the values of the other components.

Gibbsite Dissolution

At a temperature of 25° C and below a pH of 5 Al^{3+} is the dominant aluminum species in solution (Langmuir, 1997). Under this circumstance the dissolution of gibbsite is given by the reaction $\text{Al}(\text{OH})_3 + 3\text{H}^+ = \text{Al}^{3+} + 3\text{H}_2\text{O}$ (reaction 1). At a temperature of 25° C the equilibrium constant for this reaction is $10^{8.11}$. At equilibrium, $10^{8.11} * [\text{H}^+]^3 = [\text{Al}^{3+}]$. In this case a small increase in the activity of H^+ (a small drop in pH) will cause a dramatic rise in the activity of dissolved Al^{3+} via the dissolution of gibbsite.

At a temperature of 25°C, the reaction $\text{Al}^{3+} + \text{H}_2\text{O} = \text{AlOH}^{2+} + \text{H}^+$ (reaction 2) has a K value of 10^{-5} , thus as the pH approaches 5 the activity of the dissolved species AlOH^{2+} increases. At a pH above 5.0 AlOH^{2+} becomes the dominant aluminum ion in solution and the solubility of gibbsite is no longer controlled by the reaction 1, but by the sum of reaction 1 and reaction 2: $\text{Al}(\text{OH})_3 + 2\text{H}^+ = \text{AlOH}^{2+} + 2\text{H}_2\text{O}$ (reaction 3), which has a K value of $8.11 - 5.0 = 3.11$. As the pH rises above about 5, these hydroxide complexes cause gibbsite to become very insoluble.

As mentioned above, these reactions are also a function of temperature. If the Vann't Hoff correction for temperature (to 80° C) is applied to the equations above, the pH where gibbsite becomes more soluble (Al^{3+} dominates in solution) drops to about 3.7,

the K value of gibbsite drops to 5.5, and the resulting K value for reaction 3 drops to 1.8. This means that 80° C gibbsite is highly insoluble down to a pH of about 3.7, at which point it becomes highly soluble.

The springs in these systems have been shown to have rapid fluctuations in pH and temperature. These fluctuations have been shown to be greater than two pH units and 20° C in a short period of time. These types of changes in conditions clearly have the capacity to cross thresholds such as the 3.7 pH threshold for a dramatic increase in gibbsite solubility (at a temperature of 80° C). Once that threshold is crossed, the dissolution of gibbsite will consume acidity until a new equilibrium condition is established or until the gibbsite is completely dissolved. This threshold pH value (calculated for conditions in Rabbit Creek spring 1) corresponds almost exactly with the observed increase in aluminum and iron on the sampling date where the pH had dropped to 3.6.

Alunite Precipitation

The reaction for the precipitation and dissolution of alunite, $\text{KAl}_3(\text{SO}_4)_2(\text{OH})_6 + 6 \text{H}^+ = \text{K}^+ + 3 \text{Al}^{+3} + 2 \text{SO}_4^{-2} + 6\text{H}_2\text{O}$ (reaction 4), has a log K value of -1.4 at 25° C, and is dependant on the pH, and the concentration of dissolved aluminum, potassium, and sulfate. The temperature dependence of this reaction is negligible in the range appropriate for these systems.

If the potassium concentration is fixed at 50 mg/L and the aluminum concentration is fixed at 2 mg/L (representative of Ragged Hills spring 1) and a pH of 2.7, this reaction will buffer the concentration of dissolved sulfate around 0.07 molar,

which is well below the observed *Sulfolobus* growth inhibiting concentration of about 0.64 molar (see Table 2).

However, if the potassium concentration is fixed at 5 mg/L, the aluminum at 0.7 mg/L (representative of Rabbit Creek spring 1), and pH at 2.7, this reaction will buffer the concentration of dissolved sulfate at about 1.06 molar. This is well above the 0.64 molar growth inhibiting concentration of sulfate. This reaction is highly dependant on pH. As the pH decreases, the ability of alunite to buffer a solution with respect to aluminum, potassium and sulfate decreases dramatically. The abundance of alunite at the Ragged Hills site indicates that these precipitation reactions are indeed buffering the concentration of sulfate in that system. However, the lack of any alunite observed in Rabbit Creek indicates that this reaction is apparently not exerting any influence over the chemistry at that site.

Kaolinite Dissolution

The clay mineral kaolinite was nearly ubiquitous in samples from Rabbit Creek and Ragged Hills (see next section). The reaction for the precipitation and dissolution of kaolinite is given by $\text{Al}_2\text{Si}_2\text{O}_5(\text{OH})_4 + 6 \text{H}^+ = \text{H}_2\text{O} + 2 \text{H}_4\text{SiO}_4 + 2 \text{Al}^{3+}$ (reaction 5). This reaction is dependent on temperature, pH (to the sixth power), activity of aluminum (squared), and activity of silica (squared), and has a K value of 7.44 at 25° C and a K value of 3.40 at 80° C. When kaolinite becomes undersaturated due to a decrease in pH, the dissolution of kaolinite will buffer the pH of the springs.

Using a temperature of 80° C and fixing the other variables based on conditions observed in Rabbit Creek spring 1 on 6/01/01, the dissolution of kaolinite would buffer

the pH at 3.1. As the dissolved silica and aluminum concentrations increase, the pH at which kaolinite buffers the system decrease. The highest silica concentration was observed in Ragged Hills spring 1 on 9/16/02. Fixing the silica and aluminum concentrations at the values measured on 9/16/02, kaolinite dissolution would buffer the pH at 2.7. In the range of temperature, dissolved silica, and dissolved aluminum common to Rabbit Creek and Ragged Hills, a drop in pH will be buffered by the dissolution of kaolinite in the pH range of 2.7 to 3.1 until the reaction equilibrium is established or the kaolinite is consumed.

Figure 21 is a 3-D plot of the kaolinite saturation surface at a temperature of 80 °C. Samples from Rabbit Creek and Ragged Hills are plotted on that surface and appear as depressions where kaolinite is undersaturated and mounds where kaolinite is supersaturated. The only springs that are undersaturated with respect to kaolinite have a pH in the range of 2.6 to 3.1.

Figure 22 is a view along strike of the kaolinite saturation surface from Figure 21. It clearly shows that all of the springs plotting below the saturation surface for kaolinite have high Al^{3+} activity and low pH. In fact, the spring with the highest Al^{3+} activity (Rabbit Creek spring 7) is actually undersaturated with respect to kaolinite, while the spring with the lowest Al^{3+} activity is supersaturated with respect to kaolinite. This is due to the inverse relationship of pH and Al^{3+} discussed earlier. Within the range of silica concentrations observed in the Ragged Hills and Rabbit Creek sites, there appears to be a threshold pH between about 3.5 and 3 (depending on the temperature and Silica

concentration) where kaolinite dependency on pH outweighs that for Al^{3+} , causing kaolinite to become undersaturated.

3-D Stability Diagram for Gibbsite, Kaolinite, Quartz, and Opal

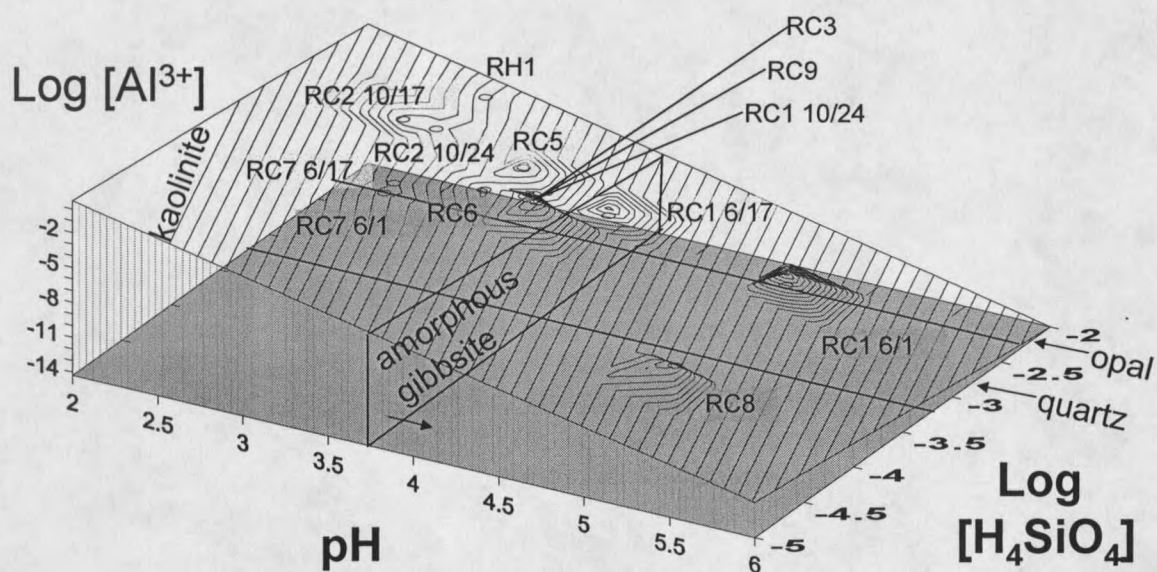


Figure 21: 3D stability diagram for gibbsite, kaolinite, quartz, and opal. The base of the diagram shows the stability fields for quartz and amorphous silica. Amorphous gibbsite is stable to the right of the vertical boundary at $\text{pH}=3.7$. Kaolinite is stable above the striped sloping surface. The composition of select springs is plotted on the diagram. Springs plotting above the kaolinite surface are contoured as mounds on the surface and springs plotting below the kaolinite surface are plotted as depressions in the surface. (Fields created using data from Parkhurst *et al.*, 1997)

Amorphous SiO_2

The saturation with respect to the pure silica phases is shown in Figure 23. The only sample supersaturated with respect to amorphous silica is Ragged Hills spring 1.

The only springs not supersaturated with respect to quartz are Rabbit Creek spring 7 and

Rabbit Creek spring 8. The remainder of the samples are above saturation with respect to quartz, but below saturation with respect to amorphous silica.

View Along Strike of Kaolinite Solubility Surface

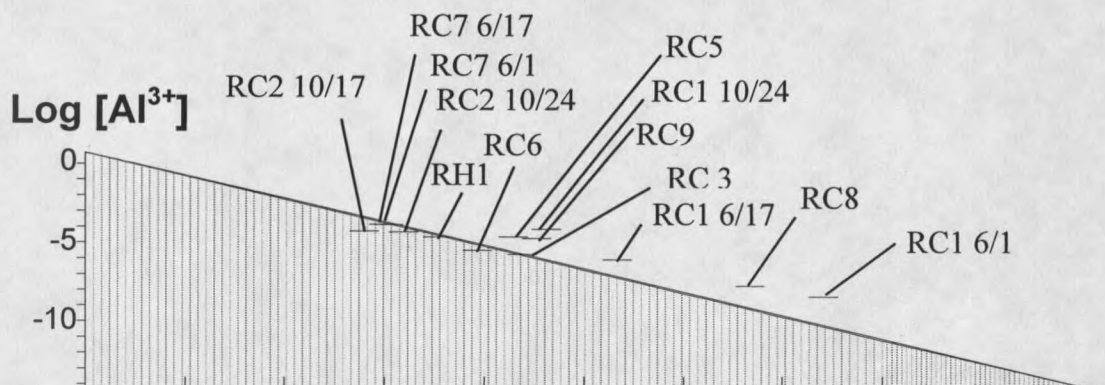


Figure 22: View along strike of the kaolinite surface from Figure 21, such that the Al^{3+} value on the vertical axis represents the concentration in equilibrium with kaolinite independent of the silica concentration. Kaolinite is supersaturated above and undersaturated below the hypotenuse of the triangle. Kaolinite is stable in all of the springs with a higher pH, and it is unstable or right at saturation in the springs with a pH below about 3.3, even though the aluminum concentrations are higher at the lower pH. (Fields created using data from Parkhurst *et al.*, 1997)

Below a pH of about 10, quartz and opal equilibrium is independent of pH (Langmuir), it is, however, highly temperature dependant. At 0° C, an approximate temperature for snowmelt in equilibrium with snow, the equilibrium Si concentration with respect to amorphous silica is approximately 34 mg/L. The average Si concentration of the acidic pools at Rabbit Creek was 29.1 mg/L (excluding Rabbit Creek spring 2, the one pool near opal equilibrium at 80° C). The dissolved silica concentrations of springs from Rabbit Creek are shown in Figure 24. These data suggest that there is a significant

