Biological Chemicals in Rock Coatings

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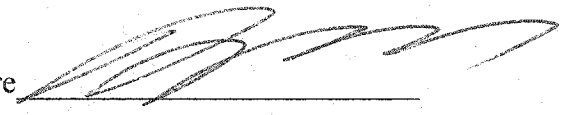
Signature 

Date 6-11-09

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Abstract

Biological chemicals in rock coatings

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Stable and unstable amino acids, DNA, nitrogen, carbon, and several polyfunctional chemicals were found along with amorphous hydrated silica (opal) in microstromatilitic desert varnish rock coatings from deserts, primarily in the US southwest. The presence of labile organic compounds, especially in deserts, requires a mechanism for their preservation. In this study the discovery of silica in desert varnish suggests that preservation may be facilitated by silicic acid ($Si(OH)_4$) or (di)silicic ($(HO)_3Si\text{-}O\text{-}Si(OH)_3$) through the formation of a variety of complexes with ions and organic molecules, including mucopolysaccharides, glycoproteins that are enriched in hydroxyl amino acids (serine and threonine), glycine, aspartic and glutamic acids. Although organo-silica complexes were not directly observed, previous work has shown that amorphous hydrated silica forms both Si-O-C and Si-O-metal complexes. Organic candidates include amino acids, some sugars, unsaturated polyhydroxy compounds, catechols (1, 2-diphenols), and other compounds with rigid structures, as well as some flexible sugar-related substances, polyols and sugar acids. It is suggested here that prokaryotic DNA cloned from coatings and amino acids may be sequestered in silica,

clay minerals, or by complexation with divalent and trivalent ions, as it is known that Fe ions interact with phosphate groups and Mn interacts with nitrogen bases of DNA. The addition of Fe ions to a DNA-Mn complex may lead to stability and Mn binding may occur irrespective of Fe-DNA interlinking. This might provide a mechanism for preserving DNA found in silica-rich rock coatings. Not only organic substances then, but metals also might participate in polymerizing, crosslinking and hardening, as small quantities of silicic acids condense and fuse by gelling. Desert varnish contains chemical and mineral components from the local environment. Polymerization of silicic acid may be the underlying process of formation of silica glazes and desert varnish and, if organo-silica processes are important in the formation of rock coatings, they also may be relevant to studies of Earth's oldest paleoenvironments, fossils (>3,400 my), and possible biotic chemicals on other planetary bodies, especially Mars.

Table of Contents

List of Figures

List of Tables

Acknowledgements

The original intent of this thesis was defined by its original title, "How Desert Varnish Forms." Since the inorganic chemistry of desert varnish had been well investigated it seemed that finding out the organic constituents might yield clues to answer this question. Past estimates of the organic components however, were less then 1% but never quantified. The tools that were primarily used to investigate varnish coatings have been the electron microprobe and the scanning electron microscope with X-ray capabilities. Both of these tools provide excellent qualitative and quantitative analyses however do not have good capabilities to survey lighter elements. The elements of organic constituents, of course, are made primarily of carbon, nitrogen, hydrogen, oxygen and sulfur. Sulfur is easily detectable but the others are not. The tools to look at the lighter elements have been available but there have been several improvements in analytical techniques during the 1990's. The original thesis concept, for solving the puzzle as to how varnish forms, came about by the lingering curiosity of John Adams and Jim Staley. Alan Gillespie, however provided the original title in part at least to say why not really solve this question. It does and did seem a tall order, but a challenge sometimes allows us to excel beyond where we might have otherwise. Alan of course knows this and I wish to express my sincere thanks for his support and encouragement. Many others have been of great help along the way, Bernard Hallet, Bruce Nelson, and John Stone in the Department of Earth and Space Sciences. Ron Sletten graciously agreed to be on my reading committee and taught me how to use Word as a tool, something I mistakenly thought I had achieved some skill with previously. Marty Fisk not only agreed to be on my committee, which involves many hours of driving from Oregon State but made it possible to voyage on Atlantis and go far beneath the sea in Alvin. John Baross, whose home department is oceanography provided most interesting discussions concerning what might seem a peripheral topic, but is not, the origin of life. Bob and Chris Carson for collecting samples from the Gobi Desert and providing excellent descriptions and images. My program however is intended to be interdisciplinary and I personally not only take this to heart but feel that the gray areas between disciplines houses interesting and unexplored ideas. Two people in the Earth and Space Sciences (ESS) department especially helped whenever I asked, Dick Stewart who sat with me for hours looking at thin sections and was always available when help was needed. Eric Cheney worked on a last minute grant proposal when it was no advantage for him to do so. Peter Ward also of ESS provided many stimulating conversations and kept me on my toes for the first year of my graduate studies. He volunteered to be my temporary advisor in astrobiology and ESS until I could arrange something more permanent. Above all else he managed to convince me that it is important to maintain focus but to work on more then one project. Peter started me on a most interesting project, the study of fullerenes and their possible role in mass extinctions. During my first year he suggested a joint project with Luann Becker who was visiting professor. She spent many hours in the lab with me, where we worked on

projects that have born fruit but are not directly part of this dissertation. We all remain friends and colleagues. In the astrobiology program there are many fine individuals. Two that I have known since the 1970's when I was a somewhat younger grad student are Don Brownlee and Jim Staley. Don and Jim both encouraged me to return to the university and just as they did many years ago encourage by their example to attempt interesting, challenging, and honest science. Jim graciously agreed to be my co-advisor, ventured into the mountains with llamas with me, and has not only been a friend and mentor but provided inspiration and sound advice. It is my fondest hope that we will continue together exploring the roads leading toward illusive truths and better explanations. A user grant at Pacific Northwest National Laboratories, Environmental Molecular Sciences Laboratories, a Department of Energy facility and a NSF IGERT Grant provided the place and the funding for this research. The NSF fellowship permitted me the opportunity to step beyond specialization and pursue interdisciplinary avenues. Karl Popper said, "specialization may be a great temptation for the scientist. For the philosopher it is a mortal sin." Wolfgang Elizabeth Krumbein, founder of the Institute for Chemistry and Biology of the Marine Environment, Carl von Ossietzky University, Oldenburg, Germany, who has always explored the beyond and attempts to instill in me history and philosophy. Special thanks to Anna Gorbushina with whom research will continue after this dissertation. Mike Engel for his friendship and many discussions and collaborations. Conversations many years ago with Russell Potter and George Rossman remain with me today. Jeremy Dodsworth (U of Washington) and Lorraine Olendzenski (Woods Hole Marine Biolgical) who attempted to instill molecular biology skills and extract DNA. Importantly Diana Maria, Mitch Sogin, Ken Nealson, Jeff Bada, Oliver Bota, Jim Haggart, Mike Brown, and many others contributed advice and support. Karin Stewart-Perry, as she always has, provided endless patience, encouragement, support, and not least editorial comments. Catherine Fosnot, who has unsurpassed curiosity, provided insightful comments and a unique opportunity to apply evolutionary concepts to learning and theories of knowledge. Three generations of Smiths: Anthony Smith for imparting philosophical notions outside the normal, Lanette, and future scientists and naturalists Sierra and Forest. Without the help and discussions, the ideas, and joint projects, many of which are included herein, with Vera Kolb of University of Wisconsin, Parkside, little of the thoughts and conclusions presented here would have transitioned to a conscious state. With all of the above one wonders if it is possible, that anyone remains unmentioned. Nonetheless there is one. All students that are fortunate enough to have a special mentor, know and understand what it has meant for me to have John Adams, both as a friend and someone that has a unique capacity to clarify important concepts. His continued quest for explanations, intellectual integrity and support have in no small part been the reason that science remains the exciting tool for discovery that it has for me since his first stint as advisor in the late seventies. Several agencies have helped enormously with funding. First, the NSF, IGERT grant, that funding my stay at the University, Washington Space Grant Consortium, NATO, and White Mountain Research, University of California, San Diego.

Dedication

To my grandmother Eva Stewart

she read to me about nature
she scratched my back
she took me fishing
she made me go to church too much
she believed in education and returned to school in her sixties
she gave me Will Durant –The Story of Philosophy
and a book on Picasso
I still write on her desk
and if she were here I would go to graduation just to watch her smile

CHAPTER 1 INTRODUCTION

'I suppose, sir,' I answered, drolling on our young gentleman's Continental education, 'it's the varnish from foreign parts.'

Wilkie Collins, "The Moonstone"

The objectives of this dissertation are: (1) to describe the organic components of the rock coating called desert varnish, particularly amino acids and DNA; (2) to test a silicic acid hypothesis for the preservation of (bio)organic components and as a mechanism of formation; (3) to explore the qualitative incorporation of microcolonial fungi (MCF) into coatings; (4) to present a simple underlying explanation for the formation of desert varnish. The motivation for this study is to better understand contemporary rock coatings and to see how this information can be extended to older environments. Desert varnish and silica glazes are ubiquitous on Earth and are of recent origin, hundreds to thousands of years old. Organo-silica compounds in paleo environments, such as silica sinter (<20 mya), and deep time Earth fossils such as the ~3.5 bya Apex Chert in Australia, may be facilitated by understanding processes in desert varnish.

Rock coatings are complex systems that occur at the interface between air, biology, and stone. The coating studied herein is desert varnish, but silica glazes as described by Curtiss *et al.*, 1985 and Farr and Adams, 1984, provide a simplified model with which to compare complex varnish coatings. Desert varnish is a relatively recent rock coating (<100,000 y). It is a microstromatilitic sedimentary deposit (~<200μm thick) found on desert rock surfaces throughout the world.

It is widely agreed that desert varnish is a coating and not a weathering product of its substrate; therefore, the source materials/elements derive by necessity from external sources such as aeolian deposits and/or aerosols. Dust lands on rock surfaces,

some components are concentrated and become adhered or "glued" together. Very importantly, unused elements and materials need to be removed in a never-ending "conveyer belt" that allows for concentration and/or formation of mineral components.

Engel and Sharp (1958) estimated that 75% of rock beds in southwest US deserts had recognizable varnish coatings. It is the black shiny nature of many varnishes that makes the desert varnish coatings distinctive and has attracted the attention of observers and investigators alike (Figure 1-1). The undersides of varnished rocks lying on soils frequently have bright red coats undercoats (Figure 1-2). Substrates on which coatings form must be reasonably stable and have some texture for varnishes to have time to form and adhere. Fine-grained basalts are an ideal substrate but varnishes can form in crevices on smooth quartz, and on some less stable and coarse grained surfaces such as granites (Figure 1-3).

Perry and Adams (1978) showed that desert varnish coatings are heterogeneous and composed of ca. micron light areas and dark layers Figure 1-4). Liu (2003) and Liu and Broecker (2000) have tied layers as climate dating tool. The surface of varnish coatings is frequently botyriodal (Figure 1-5). Dark layers within varnishes are enhanced in manganese by orders of magnitude over soils. Silicon, aluminum, and oxygen are the primary elements but, while oxides including manganese, iron, titanium, and magnesium are important, they are not the main elements in desert varnish. Variable amounts of detrital grains are embedded in varnish coatings (Figure 1-4). The coatings have been shown to have up to 9% water (Perry, 1979) and frequently have large quantities of embedded detrital grains when viewed in thin section (Figure 1-4). Differing from dark oxide rich varnishes, silica glazes on rock surfaces in Hawaii (Curtiss et al., 1985; Farr and Adams, 1984) are composed primarily of silica with only trace amounts of oxides. Desert varnish and silica glazes, found within the same matrix, have been described in Hawaii and Oregon deserts (Farr, 1981). Adams et al. (1992) noted the presence of both silica glazes and desert varnish in US deserts, as did Jones (1991) in Peruvian deserts.

Biological organisms and the remnants of their degrading cells are present in

soils and on rock surfaces. The question here: what organic substances, if any, are in coatings and, if present, what can they tell us about paleo biochemicals? Past questions, however, were centered on whether rock coatings are biologically mediated or are inorganically formed. Biological and geochemical processes are intimately mixed at the subaerial interface and the formation mechanisms of rock coatings are a complex interaction of those organic and inorganic chemicals.

What's in a name? *"Desert Varnish"*

Wheeler (as quoted in Walther, 1891 p. 453) states when writing about the California desert, "It is remarkable that over wide stretches, rocks, and rolled material are coloured black on their upper surface just as though they were coated with a black varnish." "Black" desert varnish has been called many things. *"Schutzrinde"* was introduced by Walther in 1891, according to Laudermilk as a translation of the French term *manteau protecteur.* Linck uses the term *"Schutzrinde"* to include all dark coatings including *"Wustenlack"* a thin, polished coating that may be an aeolian polished patina. *"Dunkle Rinden"*, as used by Linck, is a dark brown to black coating, possibly more similar to Sonoran and Mojave desert varnish.

Not all shiny "coatings" are indeed coatings. Polished ventifacts from Antarctica (pers, comm.. John Adams, 2004), that resemble dark shiny varnish coatings, are not coatings but rather dark wind-polished rocks, when viewed in thin section. *"Schweinfurth"* (as cited by Linck) describes black coatings on rocks in Egypt that have spread on surrounding sands. Samples collected by John Adams from western Egypt appear in thin section to be coatings that are spreading and surrounding grains in sandstone substrates. More recently, Dorn (1998) suggested the useful and general term "rock varnish" that takes into account the wide environmental and geographical zones where coatings are found. This dissertation will indeed suggest that coatings are related, but with a substantive difference. It will be proposed that dark oxide and silicon-rich coatings, desert varnish, are a special case of the more general phenomena of silica-rich glazes.

Figure 1-1. Site "X" 0.3 miles west of the eastern Death Valley boundary, Hwy 190 on the north side of the road. Rocks are solidly embedded in soils and are very stable.

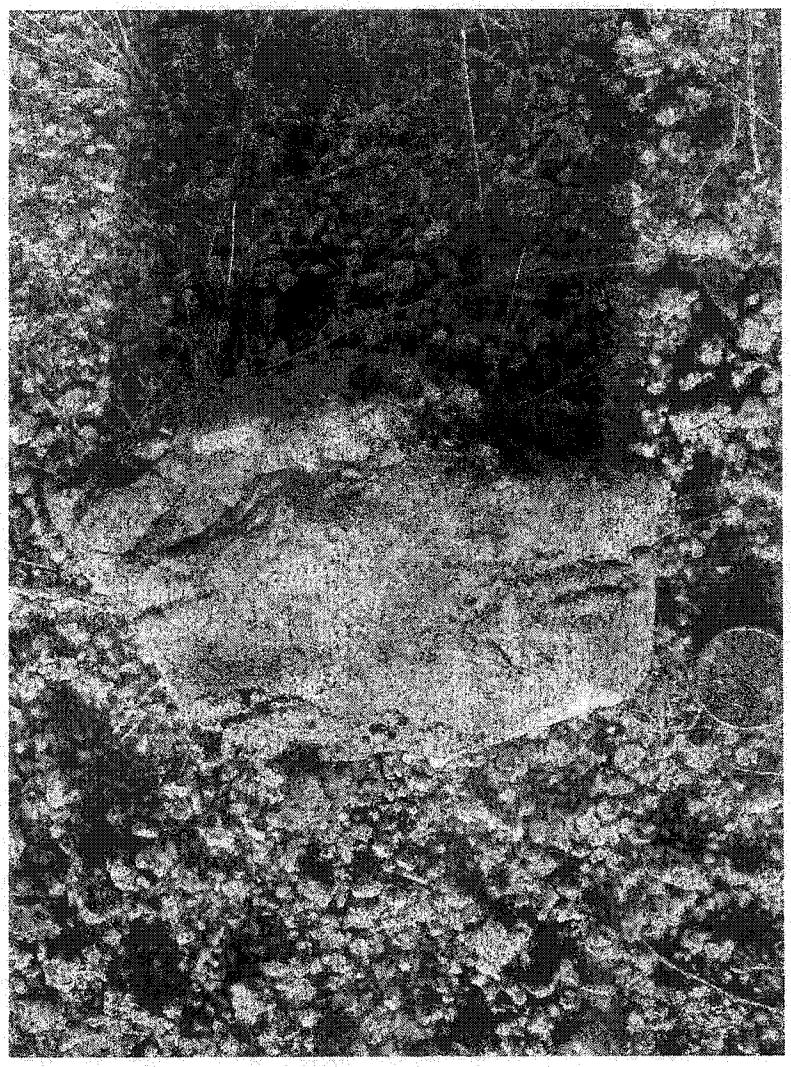


Figure 1-2. Pictured above is an overturned translucent stone from Baker, California. Algae and/or cyanobacteria are on the edge of the stone that was in contact with the soil. The glossy red under-glaze, typical of smaller stones, covers the under sides of both opaque and translucent stones.

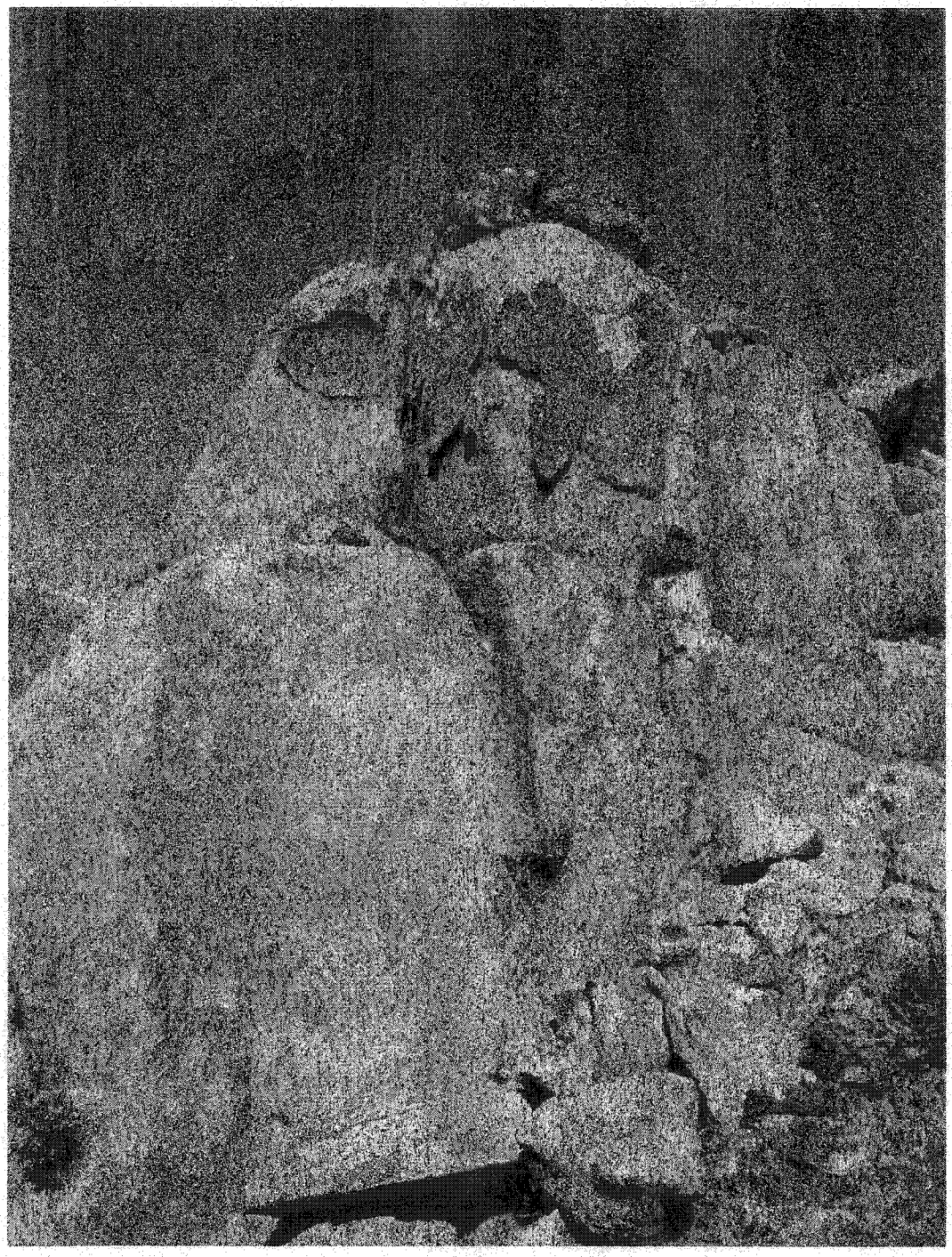


Figure 1-3. Granite with desert varnish at Lone Pine, California. Near the top of the rock outcrop, older granite is exfoliating. Desert varnish is visible on those older granite surfaces.

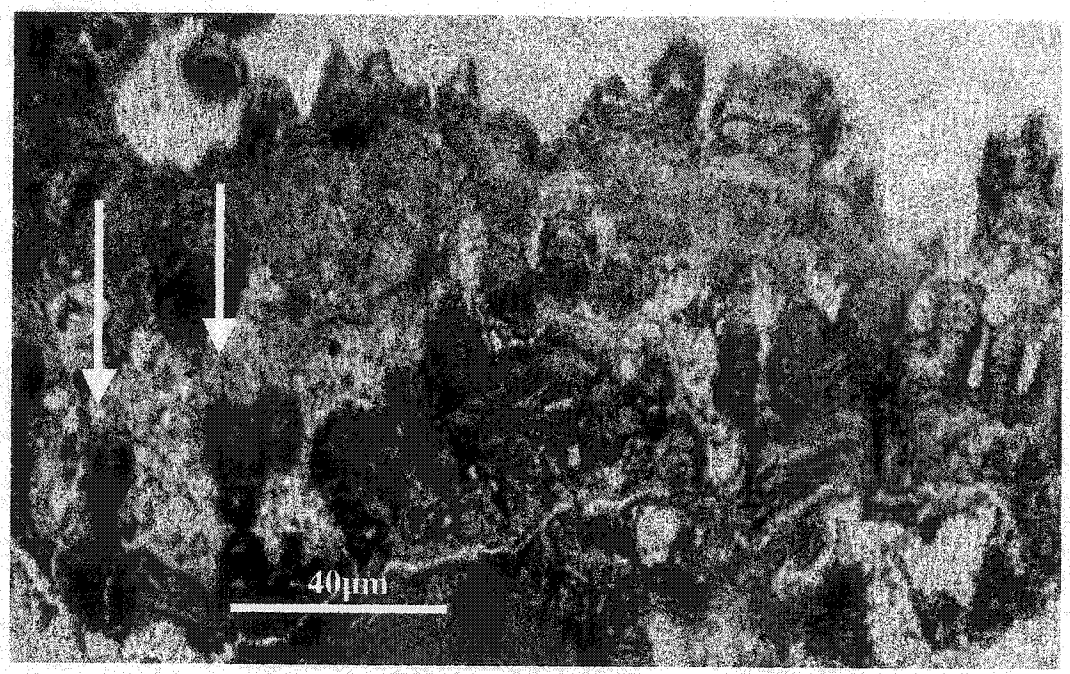


Figure 1-4. Ultra-thin section (~<10 µm) of varnish coating. White arrows point to abundant trapped detrital grains. This sample has botryoidal structures in several orientations. Dark areas within stromatolitic structures are enriched in manganese oxide. The primary element present, other than oxygen in all varnish, glazes and red bottom coats, is silicon. The silicon present in detrital grains in desert varnish is probably a significant component of the silicon present in bulk analysis. However, separating out the detrital grains allows for chemical and XRD examination of the remaining elements and minerals. After mechanical/chemical separation of sand, silt and clays, silicon is still the prevalent element after oxygen. TEM also allows for a fine scale examination of the chemistry of the varnish matrix, excluding detrital grains that are present in bulk analysis.

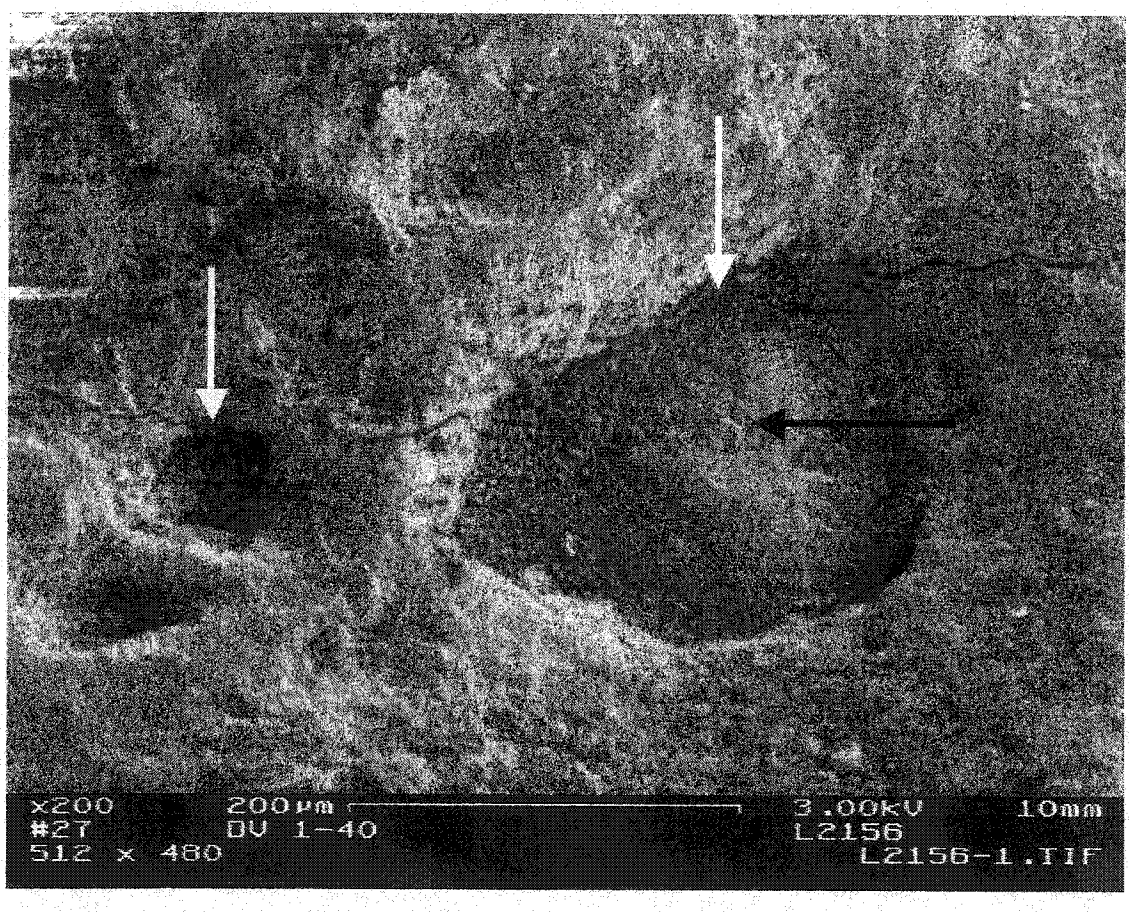


Figure 1-5. Botryoidal varnish formation in a depression on the rock surface (arrows). Note that there is no characteristic mounded texture in areas that are higher within the surface "dimple" (depicted by the black arrow). This sample is from the Panamint Springs, Death Valley, California. Micro-surface textures are variable from area to area, and not all varnish coatings display mounded surface morphologies. When it is present, botryoidal growth appears in low areas as in the above image. Botryoidal morphologies are variable from locale to locale. Surface textures from the Sonoran Desert, in this study, are similar to the above Mojave Desert botryoidal morphologies and have been well characterized previously (Perry, 1979).

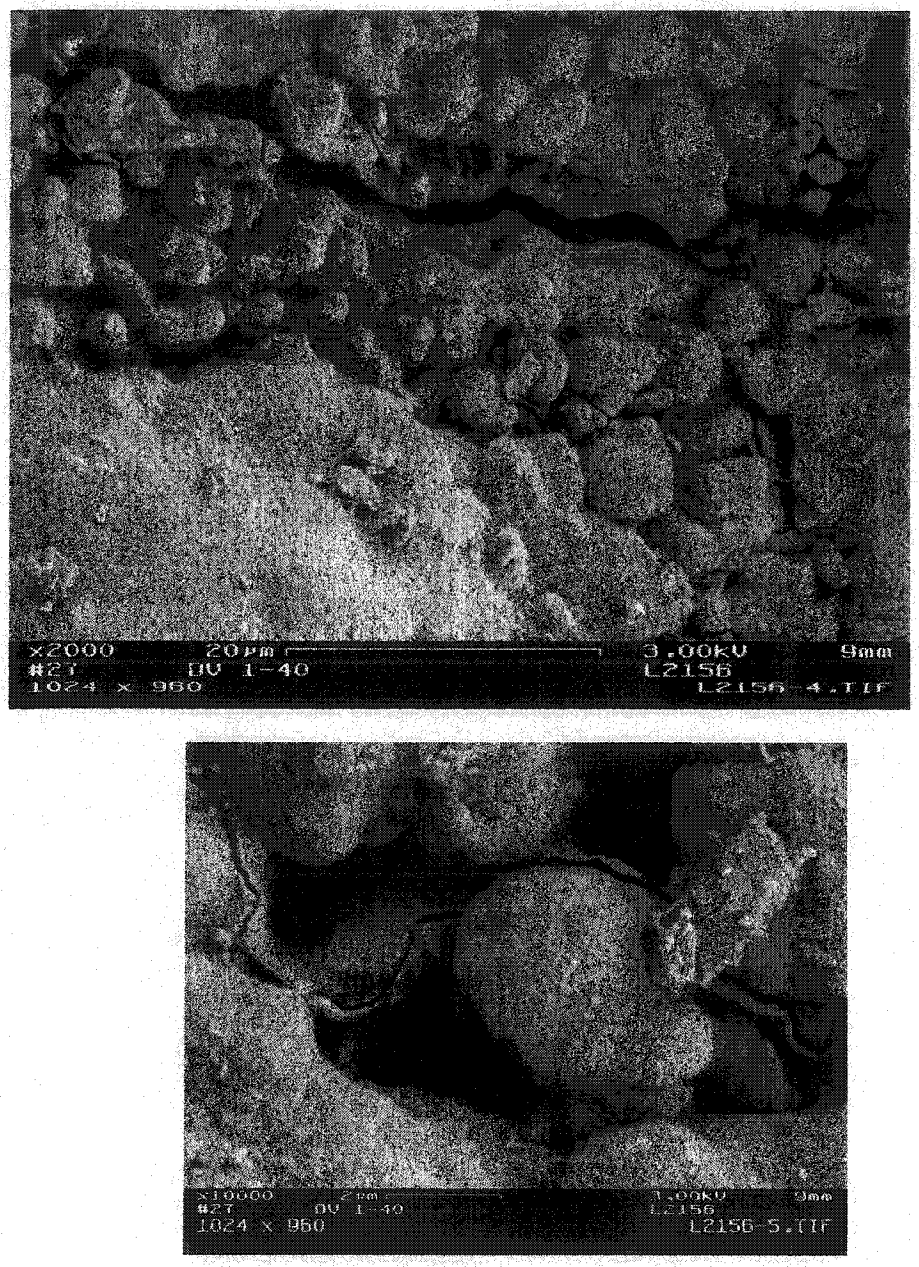


Figure 1-6. Higher magnification SEM images of the same Death Valley, California desert varnish in Figure 1-5. SEM image above is shown, with two magnifications (top image x2000 and lower x10,000). Note detrital material between and on mounds.

"Desert varnish" will be used here then to define coatings that appear dark (reddish brown, chocolate, black, and blue-black). Reasons for not abandoning desert varnish as a term are both because of its historical use and because it seems a most apt description for dark surfaces formed in subaerial conditions in hot, cold, high and low elevation deserts. Smith and Whalley suggested the same in their paper about Moroccan 'desert varnish' (Smith and Whalley, 1988). It must be emphatically stated that this definition of darkness is a macro-visual phenomena. Close inspection of varnish coatings exposes the heterogeneity of the surface composition. Areas that are not dark on varnish rock surfaces may still be coated with silica glazes.

Silica glazes can appear clear (Curtiss et al., 1985; Farr, 1984) or tinted by small quantities of oxides. Under-coatings that are normally red and often lustrous will be referred to as red under-glaze, or under-coatings and/or red bottom coats. Top surface glazes that are not dark will be referred to as rock glazes.

History

Nearly two centuries ago two great and early observers of nature noted a smooth, lustrous, black patina that glistened on rocks. The two were Alexander von Humboldt (1852) and Charles Darwin (1871). What they observed was not a weathering product of a rock substrates but rather what we now know to be a microstromatilitic coating that is separate and distinct from its substrate. The black coloration and shine of the coatings is likely what attracted their attention and still does attract contemporary investigators and observers to this day. Darwin and Humboldt pondered the same questions that are still being asked today: of what are rock coatings made, and how do they form?

The rock coatings that Darwin and Humboldt observed are common throughout the world's arid lands and the question of their origins became refined in the 1980s, but remained unresolved. Starting with Darwin and until recently, rock coatings have been extensively analyzed, but without the benefit of a new generation of analytical equipment. During the last 30 years many investigators looked for links to biology. Microbes are pervasive in all environments on Earth and researchers actively sought a

causative link to desert varnish. Primarily, these investigations entailed culture-based studies of microbes, both bacterial and fungal. The emphasis was on a search for manganese oxidizing microbes. The order of magnitude concentrations of manganese over soils has both perplexed and intrigued researchers. It was thought that understanding manganese enhancement would reveal the answers to the genesis of desert varnish. While the microbial (Dorn and Oberlander, 1981) and mineral/chemical makeup of coatings were exhaustively studied, only scant attention was paid to the study of organic components (notable exceptions Staley personal communication, 2000; Nagy *et al.*, 1991; Dorn and DeNiro, 1985).

During the last 30 years, the question of how varnish forms has been polarized with inorganic mechanisms at one pole and biological at the other. This either/or approach discounts the possibility that the coatings form in the presence of biology or its byproducts but are not necessarily the direct result of biological metabolisms. As W. Verandsky (1929) stated, "There is no phenomenon on the surface of the Earth, which is independent of life." Whereas a few researchers have suggested inorganic origins (Potter and Rossman, 1977; Jones, 1991; Smith and Whalley, 1988), many others have suggested the possibility of a biological involvement (Perry *et al.,* 2003 a; Sterflinger *et al.*, 1999; Grote and Krumbein, 1992; Raymond *et al.*, 1992; Staley *et al.,* 1992; *et al.,* Nagy *et al.,* 1991; Palmer *et al.,* 1986; Taylor-George *et al.,* 1983; Krumbein and Jens, 1981; Dorn and Oberlander, 1981; Allen, 1978). Some have gone further and suggested possible causal relationships with bacteria and fungi (Sterflinger *et al.*, 1999; Grote and Krumbein, 1992; Raymond *et al.*, 1992; Staley *et al.,* 1992; *et al.,* Nagy *et al.,* 1991; Palmer *et al.,* 1986; Taylor-George *et al.,* 1983; Krumbein and Jens, 1981; Dorn and Oberlander, 1981; Allen, 1978). The latter premise, a biological origin, while hard to dismiss, has proven elusive, with no consensus for a direct link attributed to microbes. Regardless of the importance of biology, no viable hypothesis has been presented that provides an explanation for key varnish features: its hardness, the "glue" that binds the heterogeneous components together, lamellar and botryoidal morphology, and its slow

rates of formation (Liu and Broeker, 2000).

Potter's and Rossman's (1977) view that clays cemented by oxides are the principle building block of varnish coatings has remained as the cornerstone explanation of rock coating formation. This is not to suggest that clays are not present in desert varnish, just that they are present in variable amounts and do not provide a fully satisfying explanation for coating hardness, luster, and morphology.

While the mechanism of formation has remained a mystery, some aspects, such as Fe and Mn accumulation, have been addressed (Adams *et al.*, 1992; Jones, 1991). Adams *et al.* (1992) suggested a mechanism that involves bacterial and fungal chelators to concentrate Fe oxides and oxyhydroxides. Fe^{2+} (uncomplexed) is unstable above pH ~5, which is typical of the soil, and thus precipitates. Fe is concentrated 3-4 times in varnish over typical dusts. Based on experimental simulations, Jones (1991) suggested a mechanism for the concentration of Mn in weak acids, such as carbonic (H_2CO_3) (Jones, 1991). Mn readily remains in solution as Mn^{2+} and Mn^{3+} until pH reaches ~8.5 where it precipitates as insoluble MnO_2. The fact that microbes can accelerate reaction rates of Mn oxidation, when present, must be taken into account (Sterflinger *et al.* 1999; Krumbein and Jens, 1981; Grote and Krumbein, 1992).

Historical and cultural context of varnish coatings

On a barren hillside 40 miles east of Fallon, Nevada are darkly stained rock outcrops. The sloping hillside climbs for a few hundred feet above an old and mostly dry lakebed. It is now called Grimes Point. One of the oldest petroglyph sites in the American Southwest is along this ancient shoreline of Lake Lahonton. The rock art is of three main types estimated to be from a few thousand years old to as much as 10,000 years before present. The oldest of the petroglyphs are thought to be part of ritual ceremonies where shamans incised the rock. Called 'Pit and Groove' style, they resemble small craters (Figure 1-7).

Alexander von Humboldt, a German geographer, in 1799 describes granite boulders among the cataracts near the mouth of the Orinoco River in northeastern

Venezuela as "smooth, black, and if as coated with plumbago." Plumbago is a mineral of lead and silver and Plumagine is a natural alloy of lead and silver.

Analyses by Humboldt, and subsequently Darwin, showed that the coatings contained manganese and iron. Local legends explained that these rocks were baked in the sun and were dangerous to human health. Humboldt wrote, "I have just alluded to the noxious influence on the salubrity of the atmosphere, which is attributed by the natives, and even the missionaries, to the bare rocks. This opinion is the more worthy of attention, as it is connected with a physical phenomenon lately observed in different parts of the globe, and not yet sufficiently explained..." Humboldt also described similar coatings on rocks along the Nile and Congo. The American Indians had a saying, "the rocks are black where the waters are white." Darwin later noted that the coatings on these river-worn rocks were of a different color, stating, "Here the coating is of a rich brown instead of a black colour, and seems to be composed of ferruginous matter alone."

The rock coatings in this study, from Bishop and Death Valley, California, Grimes Point, Nevada, and the Gobi Desert are black like those described by Humboldt and Darwin. However, coatings from the Mojave are deep reddish brown rather than black, but do contain manganese. Manganese is a central feature only of dark desert varnish coatings and is present in only small quantities in silica glazes (Curtiss *et al.*, 1985; Farr and Adams, 1984) and red under-glazes (Figure 1-2).

Humboldt suggested that the "Missions of Carichana and of Santa Barbara, periodically washes the granitic rocks." He stated, "The colouring matter does not penetrate the stone, which is a course-grained granite, containing a few solitary crystals of hornblende." He contrasted the rocks to the granite of Syene in Egypt. He stated, " The black crust is 0.3 of a line of thickness, it is found chiefly on quartzose parts. The crystals of feldspar sometimes preserve externally their reddish-white colour, and rise above the black crust." He noted that breaking the stone with a hammer showed that there was no evidence of decomposition of the underlying white rock, thus becoming the first to conclude that the black crusts are coatings. Humboldt quoted another local saying

Figure 1-7. Petroglyphs at Grimes Point National Monument, Nevada. The insert is of a petroglyph dated by archaeologists at ~2000 years old, while the main photo is of a style of petroglyph thought to be ~10,000 years old. The older petroglyph is re-patinated by desert varnish.

that the rocks are 'burnt' (or carbonized) 'by the rays of the sun." He went on to say, "We saw them not only in the bed of the Orinoco, but in some spots as far as five hundred toises from the present shore, on heights which the waters now never reach even in their greatest swellings." Humboldt did state the possibility of two different types of coatings, as did Darwin. The river deposits are clearly formed in a different environment from the coatings formed in deserts, even though they appear similar in appearance. But, the need for water and input and removal of source materials remains the same. It is a testament to their logic that both men looked for a solution chemistry in the form of rising waters and also addressed concepts of light and heat.

Humboldt's summary of his thoughts and his questions are nearly identical to the ones that we ask 200 years later. Do coatings include concentrated manganese and "ferriginous metals?" Are components added from the atmosphere? Humboldt sought an explanation for the uniformity of the coatings as a solution deposit from water flowing over surfaces He said, however, that "the celebrated chemist", Berzelius, examined the dark coated rocks, "and was of the opinion that they derive from subterranean sources, and deposit on the rocks in the manner of cementation…" This first notation of dark rock coatings in western cultural writing illuminated a mystery that has remained unsolved. "What is the brownish black crust, which gives these rocks, when they have a globular form, the appearance of meteoric stones?" asked Humboldt.

In 1871, Charles Darwin sampled and tested dark coatings in South America along the same cataracts as had Humboldt. "The layer is on extreme thinness; and on analysis by Berzelius it was found to consist of the oxides of manganese and iron." In the Orinoco river, Darwin noted, this coating is "of a rich brown colour, and seems to be composed of ferruginous matter alone." The coatings in the rivers are different from the oxide coated rocks, as Darwin summarized "The origin, however, of these coatings of metallic oxides, which seem as if cemented to the rocks, is not understood…"

CHAPTER 2 METHODS

I readily admit that only observation can give us "knowledge" concerning facts...but knowledge does not justify or establish the truth of any statement.

Karl Popper

Amino acid abundance

The samples collected from Painted Rocks and Deem Hills in 1990 were analyzed for their amino acid abundance by two separate methods (Perry *et al.*, 2003a). In method one (performed by Mike Engel of Univ. Oklahoma), approx. 200 mg of each varnish sample was placed in acid-clean Pyrex tubes. Ten milliliters of distilled 6N HCl was added to each tube, and the tubes were sealed under nitrogen and hydrolyzed (24 h, 100°C). Next, the hydrolyzates were separated from the residual, insoluble varnish by filtration and evaporated to dryness. The residues were then dissolved in water and desalted by cation-exchange chromatography using the method of Silfer *et al.* (1990). The samples were evaporated to dryness, dissolved in 0.01 M HCl and subsequently analyzed by high performance liquid chromatography (HPLC) using the method of Hare *et al.* (1985). Amino acid concentrations were determined by comparison of the samples with a standard amino acid mixture of known concentration that was run prior and subsequent to each sample analysis. The experimental error for these measurements is 5 to 10%.

The varnish samples were also analyzed (method 2) by a commercial laboratory (AAA Laboratory, Mercer Island, WA) using a similar procedure. The samples were hydrolyzed (20h, 115°C) in 6N HCl/0.5% mercaptoethanol to which a crystal of phenol was added prior to heating. The analyses were performed using cation exchange chromatography.

Amino acid stereochemistry

Two independent methods were employed to determine the stereochemistry (i.e. D/L values) of the amino acids in the varnish samples. The first method (University of Oklahoma) was used to analyze the samples collected from Deem Hills and Painted Rocks in 1990. Aliquots of the desalted acid hydrolyzates (see above) were evaporated to dryness. The amino acids were subsequently derivatized to N,O-trifluoroacetyl isopropyl esters (Engel and Hare,1985) for analysis of their respective D- and L-enantiomers by gas chromatography (GC). Replicate analyses of each sample were performed using a Hewlett Packard 5890A GC equipped with a 50m x 0.25 mm i.d. fused silica capillary column coated with an optically active stationary phase (Chirasil-Val III), a nitrogen phosphorus detector (NPD) and a Hewlett Packard 3390A integrator. Details of this GC method have been previously reported (Serban, *et al.*, 1988). Amino acid D/L values were calculated from peak areas. Analytical error, determined as standard deviations for the replicate analyses, was less than 0.01 %.

The samples collected in 2000 and 2001 were analyzed for their amino acid compositions and their enantiomers using HPLC (method 2). Samples were crushed to a powder. All glassware was cleaned and heated at 500°C for 3 h. Amino acid extracts were prepared by adding double-distilled water to each sample and heating for 24 h at 100°C in a sealed glass tube. The supernatant was decanted and dried and the residue hydrolyzed in 6N double-distilled HCl for 3 h at 100°C. The hydrolyzed residue was then dried and passed through a desalting column containing AG 50W-X8 resin (Bio Rad). The amino acid fraction was then eluted with aqueous NH_4OH. The eluate was dried, re-suspended in aqueous borate buffer at pH 9.4, and dried again to remove ammonia. The amino acid residue was then re-dissolved in double-distilled water and derivatized by the OPA/NAC (*o*-phthaldialdehyde/N-acetyl L-cysteine) technique. The derivatives were separated by reverse phase HPLC with fluorescence detection and identified by comparison of retention times with standards (Zhao and Bada, 1995). A control blank was processed and analyzed in parallel with the samples.

DNA sample collection- Bacteria and Archaea

A rock sample from Death Valley was brushed with a sterile brush to remove loosely attached soil *in situ*. Varnish was removed from the rock surface using a Dremel tool with sterilized bit and collected using a sterilized brush and brushing the contents onto sterile paper. The fine powder was placed in glass tubes and transported to the lab and maintained at -60°C The rock was brought to the lab and more varnish was removed and the DNA was extracted within one day. Soil from the area near the rock was also collected.

DNA extraction, PCR, cloning, and sequencing

DNA was extracted from 500 mg of varnish or surrounding soil using a Fast Soil DNA extraction kit (Bio101) and Beadbeater (Savant). Approximately 2 μg and 6 μg of DNA was obtained from 500 mg of varnish and soil, respectively. Polymerase Chain Reaction (PCR) to amplify16S ribosomal RNA (rRNA) genes was performed using DNA from soil or varnish as template with primers 27f and 1492r (Lane, 1991). PCR cycling parameters were 30 cycles of 95°C for 0.5 min, 55°C for 1.5 min, and 72°C for 2.5 min. with *Taq* polymerase. For amplification of Archaea 16S rRNA genes, primers 20f and 1492r were used. PCR amplification for Archaea was performed using the same technique as for the Bacteria.

DNA Library construction for trees

Screening and sequencing

Clone libraries of bacterial- and archaeal-specific PCR product from desert varnish were constructed by cloning product using a TOPO TA cloning kit (Stratagene) and transforming into *E. coli*. Resulting clones were screened by amplified ribosomal DNA restriction analysis (ARDRA) using restriction enzymes RsaI and MspI. Clones with similar restriction patterns in both digests were designated to a common operational taxonomic unit (OTU). Full length (single strand) sequence was obtained by sequencing the same primers used for PCR amplification. Only a subset of the screened clones has

been sequenced thus far.

DNA Phylogenetic Analysis for trees

Full length 16S rDNA sequences (DV Bact-X and DV Arch-X, with X representing the clone number) along with high BLAST hits were initially aligned and checked for chimeras using the Sequence Alignment tool and Chimera Check tool, respectively, at the RDP website (Cole, 2003). Sequences were then manually aligned using the program GeneDoc, using only those positions that could not be unambiguously aligned. Phylogenies were inferred with the program TreeCon (Van de Peer and DeWachter, 1997) using the neighbor-joining method with a Kimura 2-parameter base substitution model, and results are shown in Fig. 5. One hundred bootstrap analyses were done for each tree. Out-groups used were *Aquifex pyrophilus* for the bacterial 16S rDNA tree and *Methanococcus jannaschii* for the Crenarchaeon 16S rDNA tree.

Bacterial Diversity

Clone libraries of resulting PCR product were constructed using a TOPO TA Cloning kit (Invitrogen) and screened by amplified ribosomal DNA restriction analysis (Moyer *et al.*, 1994). Clones were divided into groups that shared the same RFLP pattern, and plasmid from selected clones in each group was sequenced with the same primers used for amplification by the UW-Seattle Biochemistry DNA Sequencing Facility. Resulting full length sequences were checked for chimeras using the CHIMERIA_CHECK program provided by the Ribosomal Database Project-II (RDP-II) (Cole, 2003), which identified that almost all of the sequences were chimeric in nature. Sub-sections of these sequences that did not appear to be chimeric in nature were determined using the CHIMERA_CHECK and SEQUENCE_ALIGNER tools provided by RDP-II. These sub-sections of sequences were assigned to a taxonomic group using the SEQUENCE_MATCH tool provided by RDP-II and by BLAST (Altschul *et al.*, 1997) if this analysis indicated similarity to more than one group, the sequence was designated as "uncertain" taxonomic affiliation.

Electron microscopy preparation techniques

A variety of techniques were used, and where appropriate, a specific technique will be credited in the text. Some samples were fixed in several locations, at White Mountain Research Center, Bishop California, the University of Washington, and the center for Geomicrobiology, University of Oldenburg. Most samples were fixed with 4% glutaraldehyde solution in a 0.1M sodium phosphate buffer (pH 7.2) and then washed. Samples where noted, were additionally treated with 2% osmium tetroxide for a minimum of 4 hours or overnight. The samples were then dehydrated in an ethanol series as follows: 15% for 30 minutes, 30% for 30 minutes, 50% for 30 minutes, 70% for 30 minutes, or held overnight or for longer periods. Before viewing the samples were further dehydrated in 90% ethanol for one hour and finally two one-hour dehydrations in full strength ethanol. Selected samples were critical-point dried in a Balzers Union (CPD 010) apparatus and sputtered in gold, palladium, carbon or osmium as noted.

Scanning electron microscopy (SEM)

SEM analyses were done at Pacific Northwest National Laboratories, a Department of Energy facility in Richland Washington. SEM imaging was performed using a LEO 982 Field Emission Scanning Electron Microscope, which is an ultra-high performance scanning electron microscope with a resolution 1 nm at 30 kV and 4 nm at 1.0 kV. The high resolution is achieved using a Schottky field-emission source, a beam booster that maintains high beam energy throughout the microscope column, an electromagnetic multihole beam aperture changer, and a magnetic field lens. The beam path has been designed to prevent crossover of beam electrons. These features result in reduced chromatic aberration, improved beam brightness, and little beam energy spread. The Leo 982 has two secondary electron detectors: below lens and in-lens for high resolution imaging. The backscattered electron detector is solid state and is optimized for short working distances.

An Oxford ISIS energy dispersive x-ray microanalysis system (EDAX) was used for chemical analyses. A SiLi detector, having 128 eV resolution, is capable of light

element analyses, elemental mapping digital imaging, microscope automation, and can combine compositional information with a secondary electron image in a software package called CAMEO. Samples were coated with gold, platinum, or carbon depending on what elements were being analyzed.

Some samples were also examined in a Hitachi S-450 scanning electron microscope located at the Geomicrobiology Center, Institute for Chemistry and Biology of the Marine Environment, Carl von Ossietzky University, Oldenburg, Germany.

Transmission electron microscopy (TEM)

TEM-EDAX: High-resolution TEM analysis was carried out on a Jeol JEM 2010F microscope with a specified point-to-point resolution of 0.194 nm. EDAX is and Oxford Link system with ISIS analytical software.

Time-of-flight secondary ion mass spectroscopy (TOF-SIMS)

TOF-SIMS measurements were carried out on a Physical Electronics TRIFT II TOF-SIMS using a $^{69}Ga^{+}$ source in a high spatial resolution mode. In this mode, a 25 kV, 60 pA unbunched primary ion beam with a 30 ns pulse width is used. Although the mass resolution in this mode is approximately m/Dm = 1000, the primary beam diameter of <200 nm affords spatial resolution of features <250 nm for chemical and elemental mapping of relative (due to variations in secondary ion yield) surface abundance. The primary ion beam was rastered over a 100 ×100 micron area using 256 × 256 pixels. A low energy electron flood gun was used to prevent charging. We note that the ion yields vary with the matXPS measurements were made using a rix as well as the ion species over several orders of magnitude, preventing quantification. However, trends in the relative surface abundance can be determined for specimens with a similar matrix. TOF-SIMS is a surface sensitive method, which samples the top few atomic layers, providing information on surface chemical composition.

X-ray photoelectron spectroscopy (XPS)

XPS measurements were made using a Physical Electronics Quantum 2000

Scanning ESCA Microprobe. This system uses a focused monochromatic Al Ka x-rays (1486.7eV) source for excitation and a spherical section analyzer. The instrument has a 16 element multichannel detection system. The X-ray beam used was a 70W,100um diameter beam that is rastered over a 1.4 mm by 0.2 mm rectangle on the sample. The x-ray beam is incident normal to the sample and the x-ray detector is at 45° away from the normal. The survey scans were collected using pass energy of 117.4 eV. For the Ag 3d 5/2 these conditions produce FWHM of better than 1.6 eV. The high-energy resolution data was collected using pass energy of 23.5 eV. For the Ag 3d 5/2 these conditions produce FWHM of better than 0.75 eV. The collected data were referenced to an energy scale with binding energies for Cu 2p 3/2 at 932.62± 0.05 eV and Au 4f at 83.96.0± 0.05 eV. A BaO cathode was used as a source of electrons for neutralization providing 1 eV energy electrons @ 21 uA. Low energy Ar+ ions was also used for specimen neutralization.

$\delta^{13}C$ Stable Isotopes

Isotope analyses were performed by elemental analyzer-continuous flow isotope ratio mass spectrometry (EA/CFIRMS), using a Carlo Erba NC2500 EA interfaced through a Finnigan CONFLO II to a Finnigan Delta XL mass spectrometer. Sample isotope ratios were normalized in each run to the values obtained for an organic standard with known isotope ratios calibrated via sealed-tube combustions versus NBS-19 at δ13 C = 1.95‰ vs VPDB and for δ15 N = 0.0‰ vs Air nitrogen. Precision in this system averages ± 0.12‰ for organic standards and homogenous natural samples. Accuracy, as measured by including repeats of a natural sample of known isotopic ratio in each run, was ± 0.10‰. All isotope ratios are expressed in delta notation, or parts per thousand deviations from the Vienna PeeDee Belemnite (VPDB) standard, where:
$\delta^{13}C = \{[(^{13}C/^{12}C)\text{sample}/(^{13}C/^{12}C)_{VPDB}] - 1\} \times 1000.$

$\delta\ ^{56}Fe$ Stable Isotopes

Varnish samples were collected using a tungsten carbide Dremel tool bit to

eliminate iron contamination. Soil samples were scooped from the surface directly into ~100 gram plastic containers. Iron isotope compositions were measured using either a Micromass Sector 54 thermal ionization mass spectrometer or a Micromass IsoProbe, a multiple-collector inductively coupled plasma mass spectrometer with a magnetic sector. Isotope analyses by thermal ionization mass spectrometry (TIMS) used a ^{54}Fe-^{58}Fe double spike to correct for instrumental mass bias. External precision (1 SD) of TIMS isotope analyses is ±0.1%/ amu (i.e., $^{56}Fe/^{54}Fe$ is ±0.2%), as determined by replicate analysis of samples and ultrapure Fe standards.

The IsoProbe is a single-focusing multiple-collector inductively coupled plasma mass spectrometer that uses a hexapole collision cell to thermalize the ion beam so that the ion energy spread is reduced to ~1 eV. Additionally, the hexapole collision cell, with a mixture of Ar and H2 gas, eliminates or minimizes argide ions that are asobaric with Fe.

Fe-isotopic ratios are reported in conventional per mil notation:

$$\delta^{56}Fe = \{[(^{56}Fe/^{54}Fe)sample/(^{56}Fe/^{54}Fe)_{E\text{-}M}] - 1\} \times 1000.$$

Soil and coating mechanical and chemical separation

Soil was weighed and 40g were put in a 250-ml plastic bottle. To deionized water, 30 ml of 0.25 M NaCO was added and the level brought to 200 ml and shaken overnight. After removal from the shaker, the suspension was allowed to settle for 4 seconds (s) per cm of suspension depth and then the suspension was decanted through a 53μm porosity sieve into a large beaker. The remaining "sand" was resuspended in deionized water, for 4s/cm and was decanted through the sieve as before. The remaining sand was then transferred to the top of the 53μm sieve and the remaining silt and clay-size fractions washed through the sieve with deionized water. The sand was then oven dried at 60°C. The sand was then weighed. The clay and silt fractions were then transferred to a large beaker with 15cm of straight wall on the sides and diluted to the 15

cm mark with deionized water. The suspension was then mixed thoroughly and the allowed to settle for 12 hours. A "J-tube" connected to a vacuum flask was used to draw off the top 10cm of suspension. The remaining was rediluted and resuspended for 8 hours and the top 10cm once again drawn into the beaker. This process was repeated until no clay remained and the top 10cm was clear. The remaining silt fraction was then dried at 60°C and weighed in the same way as the sand fraction. The clay fraction was not dried. The clay fraction was instead concentrated by adding 10 M Mg Cl to flocculate and the suspension was allowed to settle. The supernatant was drawn off. Excess salts were removed from some of the samples by placing the flocculated clay in dialysis tubing and soaking in deionized water until a Ag NO_2 test showed no chloride. The clay weight was then determined by the difference between the original soil weight (oven-dry-basis) and the sum of the sand and silt fraction weights.

Soil and coating mineralogical analysis by X-ray diffraction

Semi-quantitative estimates of mineralogy of the sand silt, and clay-size fractions of the soil and coatings were determined using a Philips automated powder diffractometer (Model 3520) and Co-K radiation. Ordinary random powder sample mounts were used for the sand and silt fractions. Clay fraction samples were mounted on glass slides by the filter-membrane peel technique of Drever (1973) to give preferred orientation and allow estimation of the layer-structure clay-sized minerals by the intensities of their (001) reflections.

Sample Preparation

Sand, silt and clay particle-size fractions were treated to remove organic matter, carbonates and free iron oxides. Desert varnish samples were not treated to remove organic, carbonate and free iron oxides, as there was too little clay-size fractions present. For best results, the clay fraction was not dried, but rather maintained as a flocculated suspension from which aliquots (< 10 ml) were taken for analysis. A McCrone micronizing grinder or a diamonite mortar and pestle was used to grind a few grams of

the sand and silt fractions to approximately fine-silt size. Duplicate slides for random powder mounts for the sand- and silt-sized fractions were made using sample holders provided by Philips.

The clay-sized fraction was transferred in 3 aliquots containing 250 mg of clay-size material and put into separate 50ml centrifuge tubes. Twenty (20) ml of near-boiling 1 N Na Ac (pH 5) was added to each tube, mixed thoroughly and let sit for 5 minutes. To two of the tubes 3 ml of 10 N MgCl was added, and to the other tube (MacEwan and Wilson, 1980) 10 ml of 2 N NaCl was added. The clays were then thoroughly shaken to flocculate the clay minerals. The sample was then centrifuged until a clear supernatant was obtained. The supernatant was then decanted. The Mg-flocculated clays were washed with 1 N MgCl once and then twice with 1 N Mg (OAc) (pH 7), then once more with 1 N MgCl. The Na-flocculated clay was washed twice with 1 N NaCl and then the same procedure was followed for the Mg-flocculated clays, but KCl and KOAc (pH 7) was substituted for the Mg salts.

The final products were 250-mg subsamples of Mg-saturated (2) and K-saturated (1) clay-sized minerals were suspended in deionized H O. The samples were mounted for diffraction analysis (Drever, 1973). The suspensions were filtered onto a 0.47-um porosity, 47-mm diameter triacetate membrane supported by a fritted-glass Millipore filter holder. The filter cake was inverted onto a 1/8-inch thick Vycor glass slide and trapped air was removed between the clay and the slide by lightly rolling over the top of the membrane a couple times with a 1-inch diameter glass vial. The membrane was carefully peeled, leaving the sample on the Vycor slide. A few drops of a 10% glycerol solution was added (in 95% ethanol) to one of the Mg-clay samples on the slide immediately after removing the filter membrane. The ethanol was allowed to stand on the slide and evaporate leaving a glycerol-saturated clay. The other two samples were equilibrated overnight in a desiccator at constant relative humidity (30-54% is adequate) until diffraction analysis.

Diffraction Analyses

All diffraction data was collected by step-scanning (0.05° steps for 2-second intervals) and stored on computer for later determination of peak intensities. A simultaneous strip-chart recording of the data was made for immediate analysis. The qualitative mineralogy of the sand and silt fractions was determined by comparison of the diffraction data with standard patterns in the JCPDS powder diffraction file, using a computerized Johnson-Vand Search/Match algorithm when necessary. To quantify the amounts of each mineral present in the silt and sand fractions, the average mass attenuation coefficient (μ) of each sample was measured. An estimate of the mass attenuation coefficient for each pure mineral phase (μ_p) was also calculated from published values of elemental μ values and from assumptions about the composition of the mineral phase in the sample. Direct measurements of μ_p can also be made from standard samples of pure mineral phases. The absolute intensity of the diagnostic peak for each pure mineral phase (I_p°) was also measured from standard samples or estimated, if necessary. These values for μ, μ_p and I_p° were used, together with the observed intensity of the diagnostic peak I_p in the unknown sample, to calculate and the weight fraction calculated for the mineral in the unknown sample (wf) by the following equation:

$$wf = (\mu/\mu_p)(I_p)/(I_p^\circ).$$

The mineralology of the clay-sized fraction was determined by comparison of the intensities of the (001) reflections after various pretreatments. Because the sum of the weight fractions of all the phases present is equal to 1, it can be shown that:

$$\mu = I/[\text{Sum of } (I_p)/(\mu_p)(I_p^\circ)].$$

Assuming, as a first approximation, that the I_p° values for the clay-sized layered silicates

are identical, the weight fraction of each silicate (wf_{LS}) may then be calculated by the following equation:

$$wf_{LS} = (I_p / \mu_p)(1/[\text{Sum of } (I_p / \mu_p) \text{ for all LS present}]).$$

If no other minerals (e.g., quartz and feldspars) are present in the clay-sized fraction, the wf_{LS} value is the weight fraction of the clay mineral in the sample; otherwise, wf_{LS} pertains only to the weight fraction among the layer silicates in the sample. A more complex equation may be derived to handle situations where non-layer-silicate clay-sized minerals are present in significant quantities. The I_p values are corrected for the Lorentz-Polarization factor and for sample width smaller than the beam width before being used in the weight fraction calculation. As time permits, mass attenuation coefficients and absolute intensities for reference clay minerals are measured and the first equation can then, hopefully, be used.

Estimates of the illite, kaolinite, smectite, vermiculite and chlorite mineral fractions wre made. The following criteria were used to arrive at these estimates:

1) illites are considered to be the fraction that gives 10° spacings in Mg-saturated subsamples and does not expand with glycerol treatment
2) kaolinites are considered to be the fraction that gives 7.2° spacings that disappear upon heating to 550°C
3) smectites are considered to be the fraction of the Mg-saturated subsamples that gives 12-15° spacings at 30-54% relative humidity, expands to 17-20° upon saturation with glycerol, and collapses to 9-10° upon heating to 550° C
4) vermiculites are considered to be the fraction that gives 12-14 D spacings in the Mg-saturated subsamples, 10-12° spacings in the K-saturated subsamples, does not expand when treated with glycerol, and collapses to 9-10° when heated at 550° C
5) chlorites are considered to be the fraction that gives 14-15° and 7-7.5°

spacings that do not change with either glycerol or heating treatments

If treatment with ethylene glycol is desired (after the initial analysis of the Mg-saturated subsamples), the slides that have not received the glycerol treatment may be equilibrated in a desiccator containing free ethylene glycol at about 60° C for a minimum of 1 hour, preferably overnight. As discussed by MacEwan and Wilson (1980, p.222-226), however, glycerol saturation provides a better separation of the vermiculite and smectite phases than saturation with ethylene glycol. The glycolated samples are then analyzed as before to distinguish between the smectite and vermiculite fractions. The K-saturated samples, following the initial analysis, wee heated to 575° C for 1 hour before being re-analyzed to distinguish the chlorite from the kaolinite fractions.

The net result of this procedure should be a minimum of four x-ray patterns corresponding to clays that are Mg-saturated, Mg-saturated + glycerol (or glycol), K-saturated, and K-saturated +575° C heat. Interpretation of these four patterns will allow distinction among the five clay mineral groups listed above.

X-ray diffraction (XRD)

The X-ray diffraction apparatus used in this study was a Philips X'Pert MPD system (PW3040/00 type). The X-ray source was a sealed ceramic tube operated at 45 kV, 40 mA (LAMBDA = Cu Ka1, 1.5406 Å). The study specimens were examined in Bragg-Brentano parafocusing geometry on a 220-mm THETA-THETA goniometer radius using incident- and diffracted-beam soller slits (0.04 rad), automatic divergence and anti-scatter slits, and a 0.2-mm receiving slit. Wavelength selection was achieved with a curved graphite diffracted beam monochromator, and the detection apparatus was a Xe-filled sealed proportional counter.

The diffractometer was controlled using the Philips X'Pert software suite (X'Pert Data Collector, V1.3d). Analysis of the experimental pattern was accomplished using Jade V6.5.7 (Materials Data, Inc., Livermore, CA) and the Powder Diffraction File database (PDF-2, 2002 Release, International Centre for Diffraction Data, Newtown

Square, PA).

Electron microprobe

The University of Washington JEOL 733 Superprobe has four wavelength spectrometers (each with two diffracting crystals; TAP, LIF, PET, LDE1, LDEC) and one energy dispersive spectrometer. A range of beam currents (10's of picoamps to 1 microamp), accelerating voltages (5-50 kV) were used in analyzing samples for either quantitative, SEM imaging capabilities, secondary, backscattered, topographic, cathodoluminescence, X-ray, Y-modulated and mixed SEM. The secondary, backscattered, cathodoluminescence and x-ray signals were digitized in real time using the GATAN DigitalMicrograph system or the GELLER dPict program.

The JEOL 733 is computer controlled, featuring control of spectrometer positions and crystal flipping, measurement and display of X-ray intensities, and stage (X-Y-Z axis) positioning. The computer automation system is composed of the dSspect hardware and the dQant32 software from GELLER MicroAnalytical. The Automation systems runs from Windows 98 on a 450 MHz DELL Optiplex that has 128 Mb RAM and 10 Gb Hd.

Mineralogical thin section preparation

Centimeter-size chips of rock with coatings were cut normal to the varnish surface as described by Perry, 1979. The cut samples were the paced in containers, a mixture of Epon 812 (50 ml) and dodecenyl succinic anhydride (80 ml) was added to a mixture of Epon 812 (80 ml) and nadic anhydride (70 ml) in a ratio of 1:1. One-third of this mixture was combined 1:1 with propylene oxide, added to the sample containers and allowed to stand at 23° C for four hours. The remaining two-thirds epoxy was then combined with 6 ml BDMA accelerator and exchanged with the epoxy remaining n the sample containers. This was allowed to stand overnight before polymerizing for twelve hours at 60° C. Samples were then cut and polished to either 30μm for standard light microscopy or polished with 6μm diamond grit to ~20μm and then to ~10μm using 0.25

diamond grit.

Microbial culture techniques

Two mediums were primarily used for cultures of eukaryotic microorganisms. Colonies on MCF were placed on culture plates either by removal with a sterile needle or by placing small chips of rock directly on and in the agar medium. Krumbiein's K2 medium, as described in Gregory *et al.*, (1980) and Perry (1979) containing 0.2% Bacto Malt Extract (Difco), 0.05% yeast extract (Difco), 0.02% $MnSO_4$-H_2O, 1.5% agar (Difco) mixed with distilled water (pH 7) before autoclaving. A variant called K1 medium (Perry, 1979) containing 2g peptone, 0.5g yeast extract, 0.29 Mn So_4, 15g agar, 1000 ml distilled H_2O and buffered to pH 7 before autoclaving was also used.

Dual Beam

The LeoXB Crossbeam was used for making TEM sections. The system employs a ultra high resolution GEMINI field emission column with the high performance Canion FIB column into on system. The system has both EDS and WDS dedicated spectrometers. The SEM is a thermal emission type. The FIB is a Ga liquid metal ion source (LIMIS). The specimen stage is 6 axes adjustable with -10° to 60° tilt. SEM magnification is 20x to 900kx and the FIB is 600x to 500kx.

Total Organic Carbon (TOC) and Methodologies

Analysis

Samples were analyzed for TOC by Leco TOC by Humbolt Geochemical Services, Houston, Texas. Approximately one hundred milligrams of varnish powder and/or soil were analyzed. Leco TOC analysis requires decarbonation of the rock sample by treatment with hydrochloric acid (HCl). This is accomplished by soaking the sample in 6N HCl for 24 hours following by rinsing and filtering with water. Analytical data are checked selectively and randomly. Selected and random checks are completed on approximately 10% of the samples. A standard is analyzed as an unknown every 10

samples. Rock samples are combusted in the TOC oxidation oven ~ 900°C+. There can be some loss of organic carbon during the decarbonation rinse process prior to Leco TOC analysis. This generally is only relevant for very low maturity samples such as recent muds and soils.

Calibration

Instrument calibration is achieved using a rock standard. Its values were determined from a calibration curve to pure hydrocarbons of varying concentrations. This standard is analyzed every 10 samples as an unknown to check the instrument calibration. If the analysis of the standard ran, as an unknown does not meet specifications, those preceding data are rejected, the instrument recalibrated, and the samples analyzed again. However, normal variations in the standard are used to adjust any variation in the calibration response. The standard is considered acceptable under the following guidelines:

Tmax: ± 2°C

S1: 10% variation from established value (0.17 mg HC/g rock)

S2: 10% variation from established value (8.41 mg HC/g rock)

S3: 20% variation from established value (0.41 mg CO_2/g rock)

TOC: 10% variation from established value (3.11 wt.%)

***In vitro* protocol**

Palmer, Adams and Staley initiated a laboratory study in 1992 (unpublished) with the objective to grow varnish resembling natural varnish. Frosted glass squares 1 cm^2 were sterilized and placed in mounts in sterile petri dishes. Slides were then inoculated with bacterial strains listed in Table 1 in various combinations with soil extract medium (Table 2). Soil extract (SE) was prepared using soil from an area of desert-varnished rocks near Phoenix, Arizona. (Taylor-George *et al.*, 1983) Five hundred grams of soil was mixed with 500 ml of water, autoclaved at 121°C for 1 hr, and filtered through Whatman #2 filter paper. The medium was composed of 0.005% sodium

acetate, 0.02% $MnSO_4 \cdot H_2O$, 0.02% $FeSO_4 \cdot 7H_2O$, 0.0001% cupric citrate, 6.5% bentonite in soil extract, final pH 6.8 after autoclave sterilization. Slides were allowed to dry, washed gently with 0.01 N $NaHCO_3$ and placed in a desiccator to dry. After drying, 0.05 ml soil extract was added to each. Usually about 24h was required for the nutrients to dry, after which the slides were washed and dried again as described above. Uninoculated sterile controls received the same treatment. Iron, manganese, clays, other varnish components, and nutrients for the activity of bacteria were available in the soil extract. To the soil-extract medium either $FeSO_4 \cdot 7H_2O$, 0.005% or $MnSO_4 \cdot H_2O$, 0.005% or both together was added. The pH was adjusted to 6.9. Sterile montmorillonite (0.05ml of 1% suspension) was added at intervals to supplement the clay in the soil extract. The procedures described above (innoculation, drying, washing, and drying) were followed over 26 months with two 6-month periods when the slides received no treatment. The slides were heated to 80°C for 10-12 h and reinoculated 7 times over the 26-month period. There were 72 cycles of feeding, drying, washing, and drying. The use of sodium bicarbonate between applications of media/bacteria possibly raised the pH (the ending pH increased to ~9.4). It is possible that the pH increase precipitated the coatings, as all samples analyzed showed Ca in the deposits. Fig. 5 shows large amounts of Ca in Inv 7 (inoculi had 4 strains of bacteria) and a spheroid deposit (Fig. 4, Inv 12) enriched in Ca and somewhat enriched in Mn and containing Mg, Na and lesser amounts of Si, P, S, and Cl. Inv 12 had Mn and SE but no bacteria added. Light and humidity were not controlled during the ~ two year experiment.

Varnish collection technique

General

Desert varnish samples were primarily collected *in situ* in the field. A Dremel tool with various bits was used. Selected samples were transported to the laboratory for processing and/or analyses. Aseptic techniques were used at all times. Sterile gloves were worn. Tools and brushes were autoclaved and bits were treated with ethanol in the

field between sample grindings. Loose surface material was brushed from the surfaces before grinding. Grinding was done with light pressure in an attempt to collect as much varnish and as little substrate rock material as possible. Collecting varnish in the field prevents contamination from soils during transport. Each analysis requires variations in collection technique as listed below.

Iron isotopes

Varnish samples were ground in the field using tungsten carbide (TC) bits. The bits contain no iron however, generally penetrated deeper then bits used for other analyses. An initial sample was collected using an aluminum oxide grinding bit and is noted were results are shown.

Surface analyses (TOF-SIMS and XPS)

Small chips were collected in the field and placed in glass containers and brought to the laboratory. Initially rocks were brought to the laboratory and chips removed. The rocks were placed in zip lock bags.

Biofilms

Small chips of varnished material were collected in the field and were fixed with 4% glutaraldehyde solution in a 0.1M sodium phosphate buffer (pH 7.2) for 4 hours and then washed in sterile water. Samples where noted, were additionally treated with 2% osmium tetroxide for a minimum of 4 hours or overnight. The samples were then dehydrated in an ethanol series as follows: 15% for 30 minutes, 30% for 30 minutes, 50% for 30 minutes, 70% for 30 minutes, or held overnight or for longer periods. Before viewing the samples were further dehydrated in 90% ethanol for one hour and finally two one-hour dehydrations in full strength ethanol.

Microcolonial fungi (MCF)

MCF were plucked from surfaces using sterile #25 syringe needles and also were

removed by placing rock chips in glass containers with sterile water and ultrasonicating at low speeds. The process appears to dislodge some MCF from surfaces.

Mycosporins

Liquid Chromatography mass spectroscopy (LC/MS) and LC/MS/MS analyses were performed using a Thermo Separation Products HPLC system with a UV-VIS detector and interfaced with a Thermo Finningan LCQ ion trap mass spectrometer. Sample volumes of 20 μL were injected onto a Lichrospher 100RP-18 HPLC column (5μm, 4.6 x 220 mm). The mobile phase was 0.2% aqueous acetic acid/methanol (94:6, v/v) at a flow rate of 0.7 mL/min. Traces of methanol were needed to stabilize the C18 phase of the column. The UV detector was set to monitor at a fixed wavelength of 310NM.

Collections sites

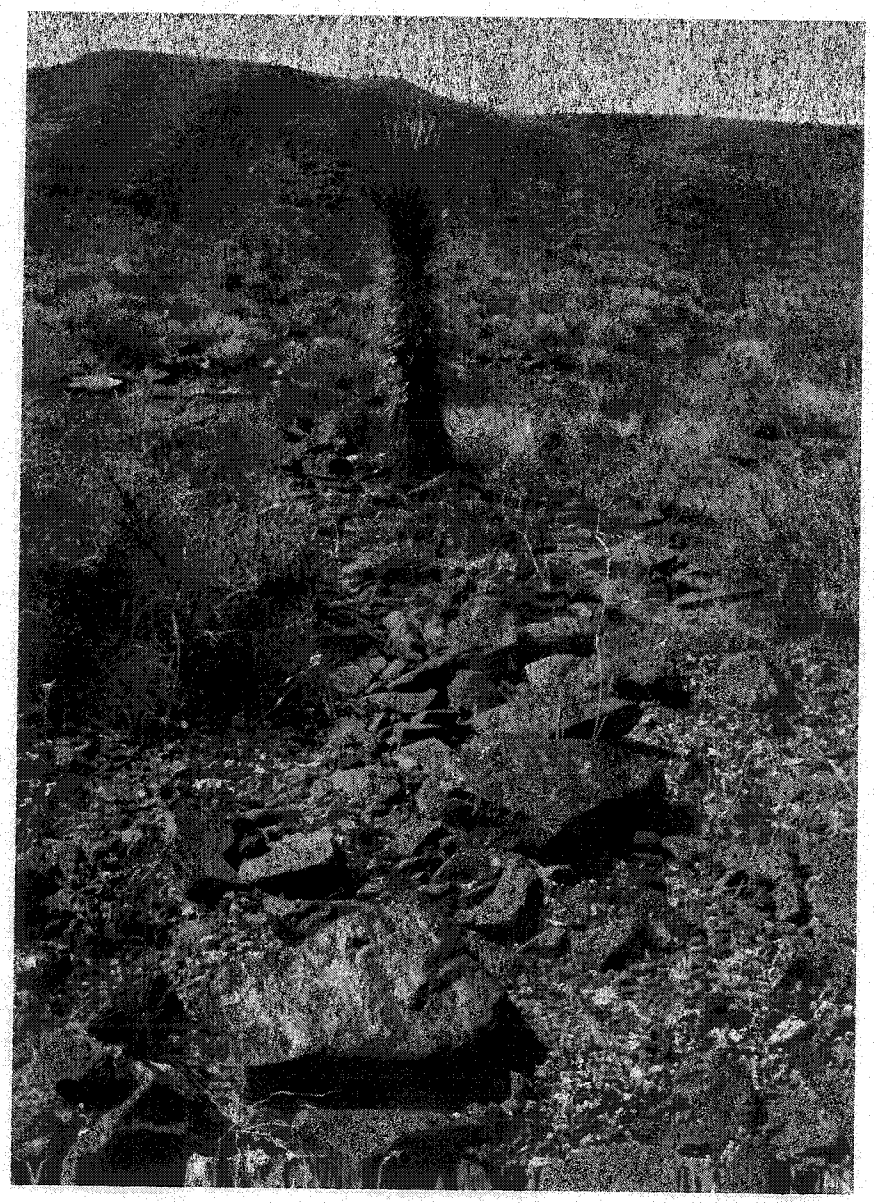


Figure 2-1. Desert varnish coated rocks in Death Valley National Park, west of Panamint Springs

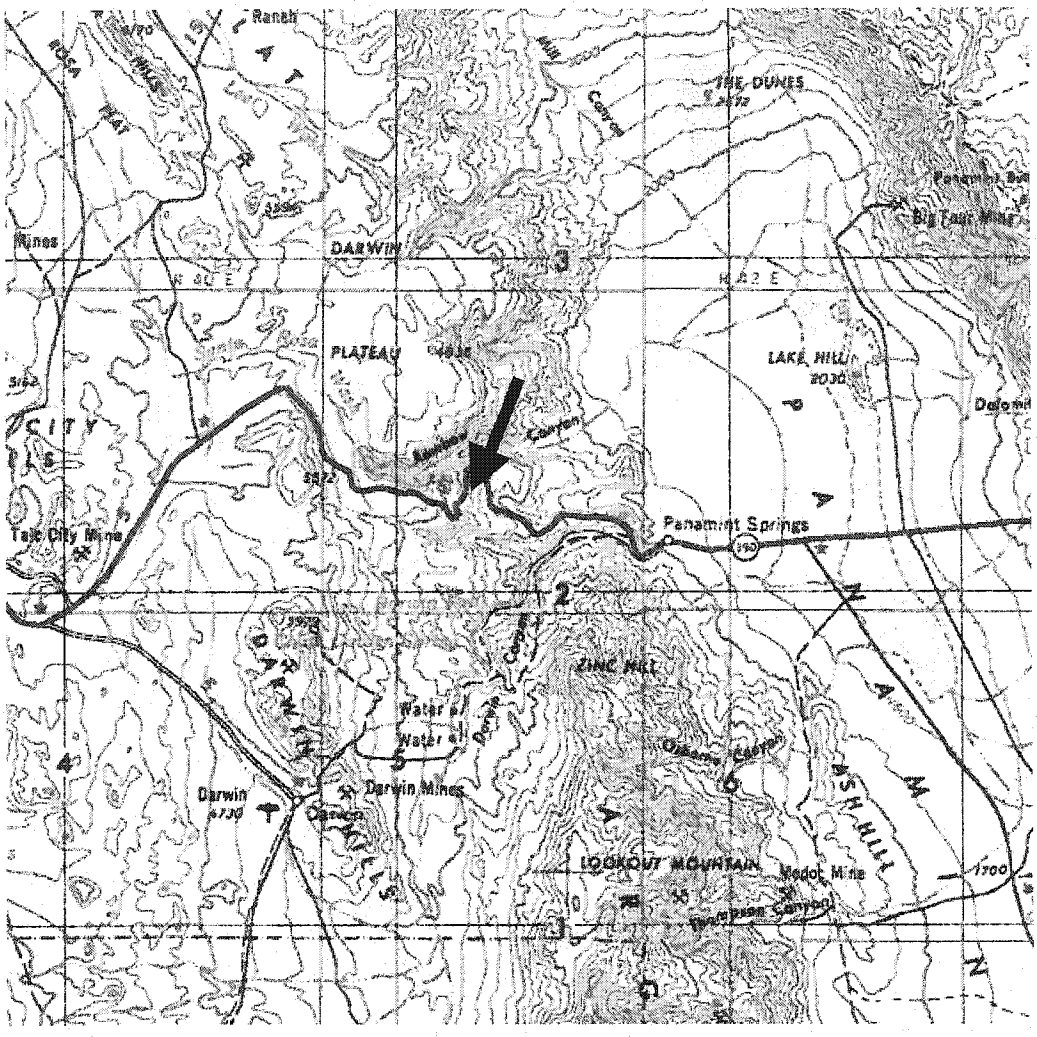


Figure 2-2. Panamit Springs,, California collection site ~14 miles west of the town of Panamint Springs.

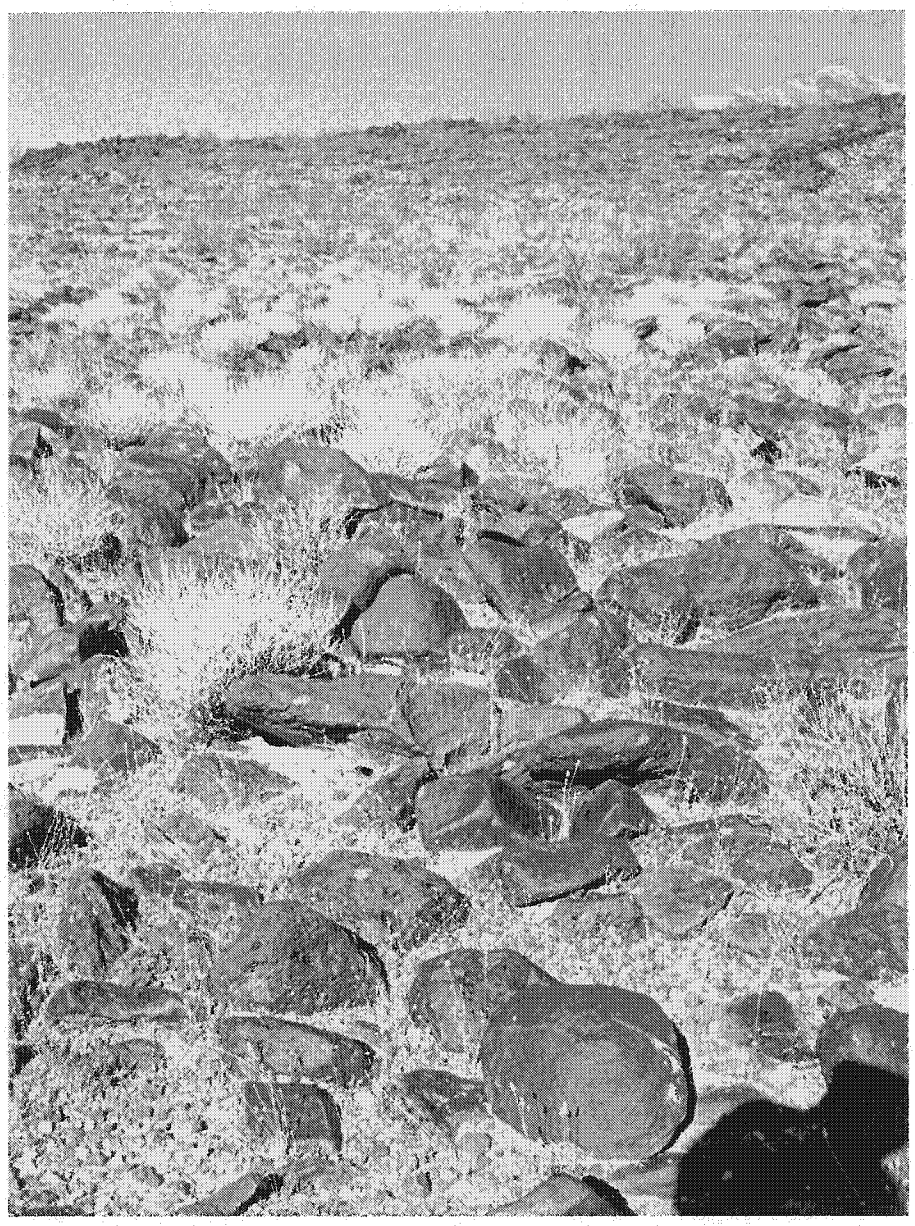


Figure 2-3. Site "X". 3 miles west of eastern Death Valley National Park Boundary Hwy 190 on north side of the road.

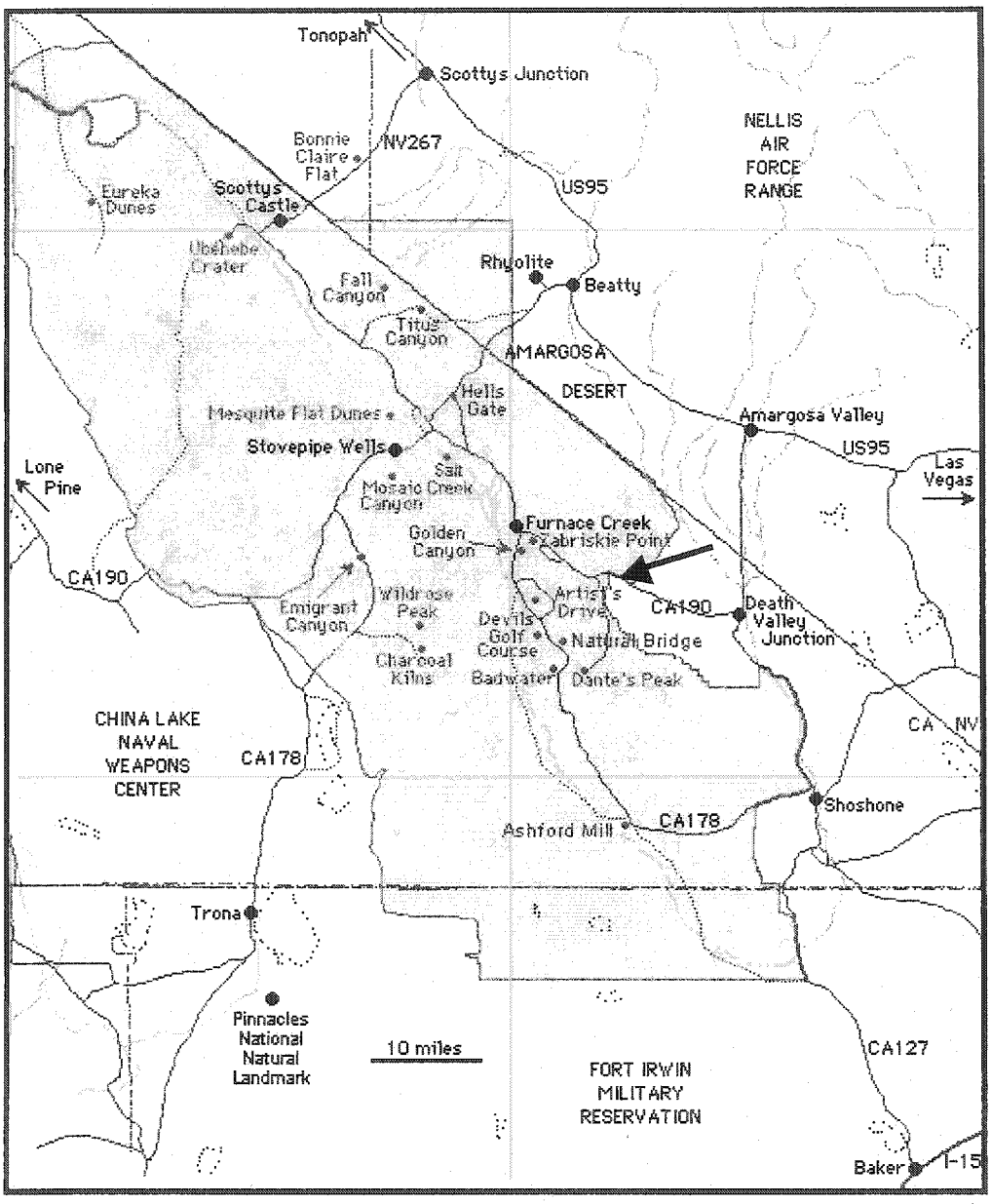


Figure 2-4. Site "X" and site "Y", Death Valley, California.

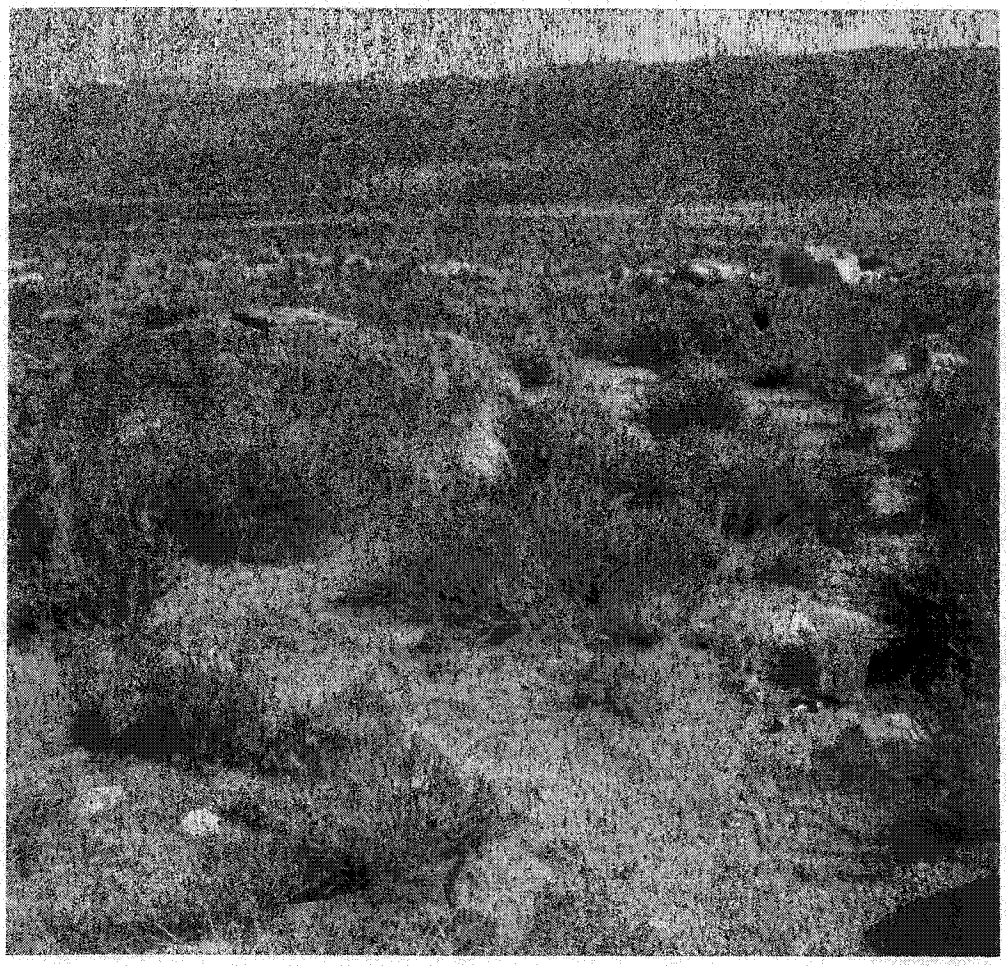


Figure 2-5. Bishop, California collection site, located 5 miles south of town on Gerkin Road west of Hwy 395.

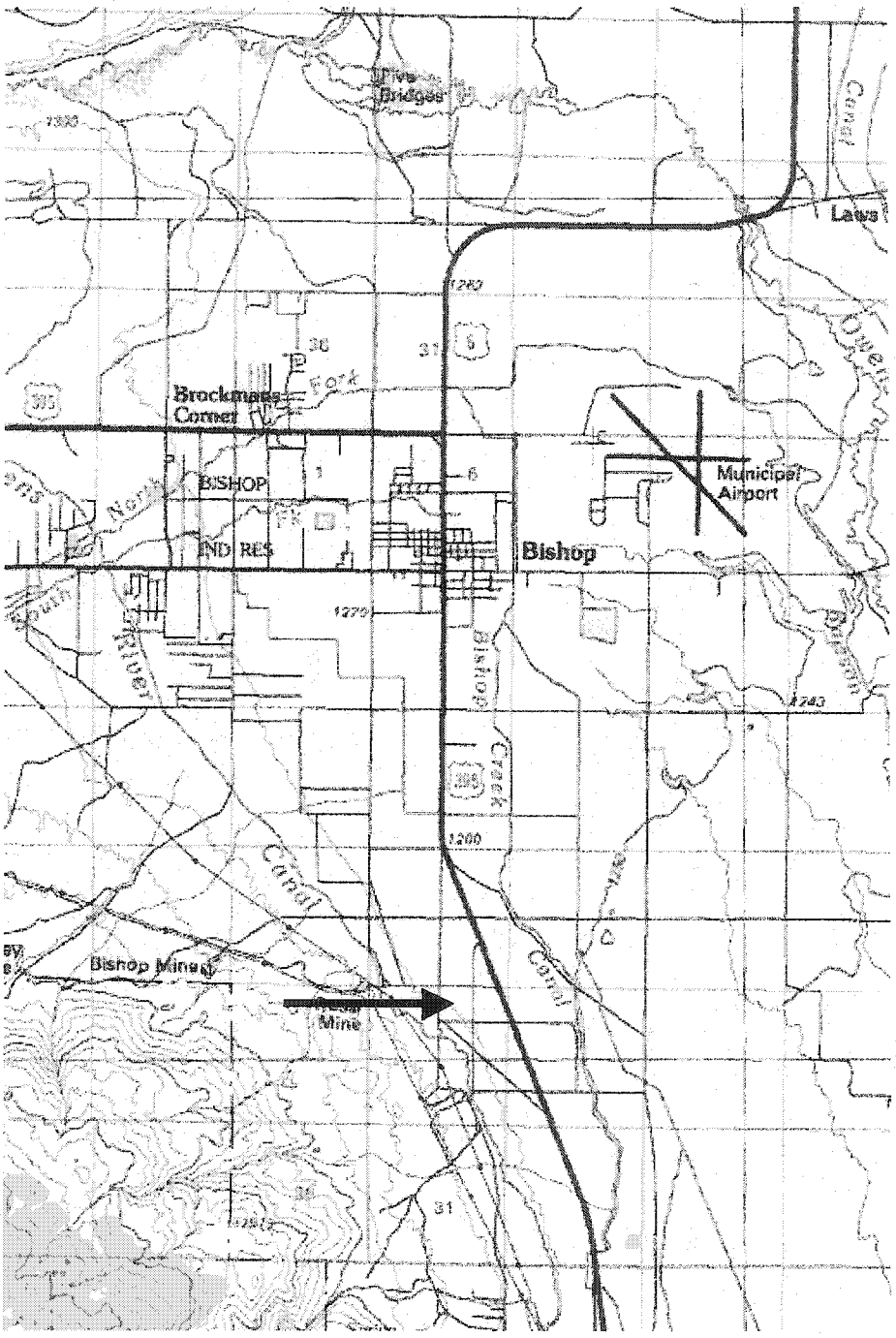


Figure 2-6. Bishop,, California collection site ~3 miles south of the town.

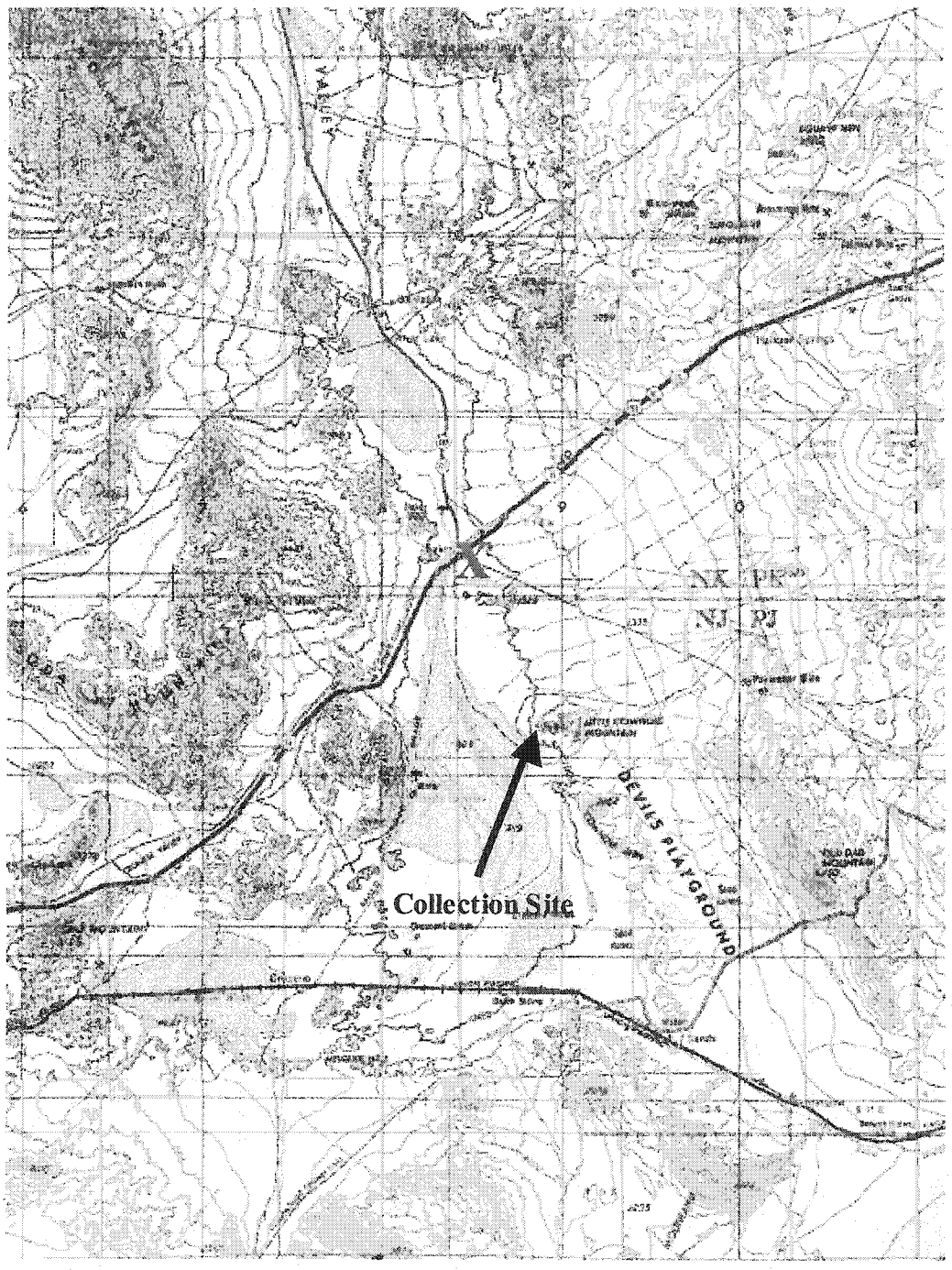


Figure 2-7. Baker, California collection site ~14 miles south of the town of Baker in the Mojave National Preserve.

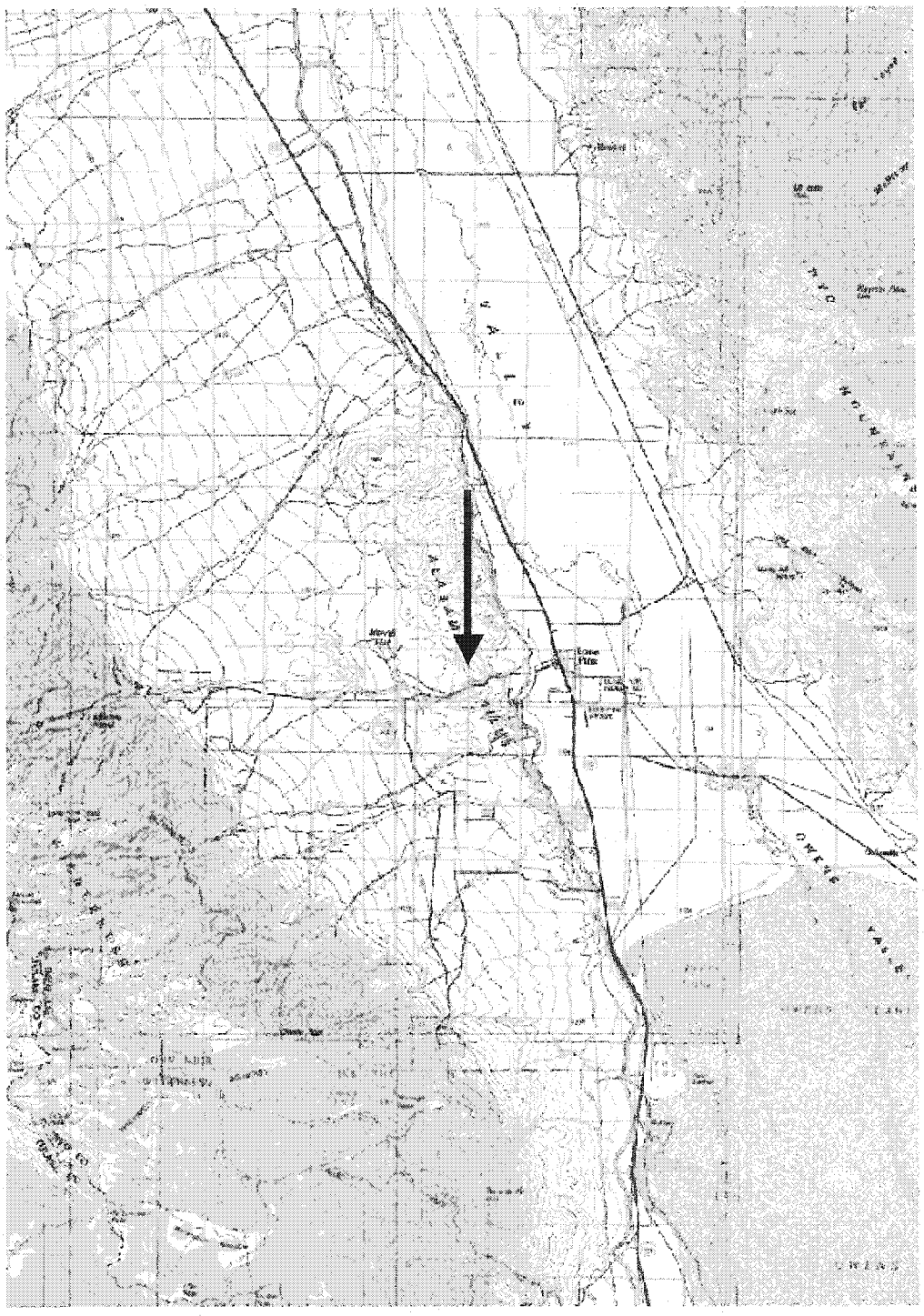


Figure 2-8. Lone Pine,, California collection site ~5 miles west of the town.

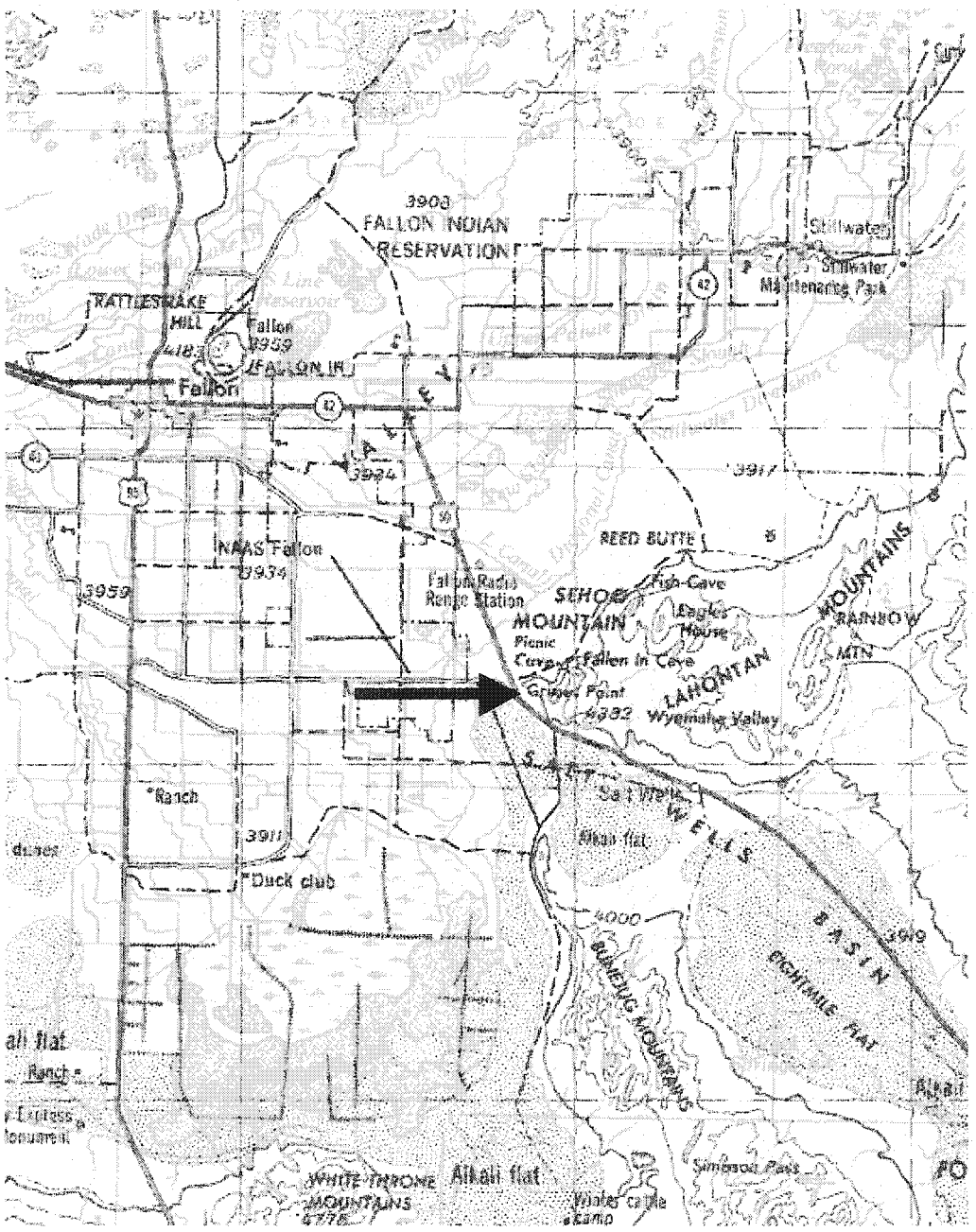


Figure 2-9. Grimes Point,, Nevada collection site.

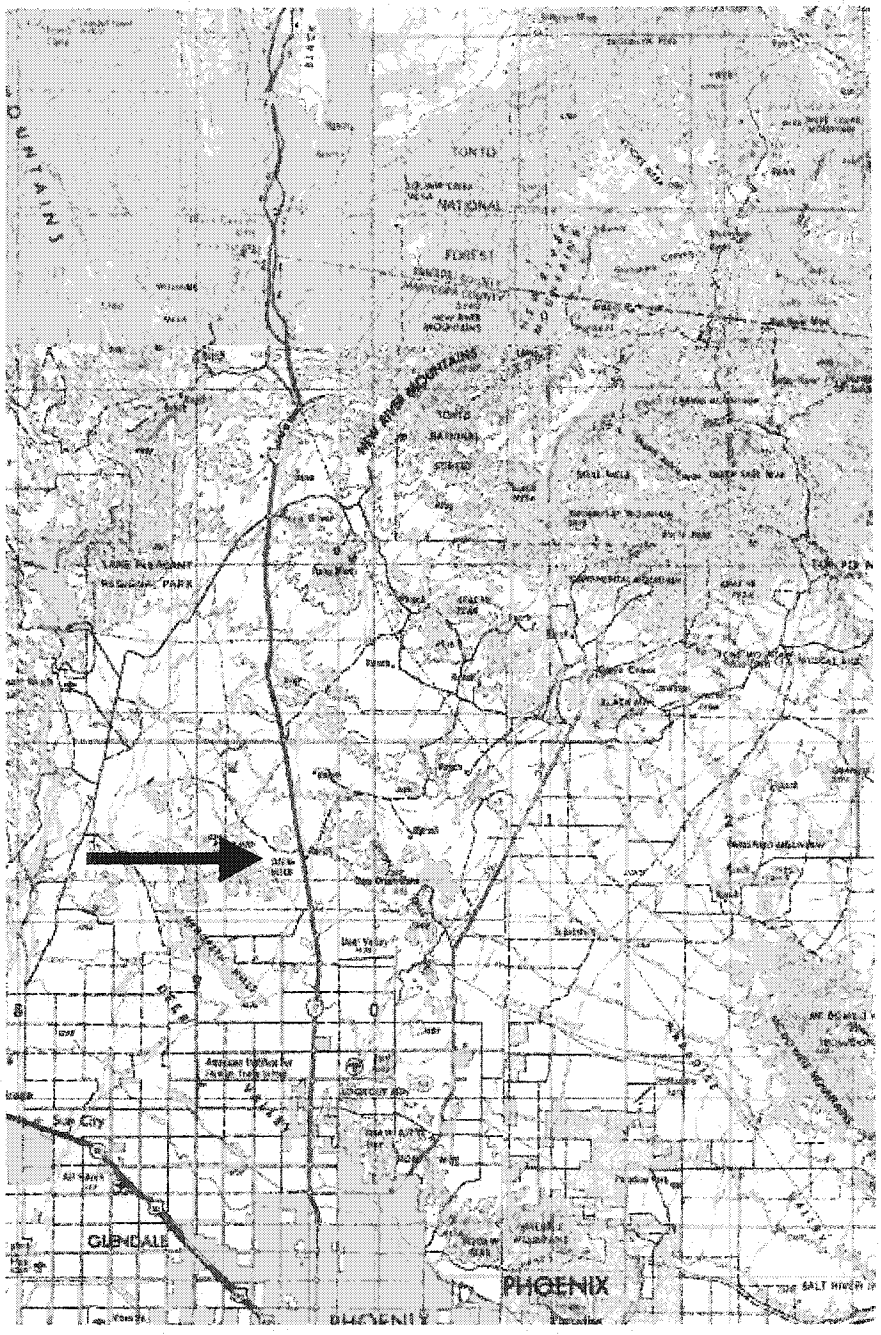


Figure 2-10. Deem Hills, Arizona collection site.

Gobi Desert collection locations

Site 1: Samples A and B

July 26, 2003

~100 m north of GPS coordinates N43°57'11.2" E 109°20'25.9" approximately 910m elevation.

Area description:

Site 1 is a gently sloping east-trending ridge. The ridge is steeper to the west and east of the samples, with a flat section approximately 50 m long. Two samples were taken approximately 10m apart on the side of this flat ridge, which slopes downhill to the northeast.

The site is approximately 20 m higher than the surrounding flat lands and might be exposed to more wind then the lower areas. Rocks here and throughout the Gobi Desert were scoured of varish on wind-exposed slopes.Ventifacts were common on the surface of desert pavement rocks and rocks appear scoured. Varnish coats appeared more developed in wind-sheltered locations. are from an exposed dyke in the southern Gobi (approx. N43°45' E109°55').

Site is within 50m of a recently active fault zone striking ~40°E of N. In the immediate area of this site the rocks are of volcanic origin, of uncertain age (mapped as lower Permian?) composed primarily of ryolites and breccias. To the southwest (downhill), on the other side of the fault are upper Ordovician marbles. Both samples are chert however.

Sample A:

3° Slope, 30° aspect.

Horizon: Av (visible vesicles, lighter in color) is 3-4 cm deep. The soil horizon is filled with angular clasts of the same rock type as the surface pavement.

See 4 pictures labeled sample A.

Sample B:

2° slope, 40° aspect.

Horizon: Av is is 2-3 cm deep.

See 1 picture labeled sample B.

Site 2: Samples C and D

July 30, 2003

Area description:

Site 2 is a wide pediment on top of soft cretaceous clastic sedimentary bedrock. The pediment is cut by a wash, approximately 60 m wide and 30 m lower in elevation. The pediment had mostly smaller clasts (<5cm) of mixed lithology. Clasts as predominately Precambrian gneiss quartzite.

Sample C:

Collected above the wash and on the older surface, it appears more exposed to the wind and ventifacts are on the surface.

GPS coordinates: N44°03'30.6" E109°36'27.5"

1.5° Slope 145° aspect.

Horizon: 5cm Av (see photo labeled "soilvesicles", then >10 cm of what Karl Wegmann called Bkw- fines with CaCO3 nodules. There are far fewer clasts than at site 1, perhaps 20% of the material in the Av and Bkw horizons.

See 3 pictures labeled sample C.

Sample D:

Taken ~100 m south, down in the wash on a younger surface, perhaps less exposed to the wind. The surface is bordered on either side by dry channels in the wash (10 m on either side of the sample).

GPS coordinates: N44°03'29" E109°36'26.2" Elevation 832m.

3° Slope 160° aspect.

Horizon: 4cm Av (see photo labeled "soilvesicles", then poorly consolidated Cretaceous clastics. Again, about 20% of the material in the Av horizon is rock, the rest is fine sediment.

See 5 pictures labeled sample D.

Notes for Mongolian Varnish Samples collected by
Bob and Chris Carson
September 7, 2003

CHAPTER 3 INORGANIC CHEMISTRY AND MINERALOGY

Thus, discoveries in one field nourish and fertilize discoveries in totally unrelated fields. The whole is more than the sum of its parts.

Michio Kaku

Inorganic data are presented in three category divisions: elemental chemistry, mineralogy, and inorganic chemistry of microcolonial fungi (MCF). The elemental chemistry of desert varnish top and bottom coats was analyzed using scanning and transmission electron microscopy, both with x-ray analytical (EDS). Electron-microprobe analyses of thin sections, cut normal to varnish surfaces, were used to investigate both varnish coatings and the varnish substrate rock. X-ray photo electron (XPS) analyses provide elemental analyses also, but of outer surface mono-layers with superior capabilities for detecting light elements such as carbon and nitrogen. Time-of-flight secondary ion mass spectroscopy (TOF-SIMS) measures outer monolayers as well but includes both positive and negative ions. The interpretation of iron stable isotopic data is subject to debate, however, preliminary results are presented along with the above inorganic analyses. Mineralogical data are primarily x-ray diffraction analyses (XRD) but, with a new twist, mechanically separating the components into three size fractions: sand, clay, and silt sized particles. X-ray analyses of bulk powder were performed but the separation, as described in the methods, provides more detailed spectra.

Background

Investigators starting with Humboldt and Darwin in the early 19th century have extensively studied the elemental chemistry of rock coatings. A two-fold question has intrigued researchers from the earliest days: what makes desert rocks black on exposed surfaces yet red on the undersides? The answer as to what elements blacken surfaces was easily answered by early chemical analyses showing high concentrations of oxides of

manganese and iron (Darwin, 1871). The question, however, that still intrigues investigators, concerns, how manganese is concentrated orders of magnitude over soils and substrate rocks. Iron is concentrated over desert soils also, but to a lesser degree (3-4 times over soils). Potter (1979) and Potter and Rossman (1977) stated that topcoats were composed of clays as are undercoats, but bottom coats have little manganese. Data presented here will show that undercoats from the Mojave Desert have varying amounts of iron making them red, and are composed primarily of clays supporting Potter and Rossman's classic study. Topcoats examined, however, have fewer clays, substantially differing from their study.

The dominant chemical elements in varnishes are Si, O, and Al, while elements that are frequently present but in lesser and variable amounts are Fe, Mn, C, Ca, Na, K, N, P, Ti, Mg, S, Ba (Perry and Kolb, 2004a; Dorn, 1998). Scientists have described the physical and elemental characteristics of desert varnish (White, 1924; Engel and Sharp, 1958; Hooke *et al.*, 1969; Potter, 1979; Perry, 1979; Jones, 1991; Dorn, 1998; Liu, 2003). It is well documented that desert varnish and silica glazes are rock coatings with source materials derived from external sources and not rock substrates (Perry, 1979).

Hooke *et al.* (1969) were the first to use the electron probe to study the chemical variations *in situ*. Their analyses showed that the chemical composition of manganese varied on rock surfaces and sides, and that little manganese was present (trace -0.6 wt % oxide) in bottom-coats. Allen (1978) and Perry and Adams (1978) also used the electron probe to characterize varnish chemistry and mineralogy. This analytical technique, as well as backscatter x-ray analysis with SEM's, has been used extensively with a comprehensive review presented in a book dedicated to and bearing the title: *Rock Coatings* (Dorn, 1998).

White (1924) noted that varnish coatings were not uniformly distributed and that the chemical composition of varnish varies not only from rock to rock but also from point to point on a single rock. In 1978, Perry and Adams also observed that varnish nucleates, grows vertically and laterally. They also presented evidence that varnish is

composed of micron-scale layers(Figure 1-4) and that manganese is concentrated in dark layers observed in ultra-thin sections (~<10 μm). The notion that varnish is not a homogenous mixture of oxides is now clearly established. White (1924) and later Hooke (1969) observed that coatings are in low spots on rock surfaces as shown in the SEM micrograph (Figure 1-5).

The following elements have been detected in most but not all varnish coatings: H, N, O, C, Si, Al, Fe, K. Ca, Mn, Ti, Mg, Na, P, S, Ba, and Pb. Less frequently, small amounts of Cr, and Cl were detected by electron microprobe, by SEM-EDS, by TOF-SIMS, and by XPS as described in following sections. Lakin *et al.* (1963) removed varnish coatings from substrates using ammonium oxalate and oxalic acid, activated by UV light. The residues were concentrated and combusted and the composition obtained analyzed by either wet chemical and/or spectrographic analyses. They identified Mn, Fe, Mg, Ca, Ti, Ag, As, B, Ba, Be, Cd, Co, Cr, Cu, Ga, La, Mo, Ni, Pb, Sb, Sc, Sn, Sr, V, W, Y, Zn, Zr and looked for associations with nearby ore provinces. It was suggested that this information might lead to a possible prospecting tool for ore bodies. Much of the correlation of certain elements depends on their association with manganese, a known scavenger of ions. Thus, elements such as Ba, La, Mb, Ni, Pb, and Y were not considered meaningful indicators of mineralized zones. It was found, however, that low manganese content correlated with increased beryllium.

Potter and Rossman (1977) calculated the clay/oxide component by removing Fe and Mn with sodium dithionite. The balance remaining was assumed to be clay minerals, "assuming that all the silicon was present in clay of the overall composition found in the extracted varnish." (Potter and Rossman, 1977, p.1447). Using this same method, 10% consisting predominantly of iron, was removed from bottom red-coats. Their conclusion was that clays comprise ca. 70% of black topcoats and ca. 90% of bottom red-coats.

The concentration of manganese in varnish has attracted the attention of biologists and sparked an ongoing debate as to whether Mn is enhanced microbially (Krumbein and Jens, 1981; Staley *et al.* 1982; Taylor-George *et al.*, 1983; Palmer *et al.*,

1986; Dorn, 1989; Adams *et al.*, 1992) or by an inorganic mechanism (Elvidge and Iverson, 1983; Smith and Whalley, 1988). Iron and manganese ratios are relevant to paleoenvironmental questions (Dorn, 1984, 1989; Bierman and Gillespie, 1991, 1994; Broeker and Liu, 2001; Liu, 2003).

While in the past, concentrations of manganese and iron have been the most intriguing avenues for investigators, silicon and aluminum, the most common elements have been largely overlooked since the investigations of Potter and Rossman (1977), and Potter in his doctoral dissertation (1979). They proposed the concept that aluminosilicates (clays), are the primary silicate components of southwestern US desert varnishes. Russell Potter's careful analyses, using IR spectroscopy and supplemented with XRD, showed that clays were present, which explained the large amounts of silicon. But, no XRD data was presented in the spectral area where silica (opal) appears. The mineralogy will be discussed in the next section, but Potter and Rossman's explanation became a fundamental tenant for varnish mineral composition, and most investigations have started with the assumption that clays cemented together with oxides, make up the bulk of desert varnish.

Non-metal elements including silicon, P, S, O, and Cl are found routinely in desert varnish coatings. Other non-metals have been detected, including B, Ge, As, Se, Br, F, Br, but are not considered in this study. Hydrogen, C, N are not discussed here since they are the elements of organic biochemistry and are the subject of Chapter IV. Oxygen has a unique role in both inorganic, organic processes, and to bio-compounds. Silicon in the form of silica compounds of $Si(OH)_4$ is abundant in soils and minerals. Oxygen-17 anomalies in sulfates have been used by Bao *et al.* (2001) to show that the O has an atmospheric component and is at least partly derived from aerosols

Elemental analyses

Elemental analyses findings

Area scans of desert varnish topcoats (SEM) show the heterogeneity of

composition. Spot analyses shown in (Figure 3-1) suggest that varnish surfaces have areas enriched in elements such as Si, Al, Mg, Ti, Ba, Mn, and Fe, but, also areas with nearly pure silicon. Backscatter electron techniques shows that the heterogeneous and homogenous Si areas are distributed randomly over surfaces.

The analysis of varnish powder (obtained by grinding the surface as described in Chapter 2) using TEM, shows that varnishes are composed of diverse minerals, similar to Krinsley, 1998. Individual areas are rich in Si (Figure 3-19), and also other silicates (Figure 3-9), and oxides (Figure 3-11). Bulk powder TEM analyses suffers from not knowing whether particles being examined are from bulk varnish coating powder, or from the varnish matrix, detrital grains within the varnish matrix, or contamination from substrates.

XPS and TOF-SIMS are bulk analyses due to larger areas scanned on surfaces (>5μm). However, the positive aspect is analyses of outer monolayers. Outer monolayers can and be removed *in situ* (~10 nm) revealing subsurface chemistry (Table 3-1). Silicon as in SEM analyses (Figure 3-1) is a dominant element (Table 3-1). However, carbon and nitrogen were also found in greater quantities (Table 3-1) than suggested by less quantitative SEM (Figure 3-3).

SEM with EDS

SEM with EDS analyses of desert varnish topcoat from Baker, California (Figure 3-1,Figure 3-2, and, Figure 3-3) show that varnish surfaces are heterogeneous, but with areas composed of nearly pure silicon. Aluminum is present in amounts that vary significantly but normally lower quantities are detected than in topcoats. Figure 3-4 is an area scan in which silicon dominates the EDS spectrum, with smaller quantities of aluminum detected. Potassium and sodium are also detected.

SEM with EDS analyses of red undercoats from Baker, CA, also show the principal element to be silicon (Figure 3-4 and Figure 3-5). Apparently, only small quantities of iron are necessary to tint layered undercoats (Figure 3-6 and Figure 2-7) undercoats red. Undercoats (Figure 1-2) appear bright red but often are just at detection

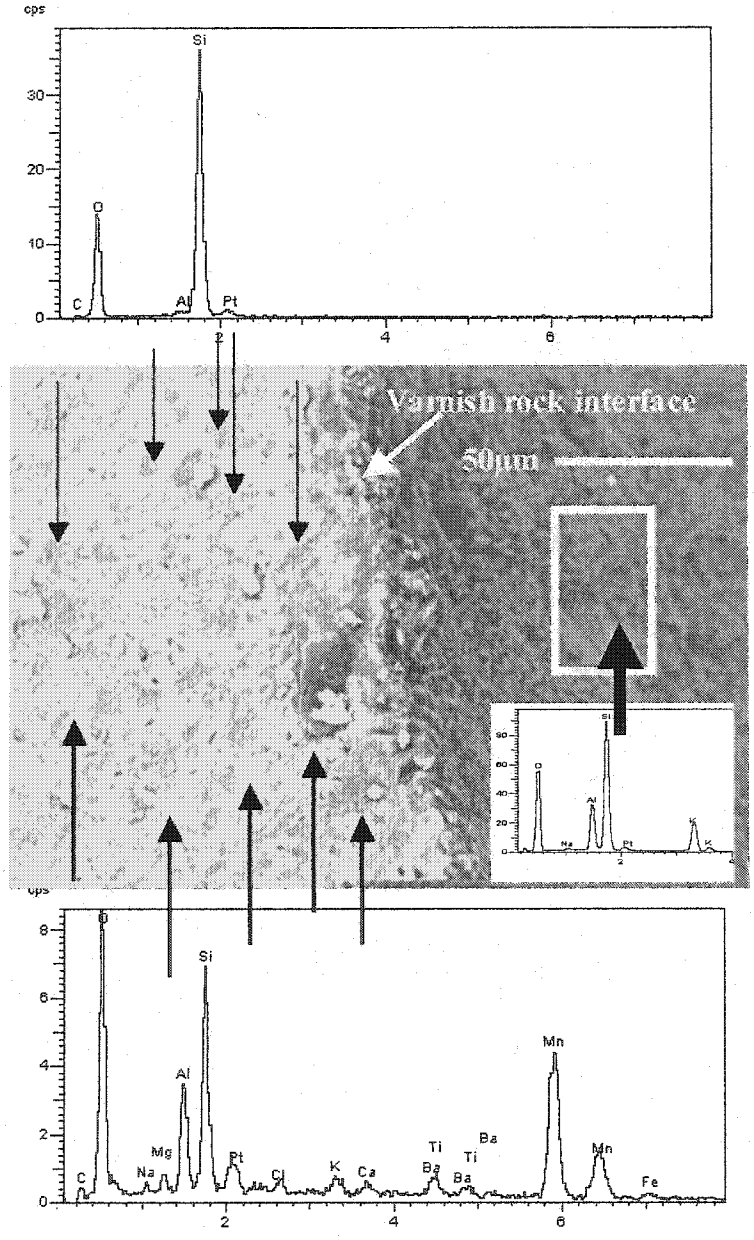


Figure 3-1. SEM with EDS,backscatter, and secondary imaging. Lighter elements appear darker and heavier elements appear lighter. Top EDS shows areas that are lighter are composed almost exclusively of Si. Lower EDS shows heavier elements. Lower spectrum is oxide-rich and also has relatively more carbon. The area EDS insert is of the rock substrate and, using electron microprobe, the rock analyses is consistent with phonolite.

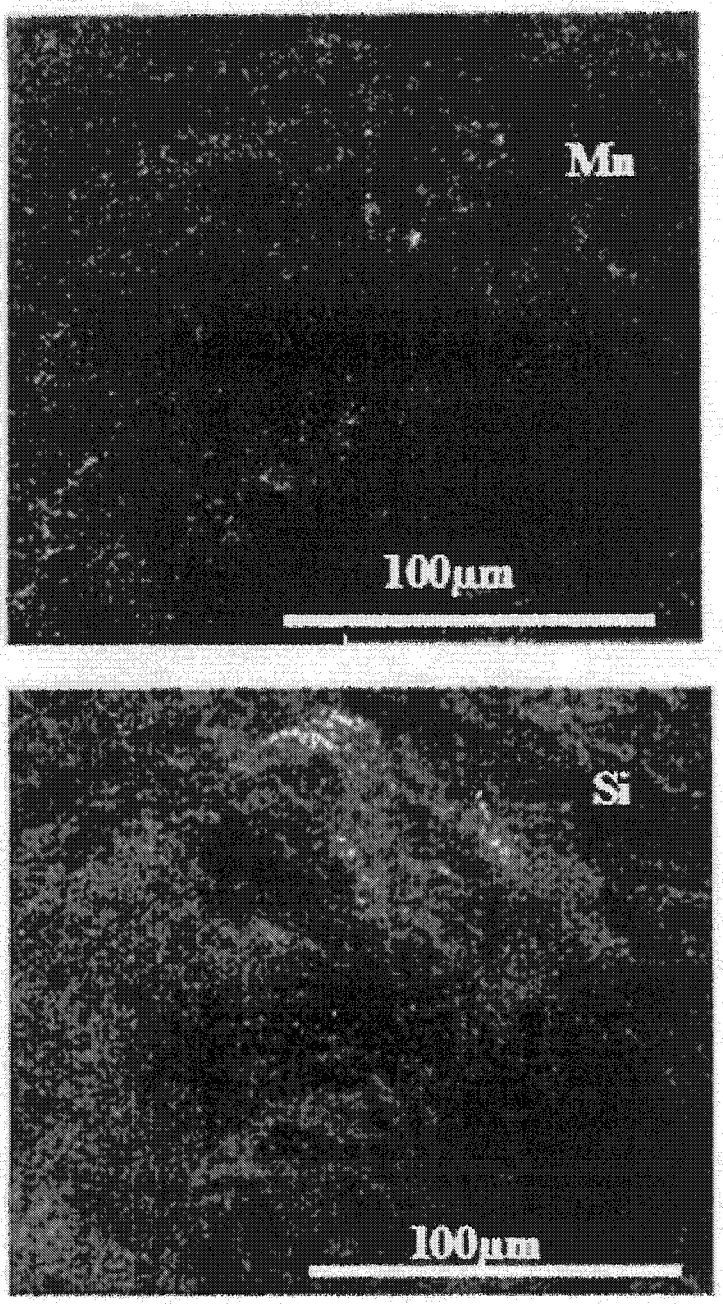


Figure 3-2. TOF-SIMS showing positive ions maps for silicon and manganese on the outer monolayer surface of desert varnish topcoat, sample#120 Death Valley, California. TOF-SIMS detects elements in the outer few monolayers. Silicon exceeds manganese in the outer (most recent) deposits in this sample. This sample result is typical for all desert varnish samples tested using TOF-SIMS. Analysis performed by Daniel Gaspar at the Environmental Molecular Sciences Laboratory, Pacific Northwest National Laboratories, Richland, Washington.

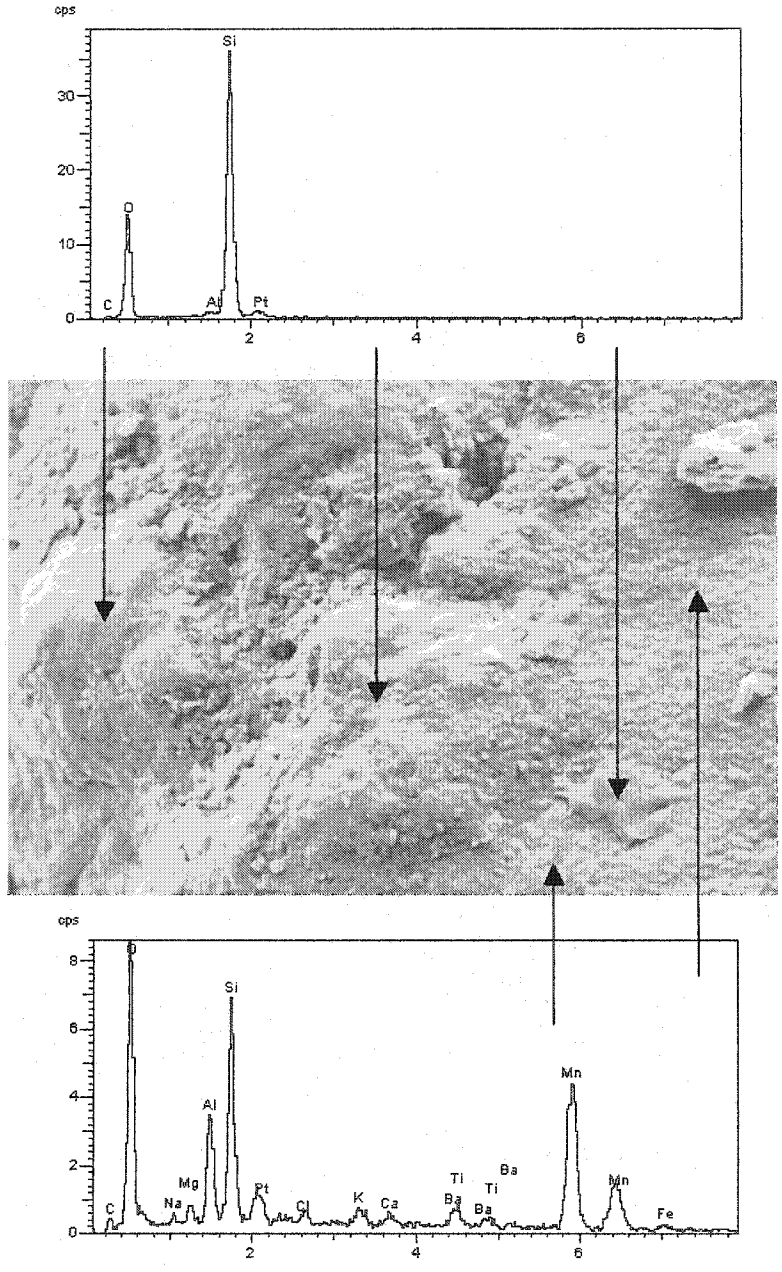


Figure 3-3. SEM image of desert varnish surface Baker, California. EDS analyses show silicon-rich areas (EDS above) and oxide enhanced regions (EDS lower). The silicon rich areas (top EDS) have detectable signals only for C, O, Al, other than Si. The Pt signal is for platinum that was used to coat the sample. The spectra vary for the different spots but, the spectra shown are representative for all spots analyzed including the ones depicted by the black arrows.

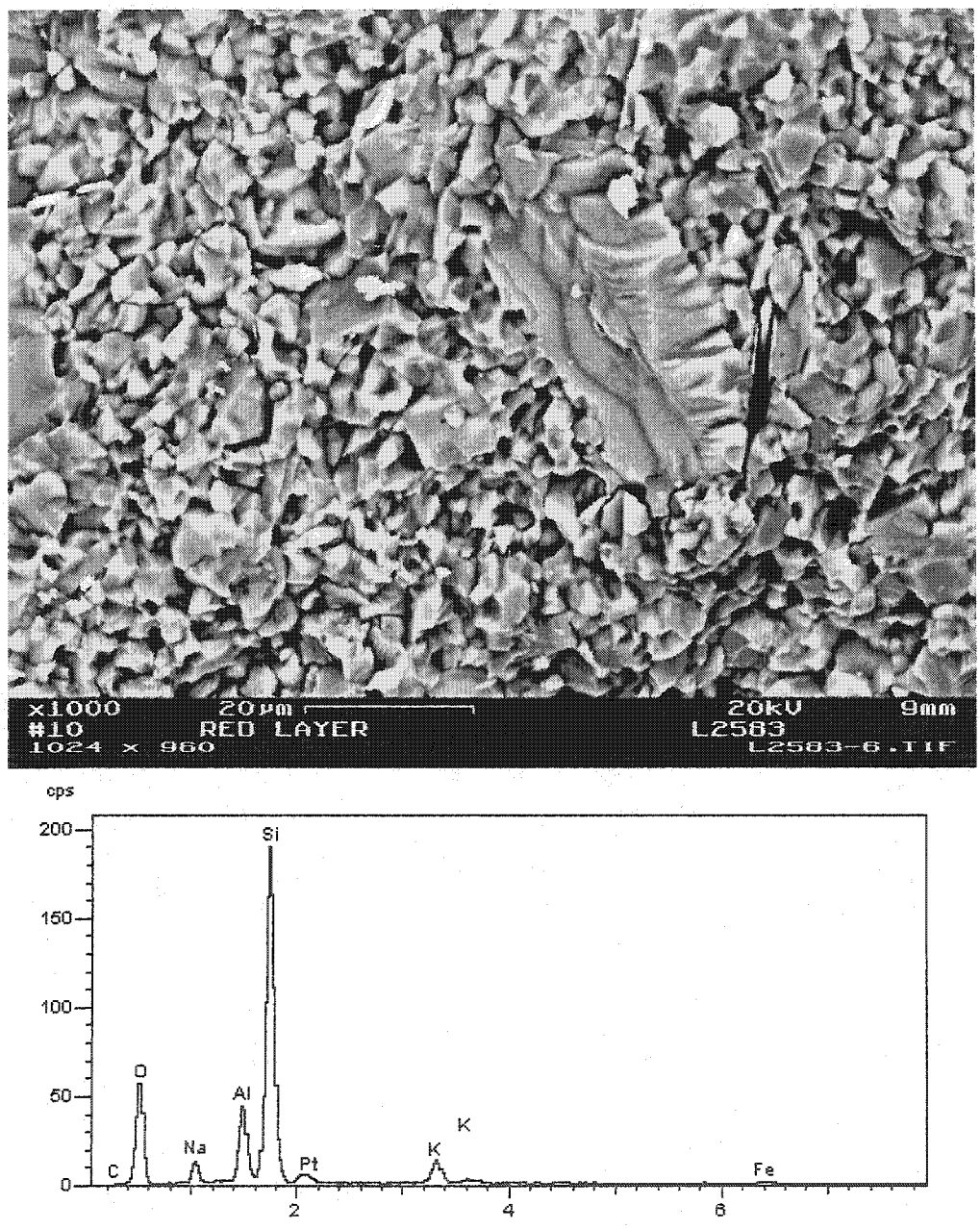


Figure 3-4. SEM with EDS area scan of red bottom coat Baker, CA VR31903. The coating looks glossy and lustrous as in Figure 1-2, however increased magnifications suggest that the surfaces are sintered in appearance. Higher magnifications Figure 3-7 are consistent with clays. XRD analysis of similar red coatings from Death Valley are primarily clays (Figure 3-21).

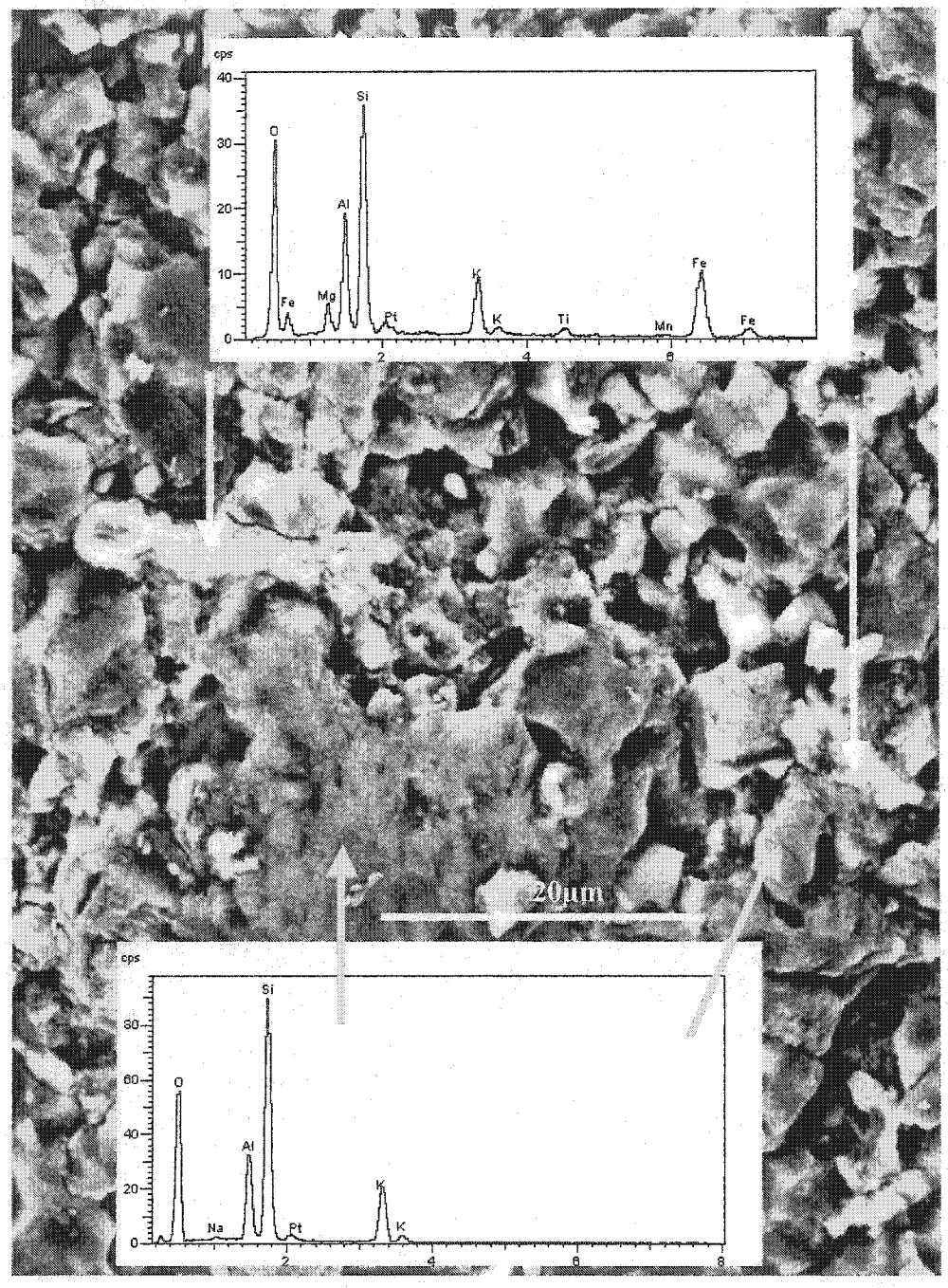


Figure 3-5. SEM with EDS of VR31903 red bottom coat Baker, California, Mojave Desert. Image is a combination of backscatter and secondary electrons. The lighter areas (upper EDS) are heavier elements, the lighter gray areas are lighter elements (lower EDS).

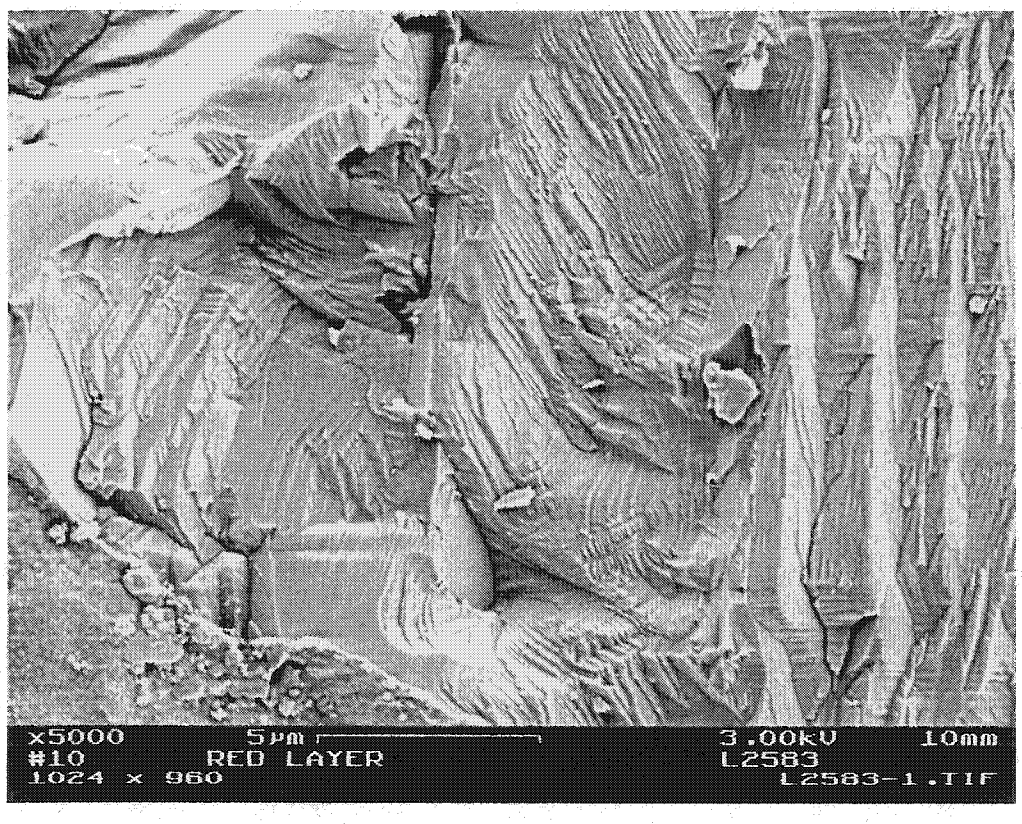


Figure 3-6. SEM image of bright red under-glaze shown inFigure 1-2, from Baker, California has a lustrous , glossy look under low magnification. However, as the magnification is increased with SEM, the morphology changes dramatically. Figure 3-4 shows a x1000 magnification of the glaze. Figure (above) shows a variety of angular textures with x5,000 magnification. The textural quality is "sintered" and angular (upper left and Figure 3-4), but also exhibits a layered morphology (Figure 3-7).

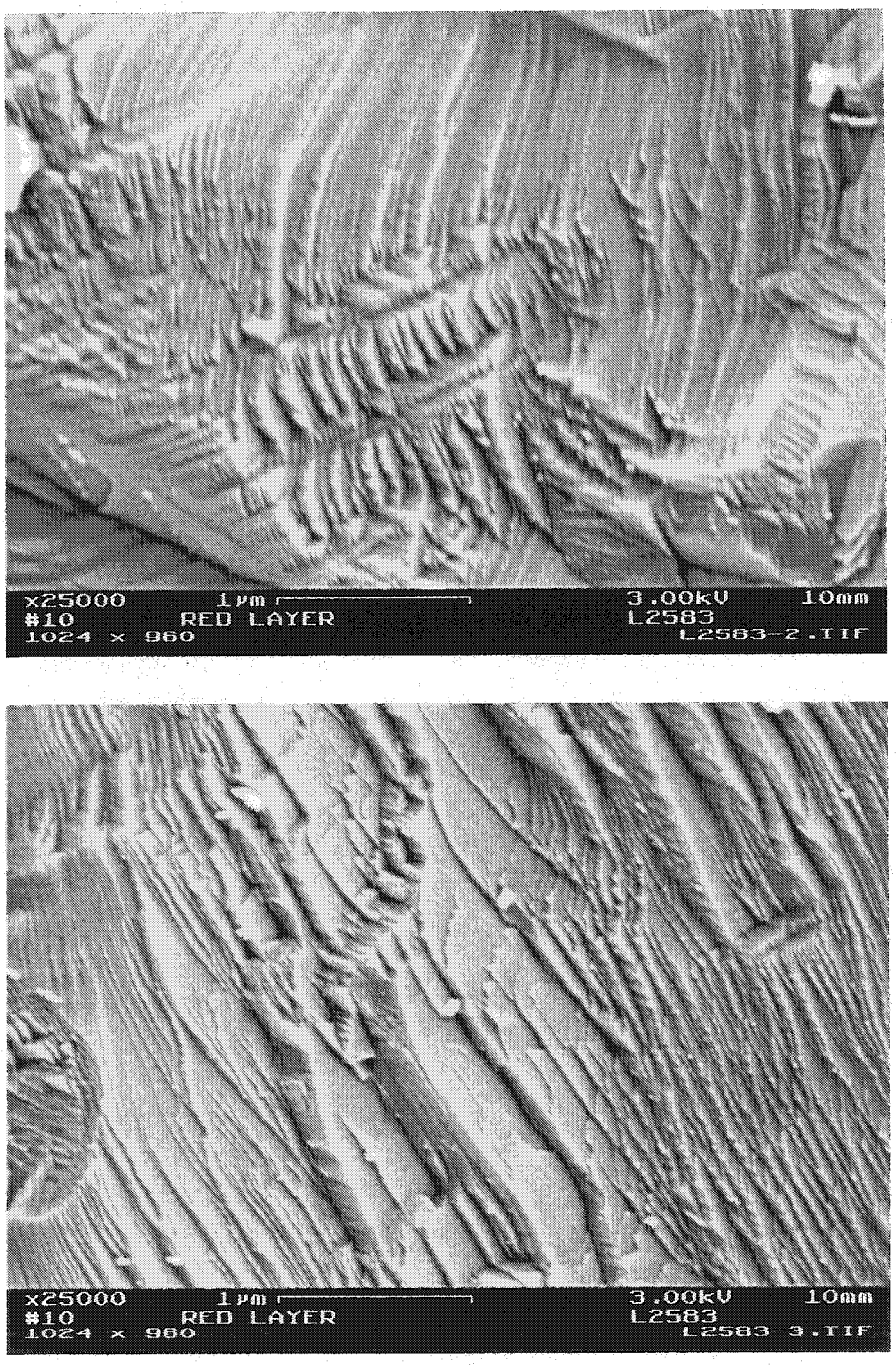


Figure 3-7. SEM image of red under-glaze on Baker, California sample as shown in lower magnification in Figure 3-6. Layered morphology is evident and supports XRD, suggesting that red under coats are composed of clays.

limits of EDS(Figure 3-4). Carbon is a consistent component of coatings, both top and bottom coats and was detected in the spot analysis (Figure 3-3). This is substantiated by other analyses including XPS (Figure 3-13) and TOF-SIMS (Figure 3-2). Potassium is anomalously high in the red bottom-coats of Mojave rocks (as an example Figure 3-5). The substrate rock is also high in potassium, and it is also high in manganese (Figure 3-1). But, manganese is present, only in small amounts in the coatings. XPS analyses (Figure 3-12) show potassium to be 0.52 and 0.56 atomic weight % in the Baker, CA bottom coat. This differs from EDS peak heights, which cannot be considered quantitative.potassium and also with other elements such as carbon which are ~20 atomic weight % using XPS (Table 3-1) but shows only a small peak using EDS (Figure 3-1).

The previous examples show the chemical diversity of red bottom glazes. Figure 3-5 employs a combination of backscatter and secondary imaging techniques electron techniques to analyze a red-coat from Baker, California. Using this technique, lighter elements appear darker and heavier elements appear lighter. In elemental chemistry overlays the image can be compared to the surface morphology. Silicon, O, Al, and K are similar over the entire surface. Carbon and Na are associated with "silicon" areas, areas that do not have enrichments of higher atomic weight oxides such as Fe. The "iron" enriched areas contain Ti and Mg but no measurable Na or C.

TEM-EDS

TEM-EDS analyses of particles on a nanometer scale range in composition from primarily Si (Figure 3-8) and various mixed phases of Fe-Mn (Figure 3-11). Mounting grids are composed of carbon and copper. Therefore, any indication of these minerals cannot be unequivocally attributed to varnish components. Silicon to aluminum ratio varies from high (Figure 3-8) to low (Figure 3-10). Aluminum exceeded Si in some spectrum. All samples analyzed, however, contained both Al and Si. Phosphorous (Figure 3-11), Cr and Cl (Figure 3-11) are primarily associated with Fe and/or Mn.

Electron microprobe

The electron microprobe sample analyzed is the same desert varnish topcoat sample as the SEM analyses in Figure 3-1 and Figure 3-3. Black topcoat, rock interior, and the red bottom coat were analyzed Table 3-2. The rock coating substrate analysis is consistent with phonolite. Notable differences between the varnish coating and the rock substrate are drops in SiO_2 from 42.69 wt.% oxide to 19.63. Manganese in the rock was 3.18 wt. % oxide and increased to 19.78 wt. % oxide in the varnish coating. FeO and P_2O_5 increased to a greater extent in the varnish coating as were other oxides to a lesser degree Table 3-2. Al_2O_3 decreased marginally from 24.95 wt. % oxide to 21.8 wt. % oxide. NaO_2 and Cl we the only other oxides besides Al_2O_3 to decrease.

The red bottom coating has substantially differences from the black top coating. SiO_2 and Al_2O_3 did not change significantly from the rock. FeO increased from 1.46 wt. % oxide to 3.84 wt. % oxide, but this was less than in top coats. MnO decreased from 3.18 wt. % oxide in the rock to 0.10 wt. % oxide in the red coating.

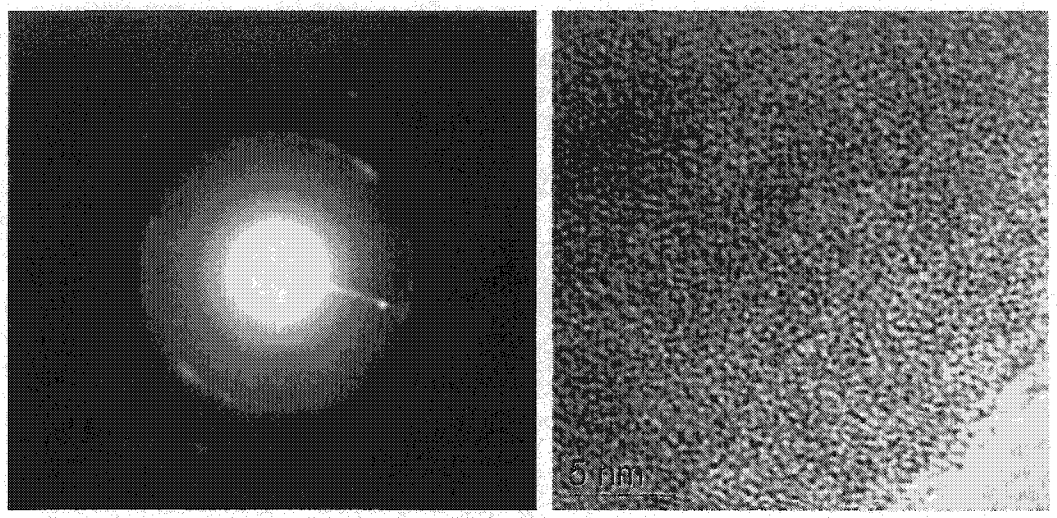
XPS surface analyses of desert varnish

Spectra from desert varnish coatings and red bottom glazes are shown in atomic weight per-cent data from Grimes Point, NV (Figure 3-14), east of Baker, CA (Figure 3-13), and Bishop, CA (Figure 3-15). Data for varnish top coats, under-glazes, and for the rock interior from Baker, CA are shown in (Table 3-1). Since XPS measures surface mono-layers, carbon aerosols are readily adsorbed. XPS measurements were made "as collected", and with 5 nm sputter removed for both black topcoats and red under-glazes removed, during analysis (Table 3-1). Ar+ ion surface sputtering was used to remove 10 nm of outers surface of coatings. Of particular interest are the high concentrations of carbon and nitrogen after sputter removal of the surface. High values for silicon compared to low values for Mn and Fe on the outer surface monolayers when compared to non-quantitative EDS. Top-coats and red bottom glazes also have a high carbon and nitrogen content (Table 3-1). Red bottom coats are higher then top black coats in silicon, have less manganese ~0.08 atomic %) as compared to ~0.65 atomic % in top-coats. The

large decreases in carbon after sputter removal, 31.33 atomic % decreasing to 10.76 atomic % in black topcoats and 20.02 atomic % decreasing to 7.97 atomic % in red glazes, are compensated for by corresponding increases in other elements. More significant increases were recorded for silicon, aluminum, magnesium, sodium, iron and manganese than other oxides. Potassium changed little in either top or bottom-coats. In red bottom-glazes similar increases were observed but with important differences. Sodium decreased from 0.3 atomic % to 0.02 atomic % after sputter removal as did manganese (0.13 atomic % decreased to 0.11 atomic %). In the topcoats, manganese increased from 1.01 atomic % to 3.34 atomic % after sputter removal of the surface.

The rock interior data (Table 3-1) is substantially different because the sample was freshly broken minutes before the analysis precluding adhesion of aerosol organics. Of particular interest is the near order of magnitude less quantity of carbon and nitrogen in the rock interior than from the sputtered coating of both top and bottom coatings. Note that manganese is 0 atomic % after sputter and 0.03 atomic %, is substantially concentrated in black coatings after sputter removal (3.68 atomic %). The above data for N, C, and Mn are summarized in (Figure 3-12) in which red glaze, black topcoat, and substrate rock interior are compared before and after sputter removal. Samples analyzed from Bishop, CA (Figure 3-15) are different from samples from other geographic area, for instance substrates are granite rather then basalt. Carbon (29.19 atomic % is higher in the black coatings than on the substrate (26.303 atomic %), as is nitrogen (1.06 on the surface of coatings as compared to 0.75 atomic % on uncoated surfaces). No sputter removal was done on these samples. Silicon (Figure 3-15) is ca. two-thirds (ratio-1.50) lower in coatings (10.53 vs. 15.78 atomic %, in uncoated).

The aluminum ratio (1.33) is less of a decline (5.67 to 4.25 atomic % in coatings). Other oxides showed little difference. However, since coatings are a surface



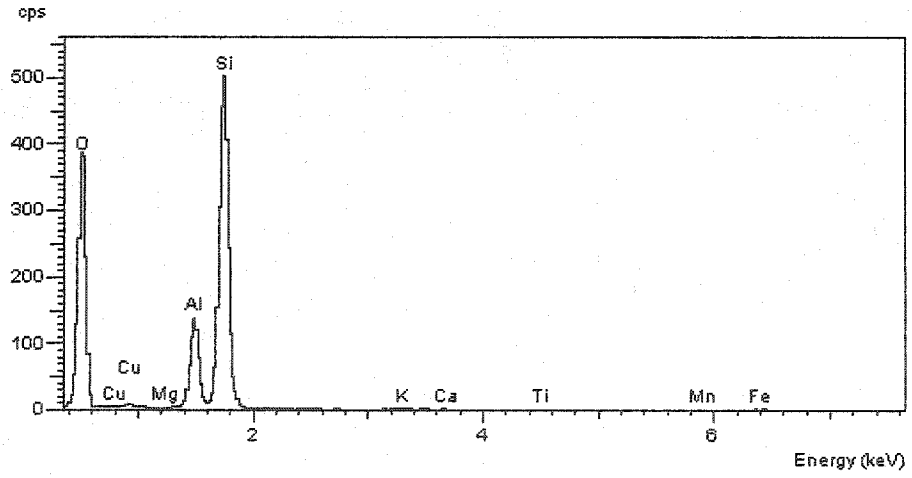


Figure 3-8. TEM image of Gobi Desert black varnish coating (upper right). EDS showing primarily Si and Al). Diffraction pattern (upper left) indicates some ordered, however the high magnification TEM image (upper right) structure suggests that the coating is amorphous.

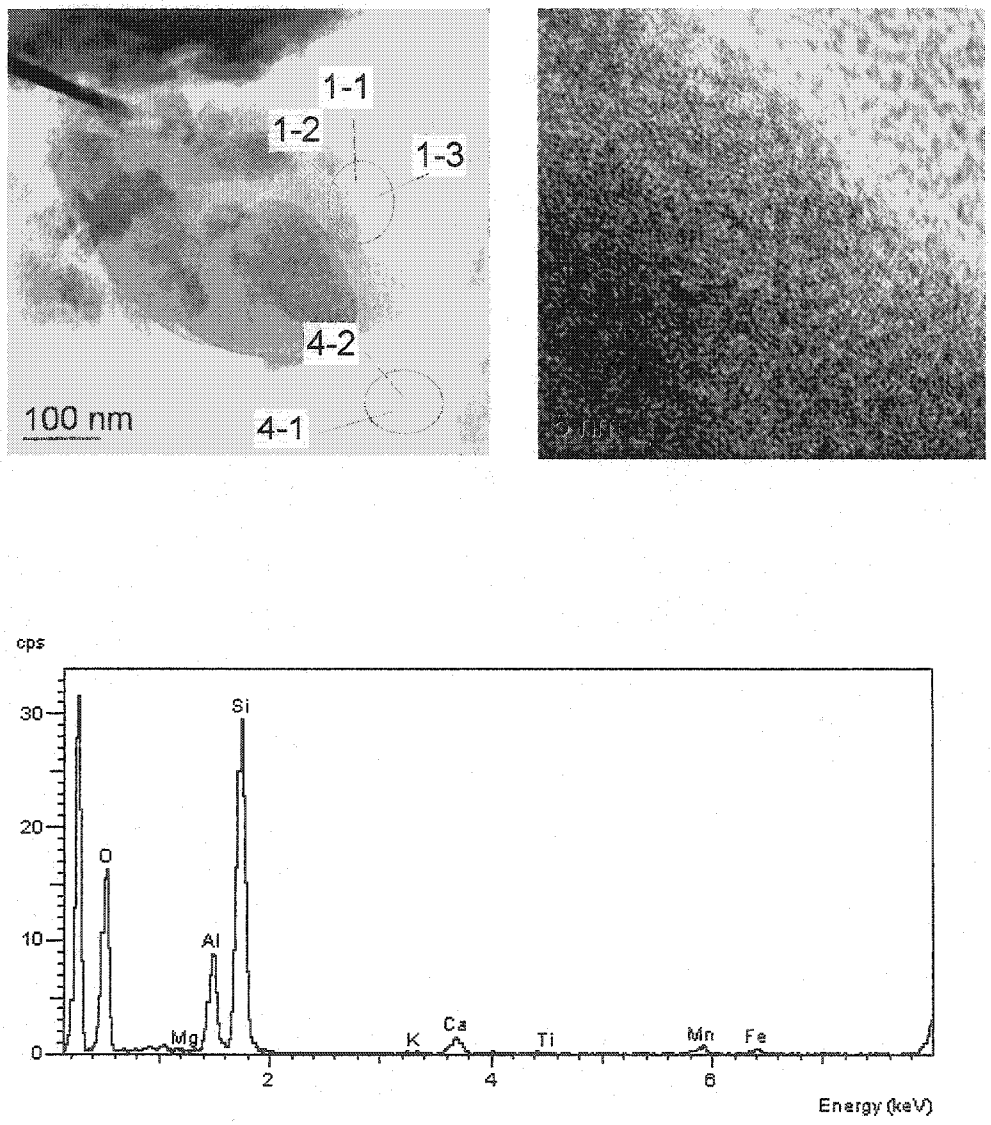


Figure 3-9. TEM image of mineral grain of powdered desert varnish from Baker, California. Image in upper right and EDS spectrum are from area "1-2" designated in upper left image. Note the crystalline structure (arrow) in the upper right image.

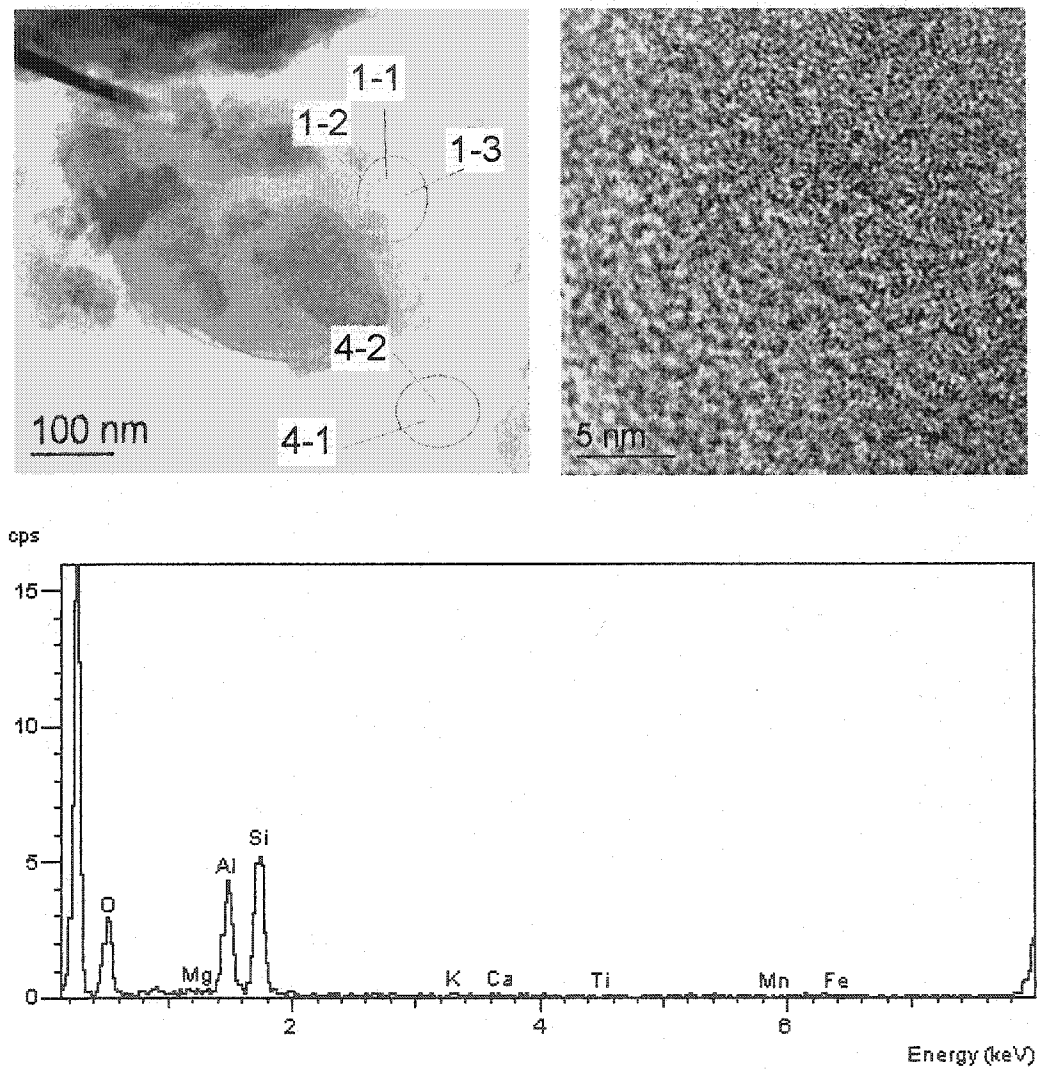


Figure 3-10. TEM image of grains of powder ground from the surface as described in Chapter 2 methods, from the Mojave Desert, Baker, California (top). Left image (top), area 4, from which the smaller scale image (upper right) was taken. EDS (below) is from area 4 showing nil amounts of Mn, Fe, or other oxides other then Al and Si.

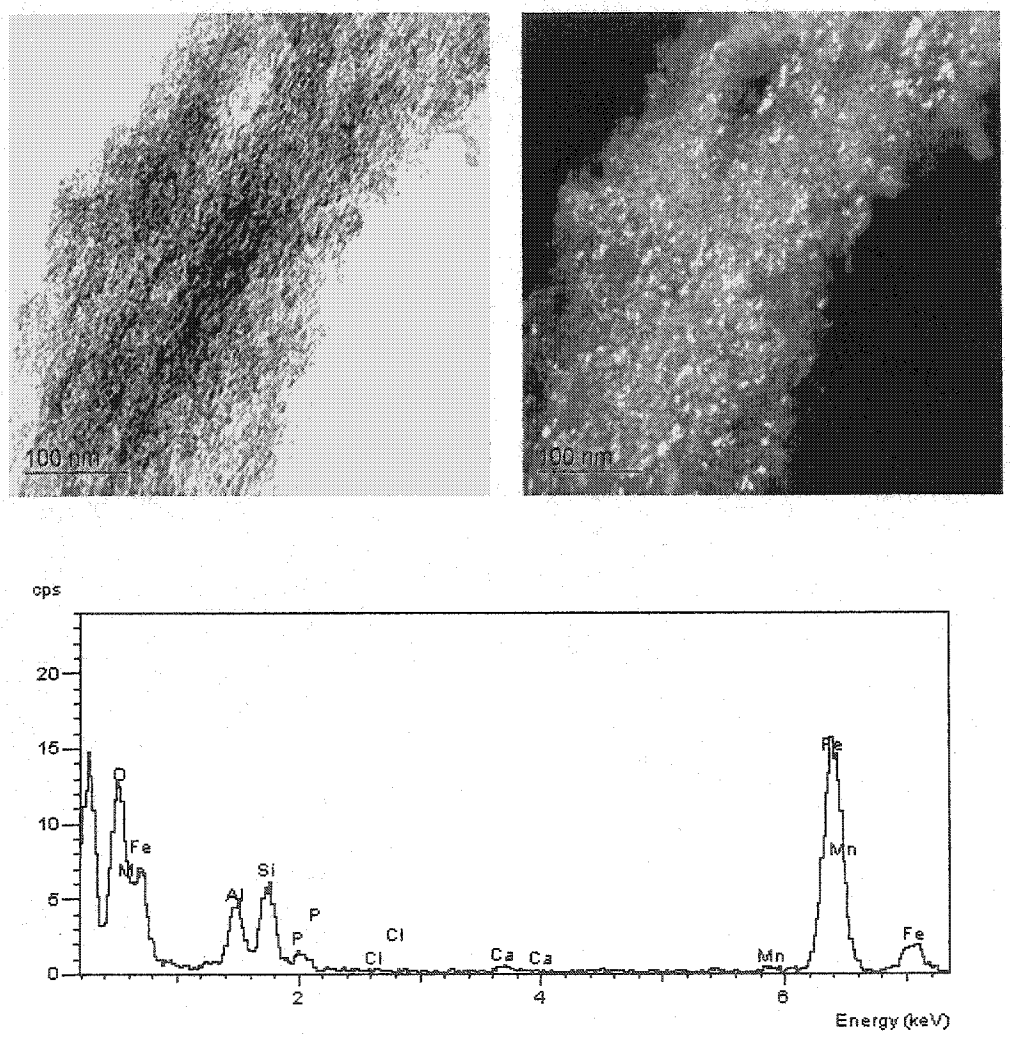


Figure 3-11. TEM image of grains of powder ground from the surface as described in Chapter 2 methods Death Valley powder, bright-field image (upper left) and dark-field image (upper right). Light areas in the dark-field are depictive of crystalline material.

Table 3-1. XPS atomic composition table of red bottom coat, upper black varnish coat and varnish substrate rock interior from Baker, California.

FileName	AreaComment	C1s [0.314]	N1s [0.275]	O1s [0.733]	Na1s [1.707]
04300301.spe	319031 red (Baker) as rec.	19.63	3.40	54.54	0.42
04300304.spe	319031 red (Baker) as rec.	18.70	3.58	54.84	0.33
04300302.spe	319031 uppercoat (Baker) as rec.	32.41	2.95	46.44	0.32
04300305.spe	319031 uppercoat (Baker) as rec.	37.44	3.30	42.40	0.20
04300303.spe	319031 inter. (Baker) as rec.	0.81	0.11	66.39	2.59
04300306.spe	319031 inter. (Baker) as rec.	0.91	0.43	66.55	2.65

FileName	AreaComment	Mg1s [1.035]	Al2p [0.256]	Si2p [0.368]	Cl2p [0.954]
04300301.spe	319031 red (Baker) as rec.	1.97	5.00	13.59	0.02
04300304.spe	319031 red (Baker) as rec.	2.01	5.14	13.80	0.07
04300302.spe	319031 uppercoat (Baker) as rec.	0.84	4.26	10.12	0.27
04300305.spe	319031 uppercoat (Baker) as rec.	0.84	3.66	9.83	0.22
04300303.spe	319031 inter. (Baker) as rec.	0.32	4.95	22.68	0.00
04300306.spe	319031 inter. (Baker) as rec.	0.07	4.85	22.35	0.00

FileName	AreaComment	K2p [1.552]	Mn2p3 [1.757]	Fe2p3 [1.964]
04300301.spe	319031 red (Baker) as rec.	0.52	0.08	0.82
04300304.spe	319031 red (Baker) as rec.	0.56	0.08	0.88
04300302.spe	319031 uppercoat (Baker) as rec.	0.60	0.67	1.11
04300305.spe	319031 uppercoat (Baker) as rec.	0.41	0.62	1.08
04300303.spe	319031 inter. (Baker) as rec.	2.05	0.06	0.04
04300306.spe	319031 inter. (Baker) as rec.	2.06	0.04	0.10

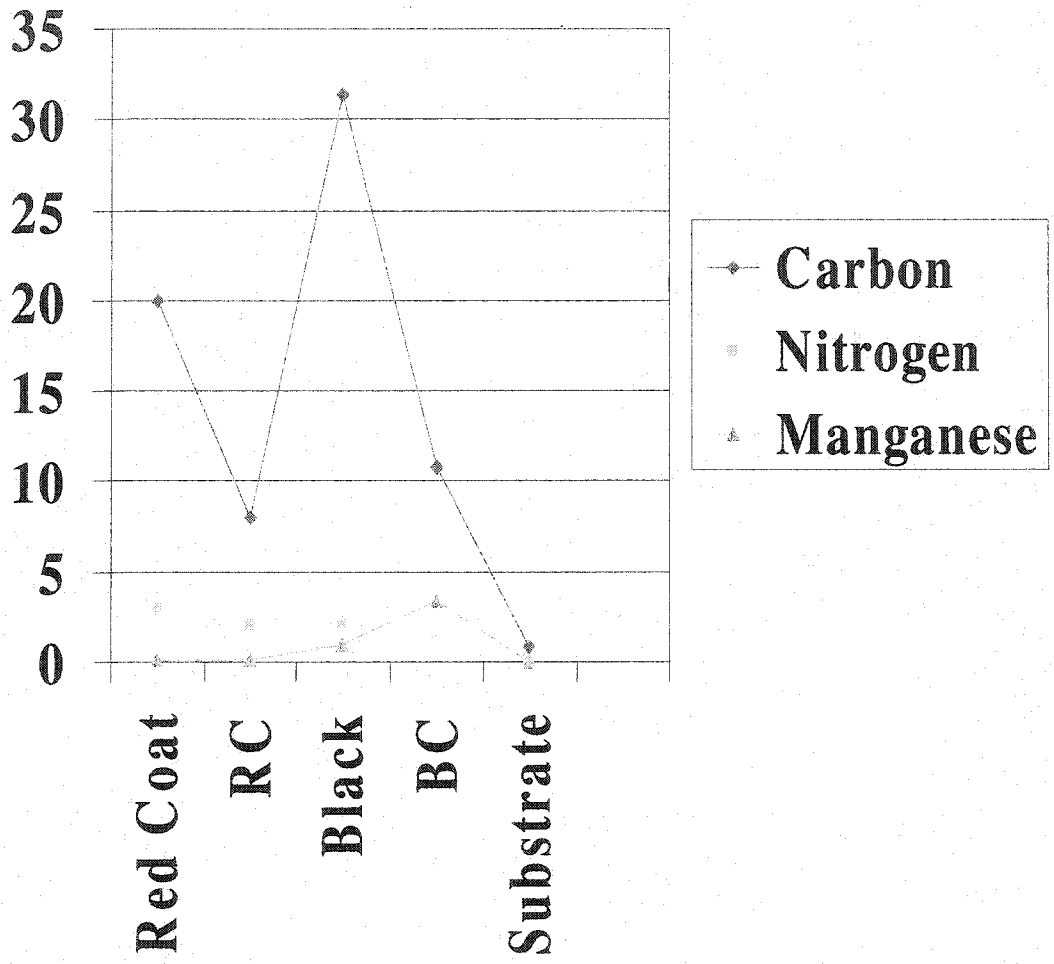


Figure 3-12. XPS of carbon, nitrogen, and manganese for red bottom coat (Red Coat), red bottom coat after a 5 nm sputter removal of the surface (RC), Black desert varnish coat (Black) and the black desert varnish coat after 5 nm sputter or the surface (BC), and the carbon, nitrogen, and manganese composition of the substrate (basalt) from the Baker, CA sample site.

deposit, not derived from the substrate, these differences may have little meaning as compared to substrates, but rather may reflect their local environments. The exceptions are carbon and nitrogen which may be deposited onto surfaces by aerosols, microbes, or dusts. The Si/Al change from 2.78 in uncoated to 2.48 in coated samples suggests different silicate phases may be present in the outer mono-layers analyzed (Table 3-1). Grimes Point, NV petroglyph site (Figure 1-7) is interesting in that there is no manganese in the outer mono-layer but more silicon than in other coatings (Figure 3-14). Carbon was lower than in any other sample analyzed (~18 atomic weight % (Figure 3-14) as compared to ~31 atomic weight % in coatings from Bishop, CA (Figure 3-15).

Binding energy (BE) spectra (all figures shown), as compared in wide scan data (low energy resolution spectra, not shown) show that several elements are present such as Cl, Pb, Co, and Ar. The Co and Ar are present after sputtering. Ar is expected, since Ar+ ions are used for the sputtering. In the "as collected" spectra (Figure 3-16) the Fe 2p3 binding energy is ~712.4 Ev after charge referencing the C1s peak to 284.8 Ev. This puts the Fe in at the high end of the BE scale for most Fe compounds. BE of ~712.4 Ev is somewhat higher than is expected for FeOOH (~711.7 Ev) and higher then Fe_3O_4 at~710.8 Ev. Perhaps a different peak for charge referencing such as Si should be considered since the hydrocarbon overlayer may not be the best choice for referencing. Caution, however is required since there are a variety of expected valence states combined in the spectra. In addition to the Fe, others detected such as K, N, O, Na, Mg, Al, Si, and Mn suggest that a large variety of compounds are present. After sputtering Fe is reduced. This is not unexpected as the Ar+ ion sputtering reduces the oxide present. The peak has shifted lower (BE ~711.2 Ev) after sputtering because of the change in the loss features. The loss feature is seen in the valley of the two main Fe2p peaks (Figure 3-16). The "as collected" (solid line) for Fe is more consistent with Fe^{3+}than "after sputtering", where there is more Fe^{2+} (dotted line). The shoulder after sputter (dotted line), suggests that Fe_2O_3 is present and not Fe_2O_4.

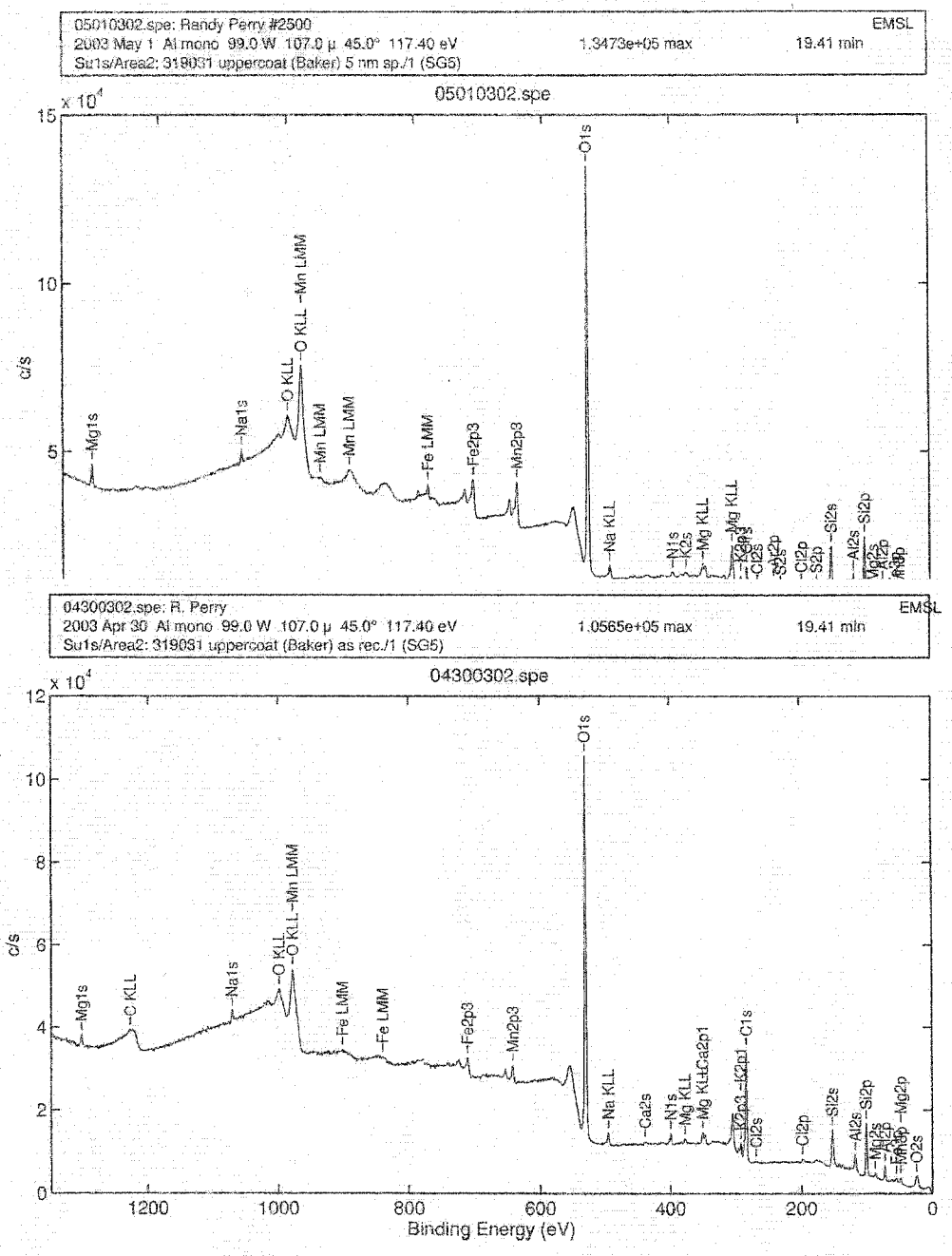
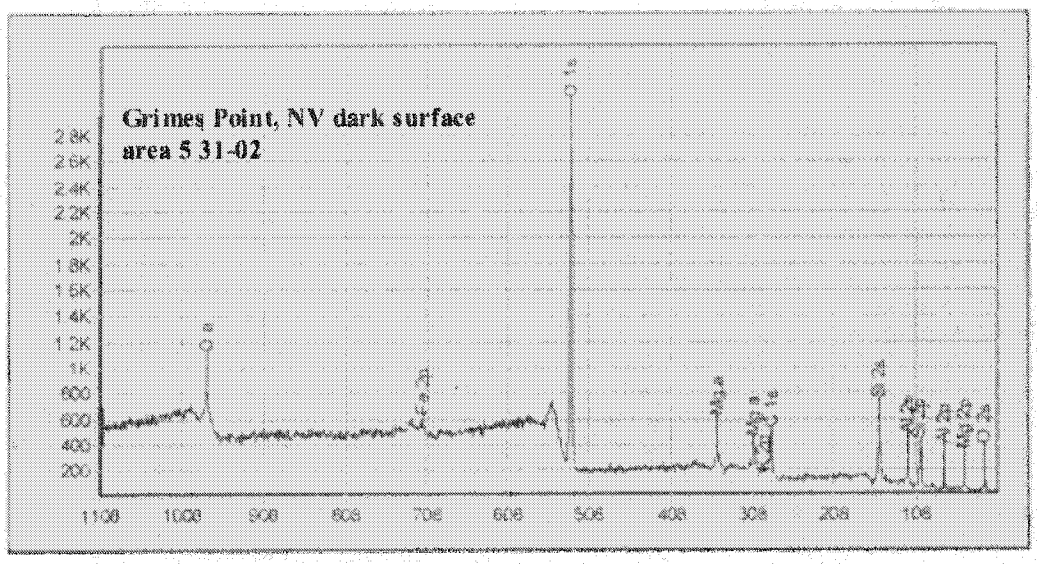
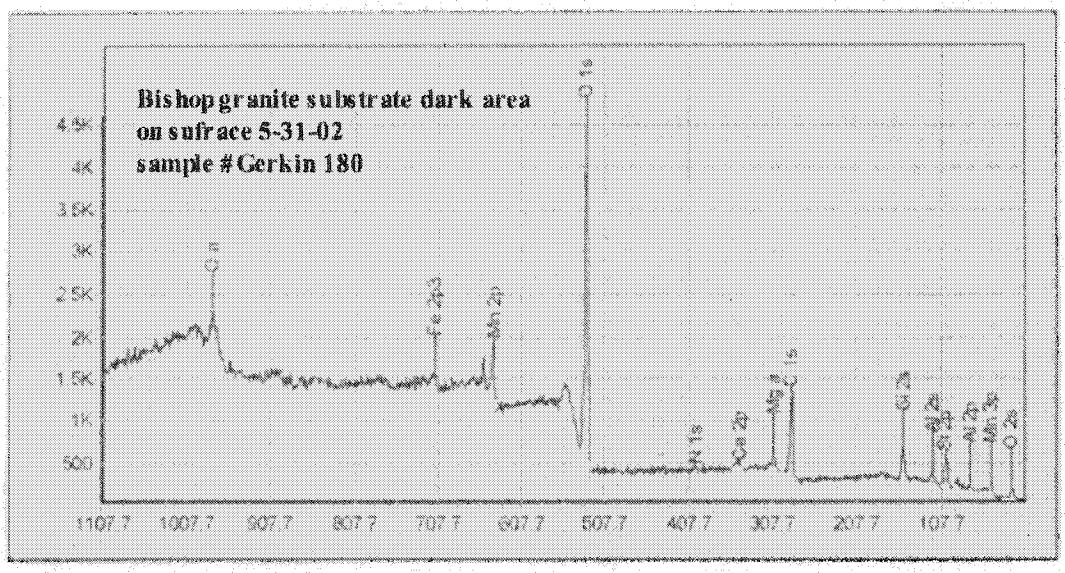


Figure 3-13. XPS of dark desert varnish top coat from the Baker, California, sample site. Top tracing is after 10 nm sputter removal of the surface. The image below is as collected and before sputter removal. Sputtering, as described in the text removes outer monolayers.



XPS Line	Adj'ed Be	Norm Area	Sen'y	Atom %
Al 2p	74.017	34.879	.59	6.212
Fe 2p	711.955	6.713	12.62	1.195
O 1s	531.133	290.905	2.55	51.810
Mg a	305.758	19.355	4.06	3.447
K 2p	293.926	3.315	3.95	.590
C 1s	284.224	101.957	1.00	18.158
Si 2p	102.086	104.366	.89	18.587

Figure 3-14. XPS tracing (top) and composition atom % (lower) of dark area on sample from Grimes Point, NV petroglyph collection site.



XPS Line	Adj'ed Be	Norm Area	Sen'y	Atom %
Fe 2p3	710.584	13.643	8.32	1.125
Mg a	305.486	29.546	4.06	2.436
Al 2p	73.780	51.517	.59	4.247
Mn 2p	641.093	37.759	11.26	3.113
O 1s	531.108	575.094	2.55	47.409
Ca 2p	346.882	10.775	4.91	.888
N 1s	399.604	12.834	1.69	1.058
C 1s	284.047	354.122	1.00	29.193
Si 2p	101.991	127.756	.89	10.532

Figure 3-15. XPS tracing (top) and composition atom % (lower) of dark area on surface of sample from Gerkin Rd, Bishop, CA.

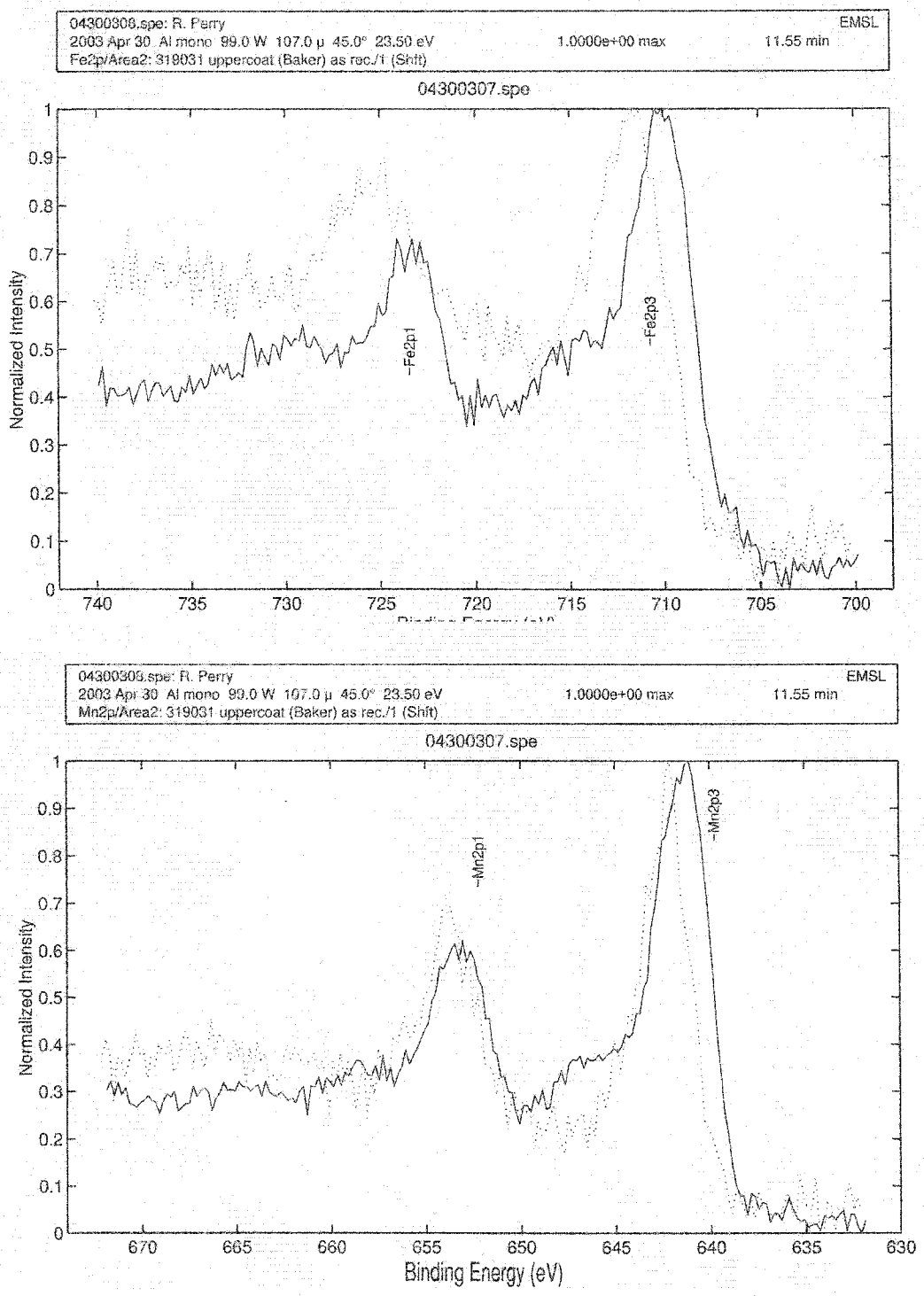


Figure 3-16. XPS of dark desert varnish top coat from the Baker, CA sample site. Top tracing shows a shift after sputter dotted line (top) for Fe 3p. The bottom tracing shows a shift for Mn 2p.

The Mn oxidation state is more difficult. All that can be said from the spectra is that there is no metallic Mn~639 Ev. The Mn in the "as collected" (solid line in Figure 3-16) is at ~643.2 Ev and is like the Fe at the high end of the binding energy scale for most Mn compounds.

Iron stable isotopes

Preliminary data for delta 56/54 stable isotopes show Death Valley soils and desert varnish to be negative values (up to -0.30), while Mojave samples for soils and varnishes are positive values (up to 0.13). Rock interior were also positive, averaging 0.12.

TOF-SIMS surface analyses of desert varnish

TOF-SIMS measures both negative and positive ions only in outer surface monolayers. Positive mass spectra of top and bottom coats have high abundances of Si closely followed by Al, and somewhat less Fe (Figure 3-17). Smaller amounts of Ca, Na, and Mn are present. Fe and Si have a correlation in overlays (Figure 3-2). No correlation appears with Mn and Si or Fe (Figure 3-2 and Figure 3-17) in samples from Death Valley, California. Abundances of Si, Al and Fe are likely underestimated since positive ionization varies inversely with first ionization energy. The red bottom coats had a high concentration of Si, with a lesser amount of Al, followed by smaller amounts of Mg, Fe, K, Ca, Na, and Mn. It is also interesting to note that the black appearance characteristic of desert varnish, in particular those imaged in (Figure 1-1) from Death Valley, California have only minor outer monolayers amounts of Mn (Figure 3-17). The Mn present appears to have no correlation with other elements (Figure 3-17). Even though top-coatings appear black or brown, Si is by far the dominant ion imaged (Figure 3-17).

Mineralogy

Previous work

Engel and Sharp (1958) reported that varnish was amorphous. Perry and Adams (1978) showed that varnish coatings have a layered botyriodal morphology and their XRD analysis did not show any oxide peaks. Potter and Rossman (1977) and Potter (1979) performed the first extensive analyses of the mineralogy of varnishes. Potter and Rossman (1977) presented infrared evidence that varnish was composed of clay and oxides of iron and manganese cemented together. The principal clay components identified by Potter and Rossman (1977) were mixed-layer illite-montmorillonite (smectite) with small amounts of kaolinite, and occasionally chlorite. Manganese and iron crystalline phases were identified as birnessite and hematite. Their primary research tool was IR spectroscopy supported with XRD and electron microprobe.

Nagy et al. (1991) stated that few clays are present in their XRD analyses and that the bulk of the measured varnish components are mostly a cyrptocrystalline Fe-Mn Figure 3-11 displays a darkfield image using TEM where both manganese and iron are present. The bulk of the material appears to be a non-crystalline. mineral phase. They concluded a mixed layer Fe-clay mineral present is probably corrensite. This mineral is usually considered to be an intermediate phase. There is a gradual change of trioctahedral smectite to corrensite and then to chlorite as is found in the smectite to illite transition. During this transition, large quantities of chlorite are produced by the loss of Fe and Mg from the smectite component when illite is produced, or by the combination of iron or magnesium with kaolinite (Velde, 1992). Corrensite does not appear to be present in Mojave coatings in this study (Figure 3-23).

Source materials vary from different locales, consequently varnishes formed in different areas are expected to have differing mineralogies. The study area for this investigation is different from that of Nagy (above) and Raymond (to follow) but similar to that of Potter (1979) and Potter and Rossman (1977). Note that there does not appear to be much smectite in Figure 3-23 and Figure 3-24, differing from Potter and.

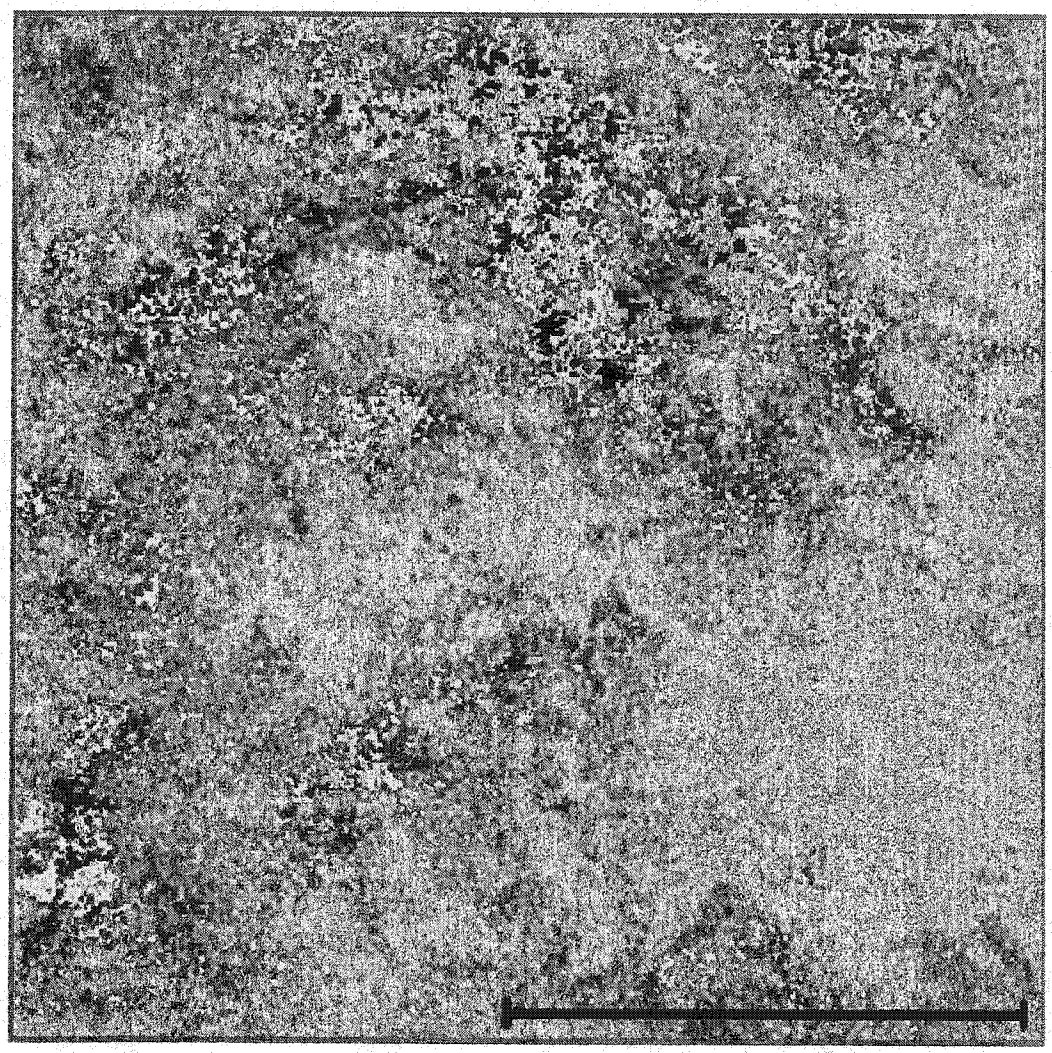
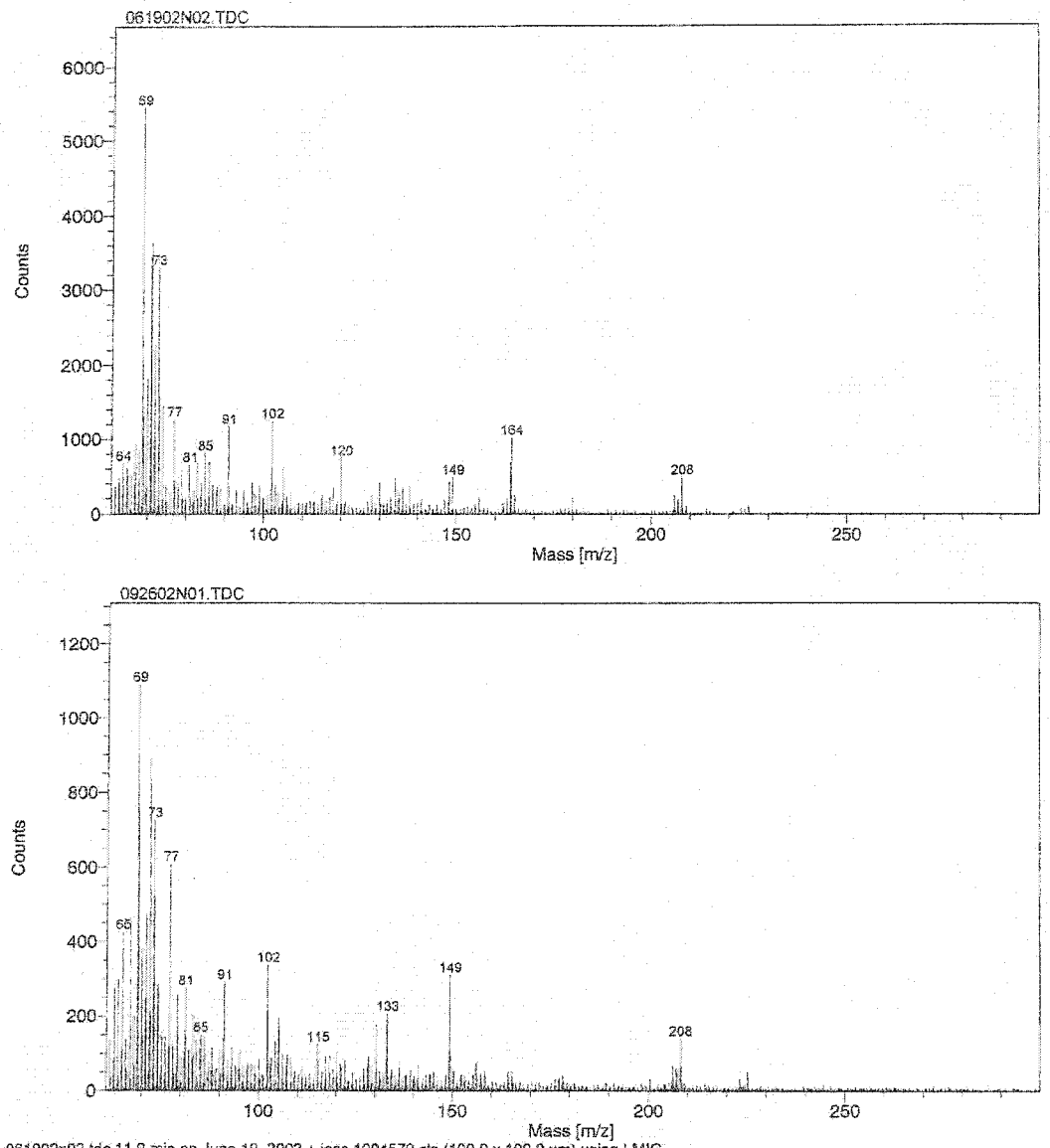


Figure 3-17. TOF-SIMS overlay of Death Valley, California varnish top coat. Concentrations of Si are represented by yellow, Mn by magenta, Fe by blue. Where the blue color, represented by Fe overlaps with the yellow color representing Si overlap, green is produced. Scale bar is 100μm.



061902n02.tdc 11.9 min on June 19, 2002 + ions 1004570 cts (100.0 x 100.0 um) using LMIG
COMMENT: Randall Perry. Death Valley #120. 2-26-02. 11.5 W of Panama Spgs. Dark side.25ga4.ins. CD#0. No energy filter. charge comp 59%.

092602n01.tdc 21.9 min on September 26, 2002 + ions 338001 cts (200.0 x 200.0 um) using LMIG
COMMENT: Randall Perry. DV 120. 2-26-02. 11.5 mi W of Panama Springs. Revisited.25ga4.ins. CD#0. 59% charge comp. No energy filter.

Figure 3-18. TOF-SIMS of two separate spot spectra of desert varnish dark top coat, sample #120 Panamint Springs, Death Valley, CA. Note lead 208 peak.

Table 3-2. Electron microprobe analyses of varnish coating from Baker, CA.

Microprobe analyses of desert varnish
from Baker, CA, sample #VR31903

Oxide [1]	Substrate *	Red Bottom Coat [ξ]	Black Top Coat [δ]
SiO2	42.69	43.36	19.63
TiO2	0.22	0.45	0.51
Al2O3	24.93	28.4	21.8
FeO	1.46	3.84	8.77
MnO	3.18	0.10	19.78
MgO	1.36	2.31	1.84
CaO	0.37	0.39	0.81
BaO	0.31	0.03	0.83
Na2O	1.14	0.04	0.20
K2O	0.28	2.25	1.43
P2O5	0.26	0.06	2.69
Cl	0.12	0	0.02
Total [2]	**76.31**	**81.23**	**78.30**

[1] All data in wt % oxide.

[2] Total Fe given as Fe_2O_3, however, some Fe^{2+} ions are present as shown in the XPS results section, however XPS data suggest that the Fe phase is Fe_2O_3 not Fe_2O_4. Total Mn given as MnO_2, however, Mn^{2+} and Mn^{3+} ions are present. Previous analyses have shown that the total H_2O to be ~10 % {Perry, 1979 #72}

* The analyses suggest the mineral matrix is phonolite

[ξ] Two spots were analyzed with the principle difference being an increase in FeO to 5.69 and MgO to 9.49 and the total oxides of 92.25 for spot 2 not shown. The composition is heterogeneous and analysis shown was representative.

[ξ] The "Black Top Coat" is heterogeneous (Perry and Adams, 1978); consequently composition will vary depending on where the analyses is taken. The spot chosen was representative for this sample.

Rossman's (1977) conclusions (same geographical area). The results of this study agree with both Raymond *et al.* (1992) and Nagy *et al.* (1991), even though they are from different geographical areas Raymond *et al.* (1992) found no crystalline manganese phase using XRD/TEM, differing from those that Potter found (1979). After chemical removal of oxides, Raymond found that the peak where birnessite appears to be chlorite 002 and/or a kaolinite 001 peak. No peak shift at 12.2° 2θ, was observed after heating both in vacuum to 200° C and in air to 500° C, as would be expected if birnessite was present, Their conclusion is that no crystalline manganese phase was present, in particular birnessite. Chlorite 002 and kaolinite 001 peaks can be confused with birnessite. The peak at 11.3° 2θ (Figure 3-23), however, is still distinct from chlorite and kaolinite. In Figure 3-24 Mg and Mg-Gly plots, this peak seems to expand to about 10-10.5 angstrom (ca. 8-10° 2θ) with glycerol. Hydrated birnessite would expand with glycerol. However, the d-spacing is larger than would be expected. This peak might also be explained by randomly interstratified smectite with kaolinite and it expands with glycerol. This hypothesis falls short since there is no bound smectite peak to complement the kaolinite peak. Organics would disappear with heat (Figure 3-24) as one would expect layered hydroxides to do. The best fit for this peak appears not to be birnessite but pyroaurite-sjogrenite or hydrotalcite, but only further and more detailed analyses will fully answer this question. Other possibilities exist, such as calcium-sulfate, potassium phosphate, or brushite. Further experiments are needed, possibly including synthesizing birnessite with different exchangeable cations, which would clarify the range of d-spacings and swelling behavior. The clays of varnish surfaces and surrounding soils were separated and then analyzed using XRD. Soil samples contained kaolinite, substantial smectite and trace cholorite (Figure 3-25). Surprisingly, varnish samples had only trace amounts of smectite and contained illite and kaolinite. Given the large amount of smectite in soils, one would expect to find it in the varnish coatings.

Raymond *et al.* (1992) did find crystalline hematite grains. Hematite was also

identified in this study. Perry and Adams (1978) said they were not certain whether grains are mixed phases (clays and oxides?) or whether they are single (unidentified) oxide-silica phase. These observations were expanded upon in Perry (1979), where no mixed oxide clay phases were found at the ca. 10-angstrom limit of the analytical equipment. Fine-grained iron was identified in this study but no conclusive crystalline manganese was observed. Small amounts of crystalline material in this study were observed in the dark field TEM image shown in Figure 3-11.

Mineralogy findings

Silica (opal), both opal A and opal CT were identified using XRD (Figure 3-24). EDS chemical analyses show that silicon is the dominant element present in all varnishes in this study (eg. Figure 3-3). This finding is consistent with amorphous silica identified in varnish coatings using TEM-EDS (Figure 3-19), and on surfaces using XPS (Table 3-1) and TOF-SIMS (Figure 3-2). XRD data of red bottom coats detected illite, kaolinite, and muscovite Figure 3-21.

XRD of bulk desert varnish powder are shown in Figure 3-22. Separations of the bulk varnish powder into sand, clay, and silt sized components followed by XRD, provides dramatically different mineralogical results (Figure 3-23 and Figure 3-24). The largest component (not quantified) was sand sized particles form the separations. Bulk XRD tracings show several silicate minerals including albite and quartz (Figure 3-22).

Soil samples contained kaolinite, smectite and trace chlorite (Figure 3-20 and Figure 3-25). Varnish samples had only trace amounts of smectite but did contain illite and kaolinite (Figure 3-23 and Figure 3-24). Given the large amount of smectite in soils (Figure 3-20), one would expect to find it in the varnish coatings if soils were the major source material for varnish coatings. Treatment with glycerol expands a peak at 11.3 degrees to ca 8-10 degrees (Figure 3-23). Heating to 550°C removes this peak and also would remove organic peaks (Figure 3-23). A layered hydroxide would react this way and the d-spacing is suggestive of a layered hydroxide such as pyroaurite or hydrotalcite rather then kaolinite and smectite interstratified layers. Hydrated birnessite might expand

similarly but as stated earlier the d-spacing (Figure 3-23) is a bit large (7.8 A).

Inorganic chemistry of microcolonial fungi (MCF)-Ubiquitous desert organisms

Background and previous work

The types of organisms that have been reported in the most extreme environments in which varnish occurs are restricted primarily to bacteria, fungi, some Eukarya, and in limited cases, lichens. These organisms generally fall into the category of extremophiles. The surface environments on rock coatings are some of the most extreme on Earth. High temperatures, low humidity, high incidence of UV light and low nutrients require microorganisms that have evolved special survival skills. MCF contain melanin, microsporines, carotinoids and probably other as yet unidentified pigments that help protect them from UV light. During the hottest and driest months, no bacteria have been observed on specimens examined from several desert regions of the world.
The survivability in these extreme conditions sets MCF apart from bacteria. Sporulating bacteria have developed survival mechanisms but must expend energy to create spores. MCF, in contrast survive and flourish where only few bacteria are present and lichens are unable to survive. Hot and dry deserts are considered to be one of the most hostile habitats for life on Earth. Drastic temperature changes, high UV levels, long periods of dryness punctuated with rain and snow, and low nutrient availability require specially adapted organisms. A seemingly unique feature of desert varnish is its worldwide association with microcolonial fungi (MCF). Black varnished rocks and black microcolonial fungi (MCF) seem to thrive in like ecological niches. Rock coatings are often black because of high concentrations of manganese and also becase of MCF black granular melanin pigments (Figure 3-26).

The phylogeny of melanized meristematic fungi (black yeasts) is quite diverse. Sterflinger *et al.* (1999) found in SSU sequences that there was a close relationship with at least three orders of Ascomycetes: *Chaetothyriales*, *Dothideales*, and *Pleosporales*. *Coiosporium sp.* species along with *Phaeococcomyces catenatus* were also identified but

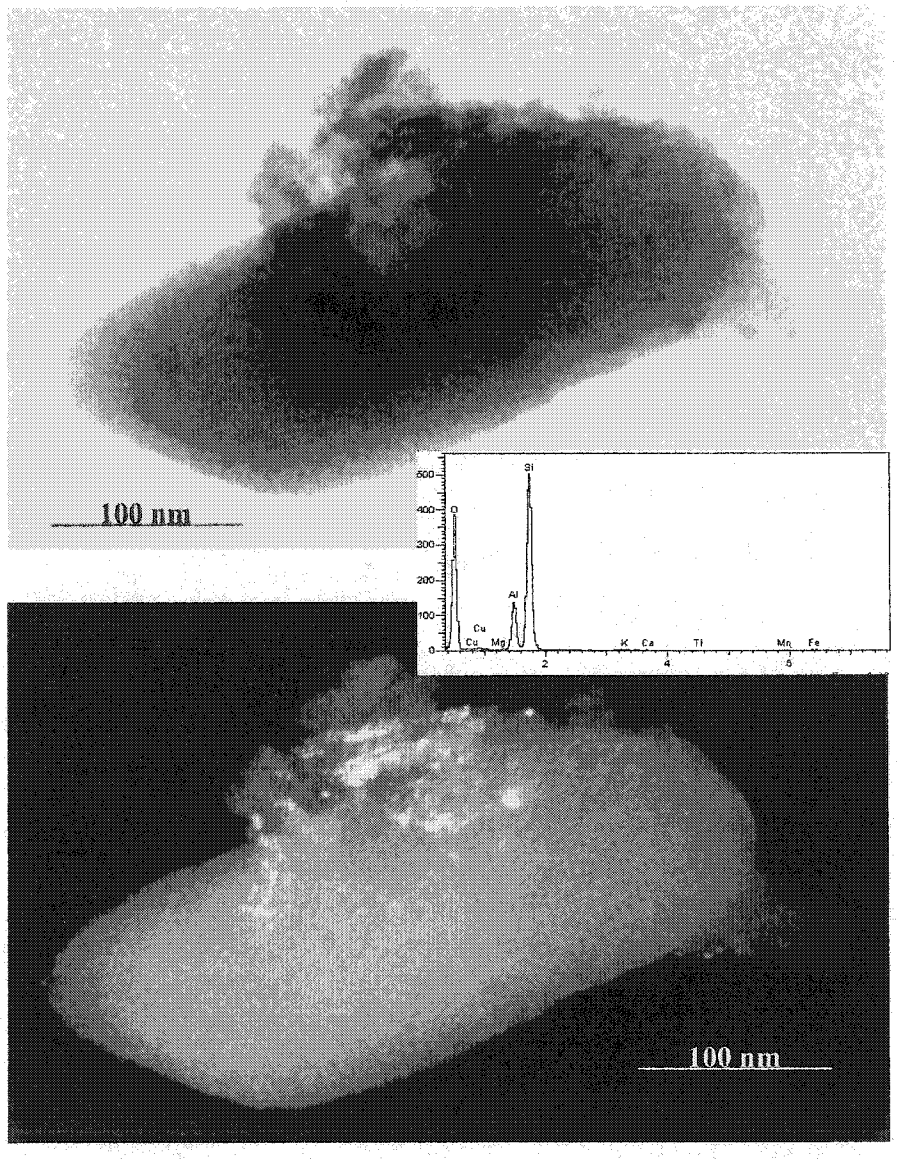


Figure 3-19. TEM-EDS bright field image of a silicon rich mineral phase (upper) and dark filled image (lower) from Gobi Desert varnish coating. Bright white areas in lower image show an ordered domain. EDS showing the presence of silicon and less aluminum, suggests that the mineral grain is primarily a non-ordered silica.

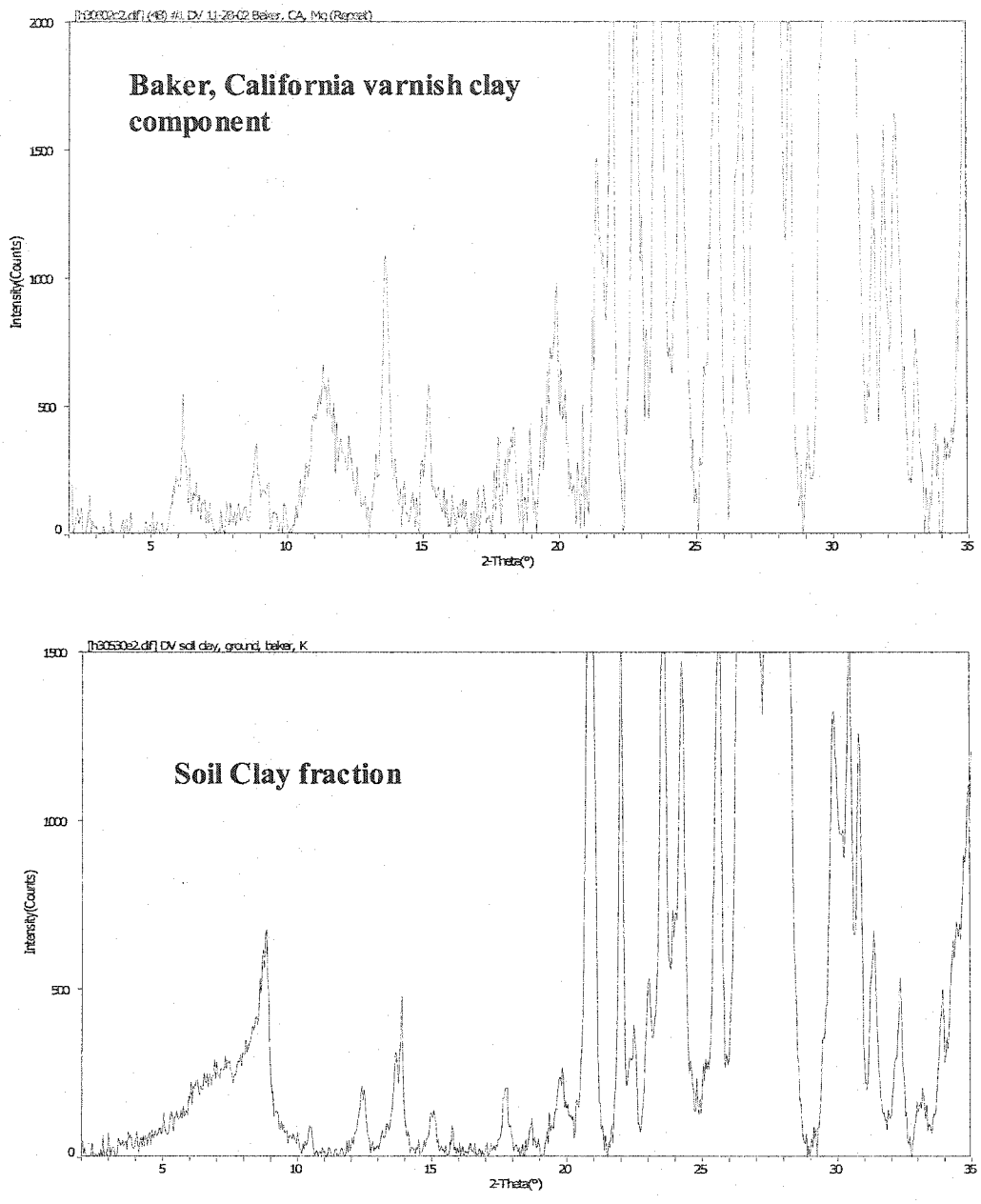


Figure 3-20. XRD spectrum of Baker, California desert varnish clay fraction. Lower spectrum is of the soil clay fraction near varnish rocks. Clay fractions were separated as described in Chapter 2 - Methods.

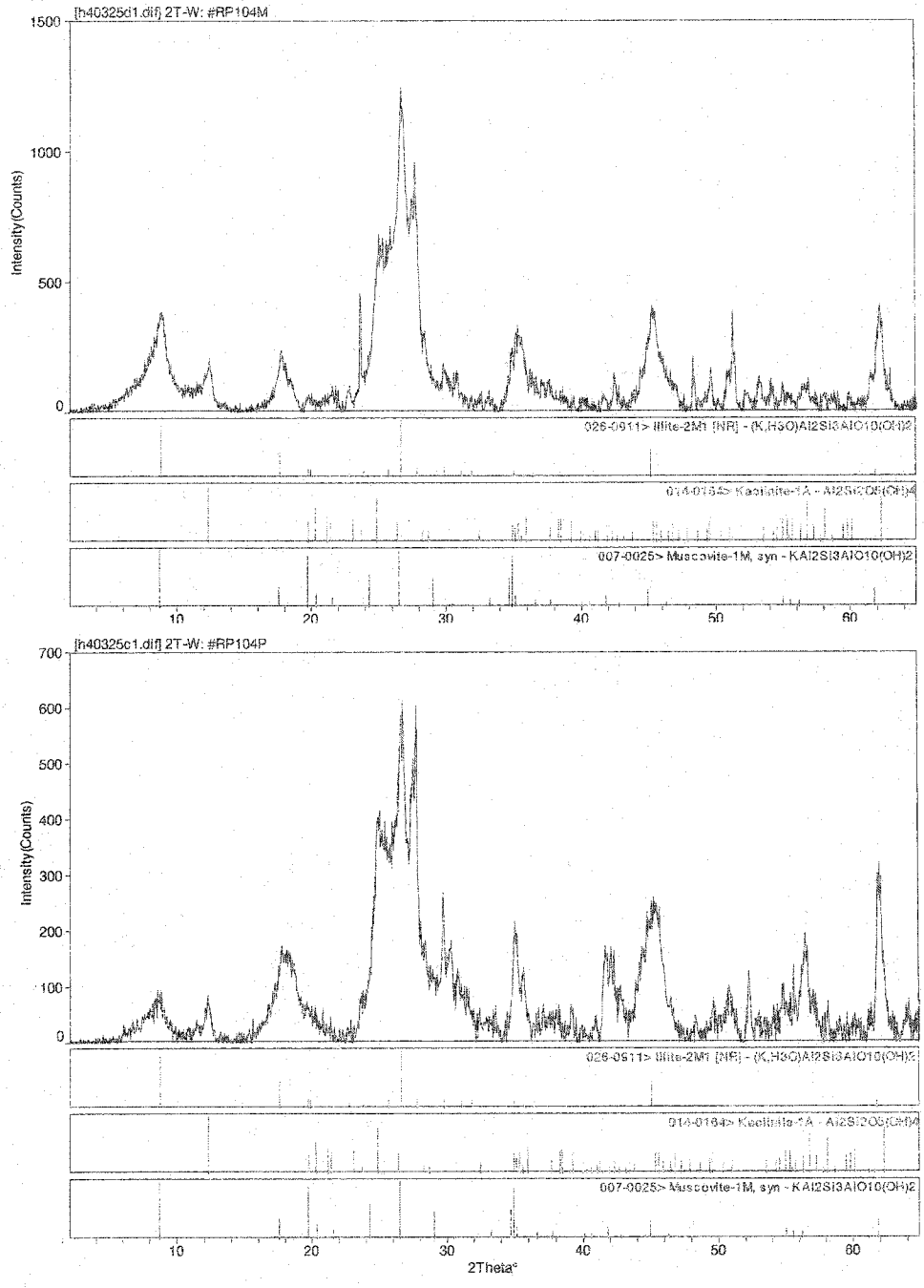


Figure 3-21. XRD of red bottom-coats from east Death Valley (Site X) shown in lower spectra. Red coat from Baker, California, Mojave Desert shown in upper image.

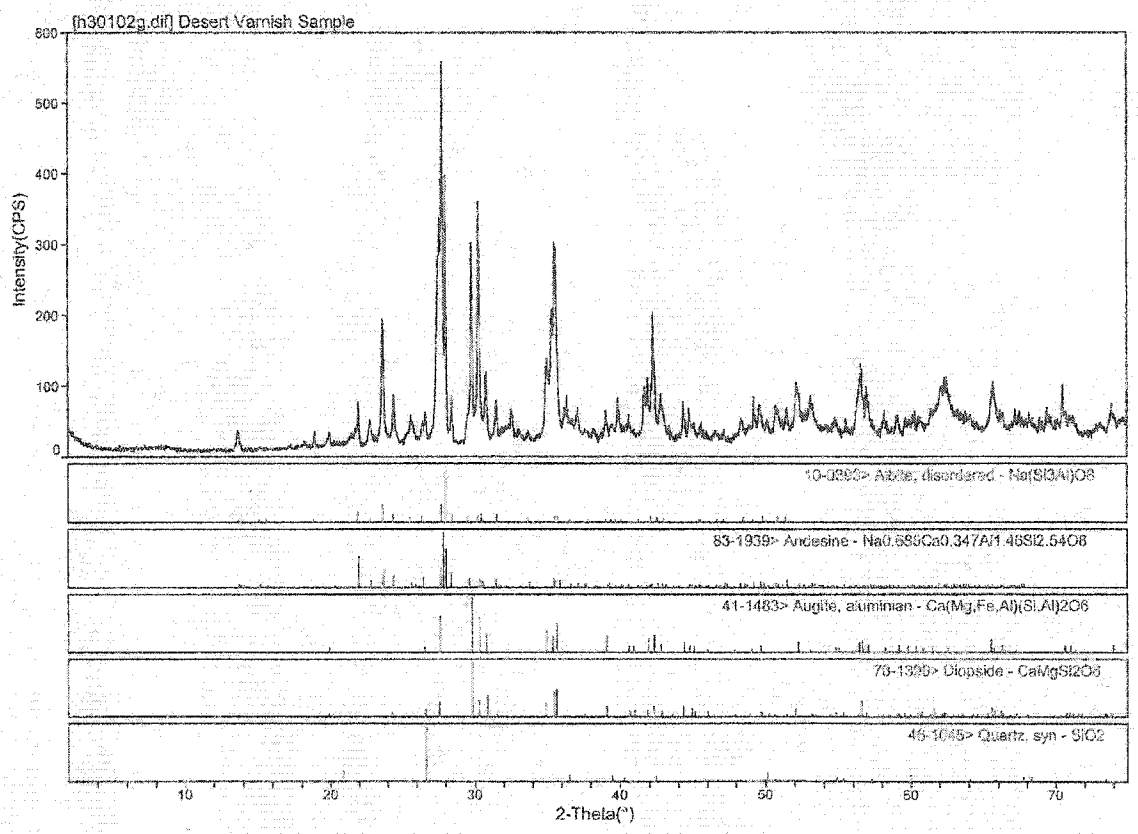


Figure 3-22. XRD of untreated varnish powder, ground from rock surfaces, Baker, California.

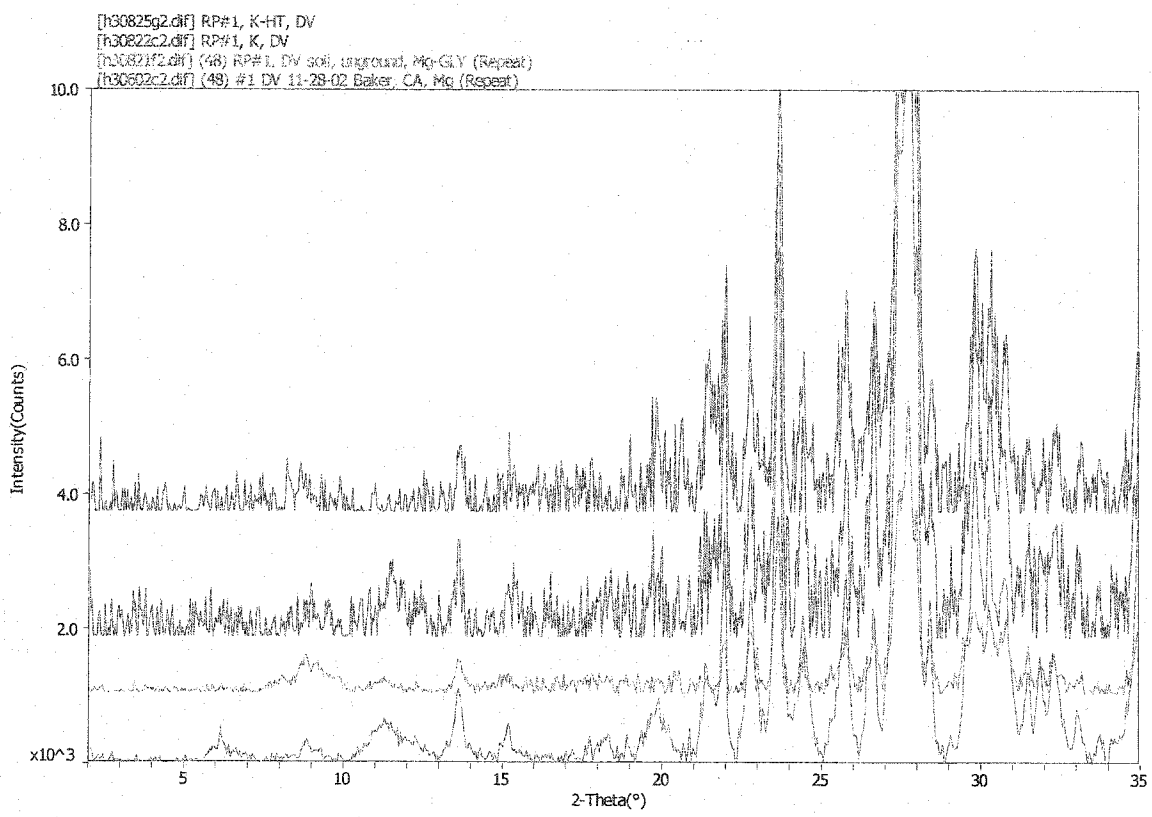


Figure 3-23. Spectra of Baker, California desert varnish (red), expandable clays showing positions change with the addition Mg-Gly (green), addition of K (blue), then heated (purple).

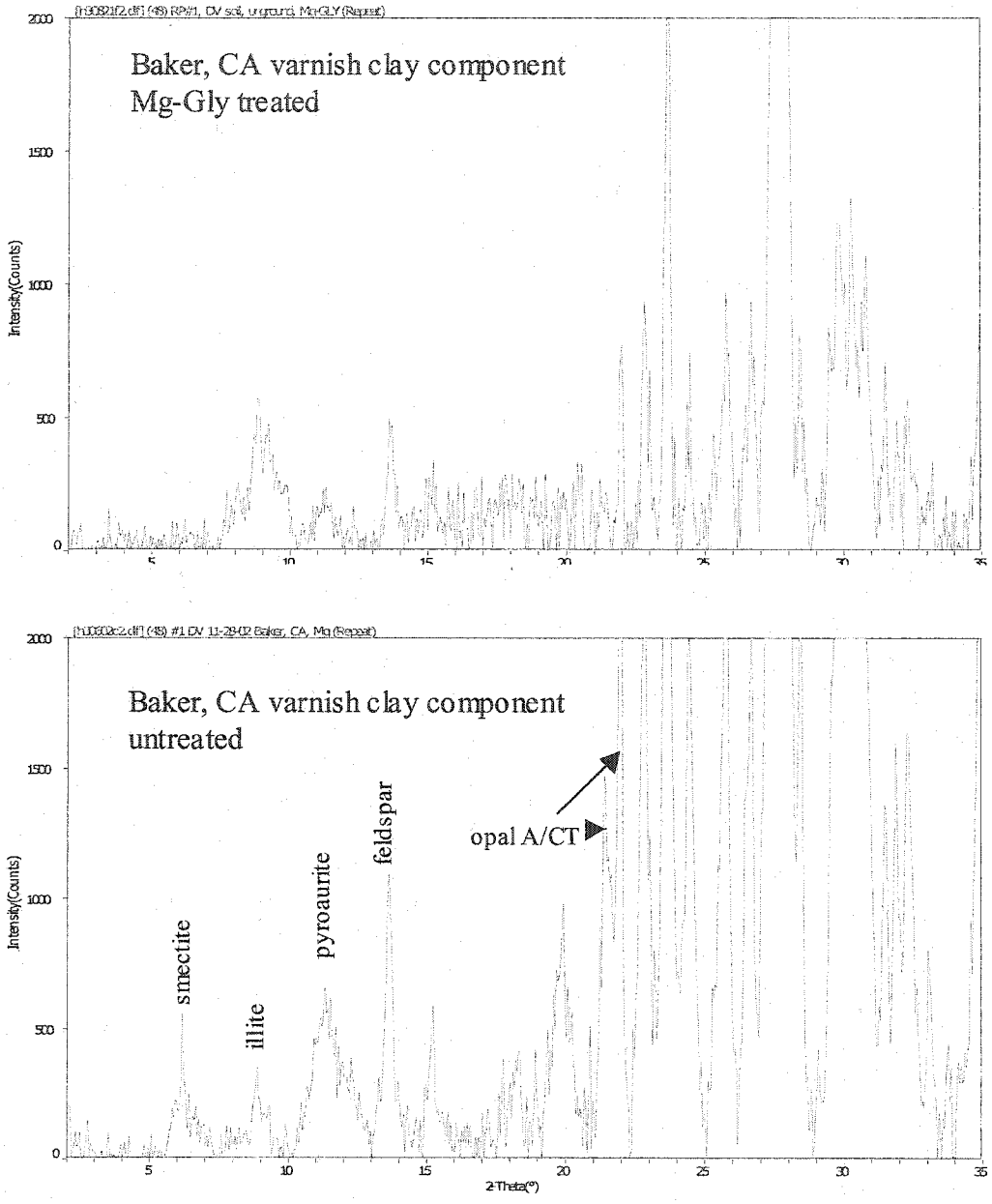


Figure 3-24. XRD of desert varnish clay sized component after separation of clay fraction from Baker, California desert varnish coating. Untreated clay extract (lower spectrum) and glycerol treated sample (above).

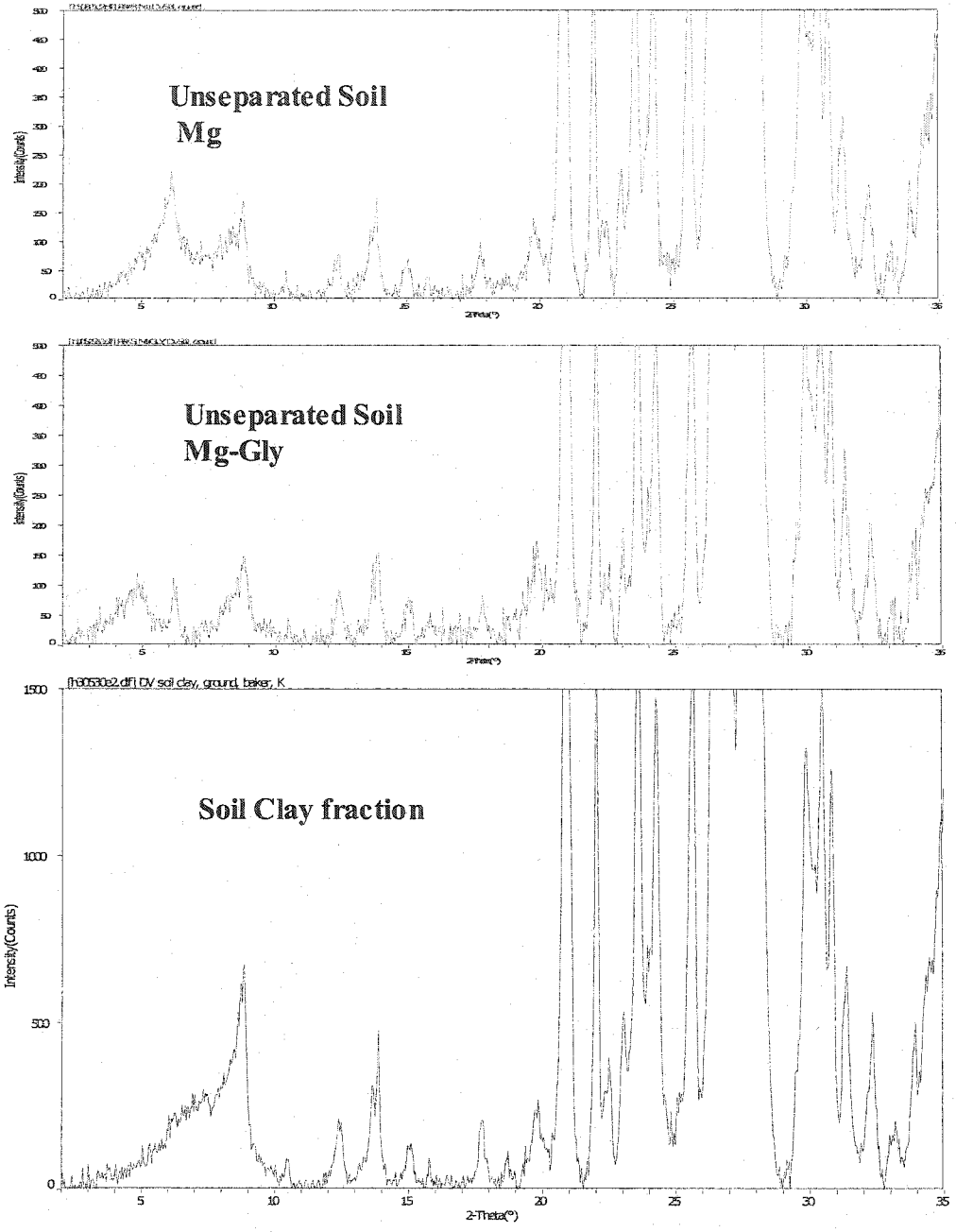


Figure 3-25. XRD of Baker, California soil near varnish rocks. The top two spectra show ground bulk soil samples before and after treatment with glycerol. Lower spectrum is of the soil clay sized fraction.

cannot be attributed to any of the known and sequenced orders of the Ascomycetes. They may, therefore, represent a hitherto undefined order of the Ascomycetes. Morphological classification is nearly impossible due to flexibility of the fungi. According to Minter (1987), the taxonomic classification of black yeasts presents acute problems, "because some of the species are believed to exist in many different anamorphic states".

The great majority of meristematic fungi have melanized cell walls. They also contain mycosporins (Volkmann *et al.*, 2003; Whitehead, 2001). Propagule liberation is sarcinic conidiogenesis or endogenous conidiogenesis. Blastic conidia may be presentand often change in budding cells. MCF have a unique ability to survive where other microorganism are rarely observed (Perry and Kolb, 2004a; Jones, 1991; Taylor-George *et al.*, 1983). The survivability in these extreme conditions sets MCF apart from bacteria.

Sporulating bacteria have developed survival mechanisms but must expend energy to create spores. MCF, in contrast, survive and flourish where only few bacteria are present and no lichens. MCF contain melanin, mycrosporines, carotinoids and possibly other unidentified pigments that help protect them from UV light. MCF produce spherical clusters ca 20-200μm (Figure 3-27 and Figure 3-28) on rock and rock coatings in arid and semi-arid deserts. Found worldwide, they have been observed in many of the most arid deserts including the Sonoran, Mojave (Figure 3-28), Gobi (Figure 3-29), and Namibian (Perry *et al.,* 2004f). The growth form of MCF are characterized by the formation of restricted microcolonies, which are visible on rock surfaces (Figure 3-27). Vegetative cells have complex intracellular division patterns or a yeast-like budding pattern. Vegetative cells within these microcolonies are highly stress tolerant and extremely long lived. They are chemoorganotrophs deriving their nutrients from aeolian-supplied organics such as pollen. They are the predominant biological form observed on desert varnish rock coatings (Perry *et al.*, 2004f; Sterflinger *et al.*, 1999; Gorbushina *et al.*, 1993; Staley *et al.*, 1982, Perry, 1979). This unique association has caused some

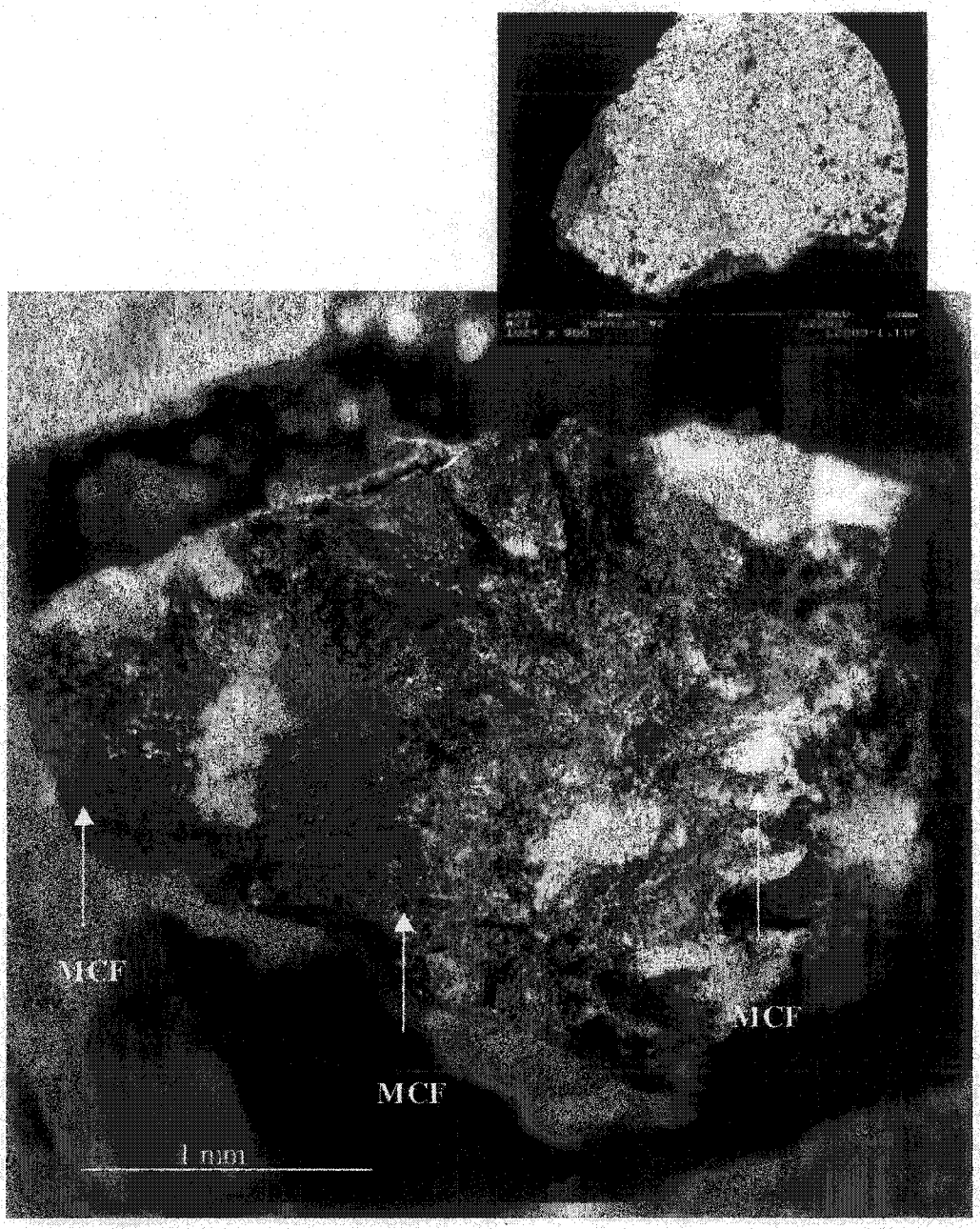


Figure 3-26. Optical image of Bishop, California varnish coated rock with MCF small black units which are difficult to see on black Mn rich varnish surfaces, but more easily seen on light mineal surfaces. The chip is mounted on half-inch circular SEM stud. SEM x20 magnification of the same sample (upper image) showing MCF as dark spots. The sample is from Grekin Road #180.

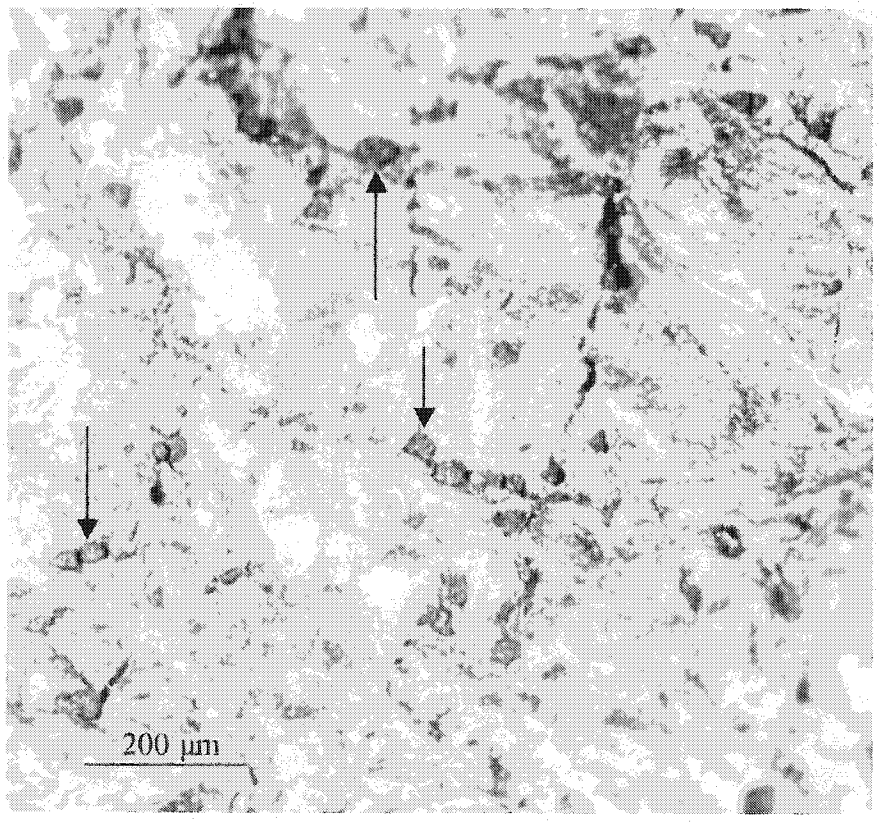


Figure 3-27. Light microscope image of MCF on rock surface from Bishop, California

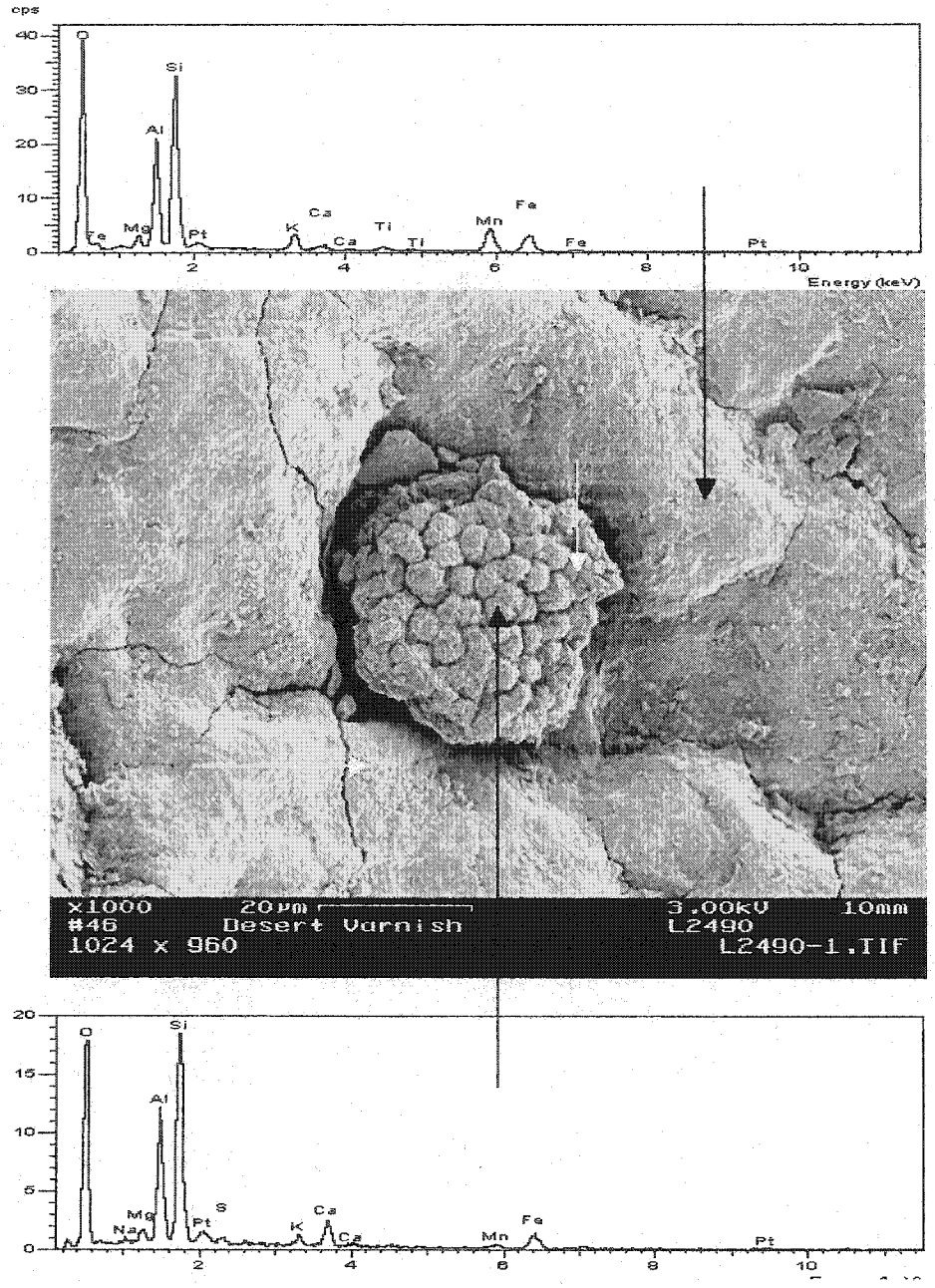


Figure 3-28. Bishop, California, MCF and EDS sample #11502. MCF are typically in depressions. As shown in Figure 3-29 the depressions are probably nearly always enhanced by MCF rather then the MCF forming in depressions. EDS shows the differences in the elemental chemistry of the MCF and the varnish coating. The white arrows depict the area analyzed for elements in Figure 3-28.

researchers to investigate them as the causative agent for desert varnish (Taylor-George *et al.*, 1983) MCF express a restricted growth pattern of yeast –like development both *in situ* and *in vitro*. Individual microcolonies appear to be size limited, rarely forming clusters exceeding several hundred spheres. It is possible that this size limitation is also a protective mechanism and that an equilibrium between exposure, nutrient uptake and size is reached. New colonies are formed with hyphae runners.

It has been suggested that spectral changes of rock surfaces that are usually attributed to metal oxidation of iron and manganese might also be staining by microbially produced organic and inorganic pigment absorbed polymers on rock surfaces or in coatings (Sterflinger *et al.*, 1999). As the colonies lyse and become part of rock coatings the organic and inorganic constituents might also be preserved. Phenol-containing melanins are probably the most stable compounds. Mycosporine-like amino acids (MAAs), frequently possessed by cyanobacteria are not present in microcolonial fungi. However, several related fungal mycosporins have been demonstrated and isolated from MCF (Volkmann *et al.*, 2003). Marc Volkmann tested one sample of varnish rock from the Baker California . No mycosporins were detected, however no MCF were observed in this particular varnish niche. This raises the question of whether or not MCF were present in the past or whether mycosporins are not stable. Further investigations are needed to test the stability of mycosporins. Fungal polysaccharides may form stable organomineral complexes with some clays (Chenu, 1989). EPS substances from MCF might also bind with clays, metal oxides and/or amorphous silica. This accumulation of inorganic substances mixed with organic compounds might be preserved in rock coatings and provide unique chemical markers for exobiological life detection (Perry and Kolb, 2004d).

MCF black coloration, particularly on black-varnished surfaces, makes them difficult to observe (Figure 3-26). Nevertheless their size up to ~200 μm and worldwide distribution makes their late discovery somewhat perplexing. MCF were first identified in the Sonoran Desert by Perry and Adams in 1977 and subsequently imaged with a

SEM (Perry *et al.*, 2004f; Perry, 1979). Morphological analysis placed them in the class Ascomycetes. For years they were referred to as blackberries and black globular units until they were more appropriately named microcolonial fungi by J. Staley (Staley *et al.*, 1982) and placed in the class Ascomycetes, family *Dematiaceae*. MCF were later found described in many deserts including the Victoria (Staley *et al.*,1983), in eastern Oregon State (Palmer *et al.*, 1990), Peru, (Jones, 1991)the Negev (Krumbein and Jens, 1991), the Sanctuary of Delos Cyclades, Greece, (Sterflinger and Gorbushina, 1997) the Namib (Gorbushina and Krumbein, 2000b; Gorbushina *et al.*, 2003) and the Gobi (Perry *et al.*, 2004).

The identification of dematiaceous fungi or meristematic fungi is difficult since different genera exhibit uniform *in situ* growth patterns under stress conditions typically found in deserts (Sterflinger and Gorbushina, 1997). They are normally black due to melanization (Staley *et al.*, 1982). Laboratory experiments and field observations (Figure 3-29) have shown that they cause decay and biopitting (Gorbushina and Krumbein, 2000b; Sterflinger and Krumbein, 1995; Krumbein, 1969).

The meristematic epilithic and endolithic fungi cause decay and biopitting of marble monuments and rocks similar to those found on rocks from the Gobi Desert (Figure 3-29). Nienow and Friedman (1993) reported meristematic fungi (epi- and edolitically) associated with Antarctic lichen communities. They at first assumed the fungi to be lichen parasites. However, *in vitro* experiments later showed that they do not form associations with lichens but produce substances inhibitory against bacteria, cyanobacteria, and algae. Meristematic fungi are found in diverse environments beyond deserts. These include inhabitants of other inorganic materials including glasses in manmade products (de Hoog *et al.*, 1997), and cauliflower-like lesions emerging from skin (muriform cells, Matsumoto *et al.*, 1984).

On desert rocks the colonies form scattered to denser: 200 microcolonies per square centimeter from the Victoria Desert, Australia while the concentrations reached 10,000 per square centimeter in samples from the Simpson Desert, Australia (Staley *et*

al., 1982). Although the colonies were not counted, MCF contribute to black coloration on rock surfaces from Bishop, CA (Figure 3-26) and also in the Gobi Desert (Figure 3-29) are black pigmented microcolonial fungi. Sterflinger *et al.*, (1999) show that red surface coloration is not only produced by iron oxides but also by pigments from microorganisms.

Elemental chemistry of MCF

The principal elements in MCF from the Mojave desert (Figure 3-28) are similar to those detected by Taylor-George *et al.* (1983). Young colonies usually contain O > Si > Al > Ca and lesser amounts of Mg >K > Na > Fe > Mn (Figure 3-30). Carbon is present (Figure 3-30 and Figure 3-31), but EDS does not provide a good quantitative measure of lighter elements, so it is difficult to say with any precision whether the above order is absolute. Comparison (Figure 3-28) with a Mn rich varnish substrate shows that Ca and C are more highly concentrated in the MCF, while Mn and Si are relatively more concentrated on the substrate. It should be noted in this investigation that there appears to be no enhancing or lessening of elements such as manganese surrounding the colonies. Jones (1991) also noted that there are no unusual concentrations of Mn surrounding MCF. Staley *et al.*, (1992) showed evidence that Mn was enhanced around MCF, differing from this study.

Taylor- George *et al.* (1983) and Perry and Kolb (2004) suggested that the colonies of MCF and black yeast microcolonies degrade and become part of rock coatings. Figure 3-31, Figure 5-11, Figure 5-12, and Figure 5-13 all show SEM images of older degrading fungi that apparently become integrated into varnish coatings. Thus, younger colonies have less mineral concentrations (Figure 3-28) and their elemental chemistry is consequently different (Figure 3-30). Similarity of the varnish surface with tht of MCF colonies is shown in Figure 3-29, where backscatter shows elemental composition is relatively uniform across the varnish surface and MCF.

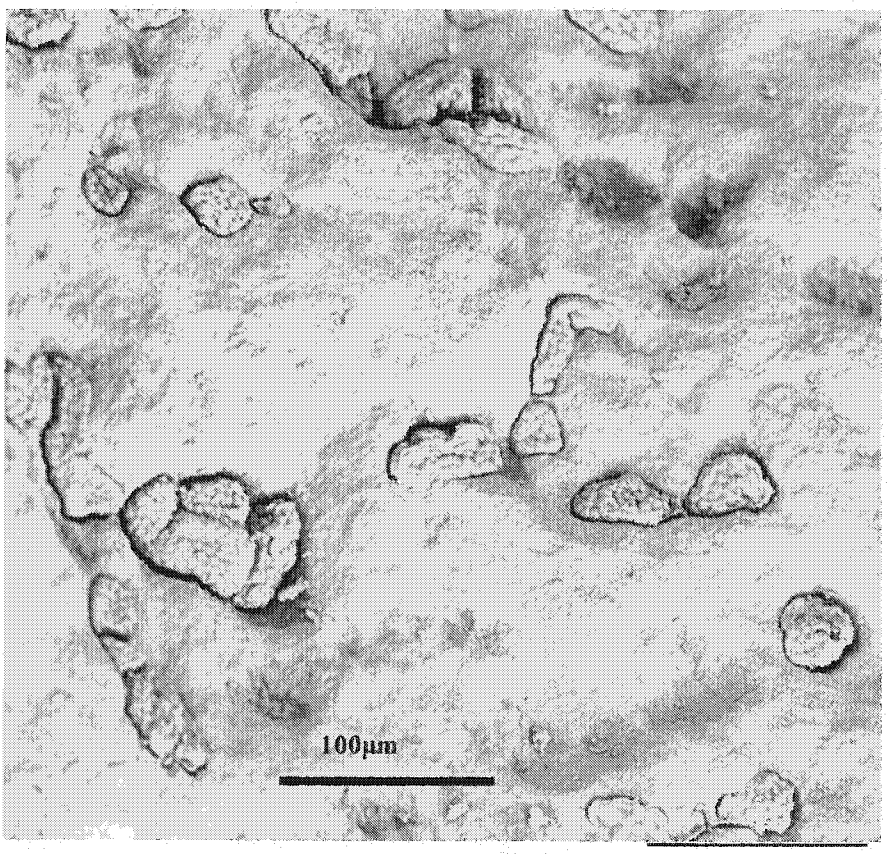


Figure 3-29. SEM backscatter image of MCF on varnished rock from the Gobi Desert, Mongolia. Heavier elements are lighter gray. Dark areas around MCF are where x-rays are blocked.

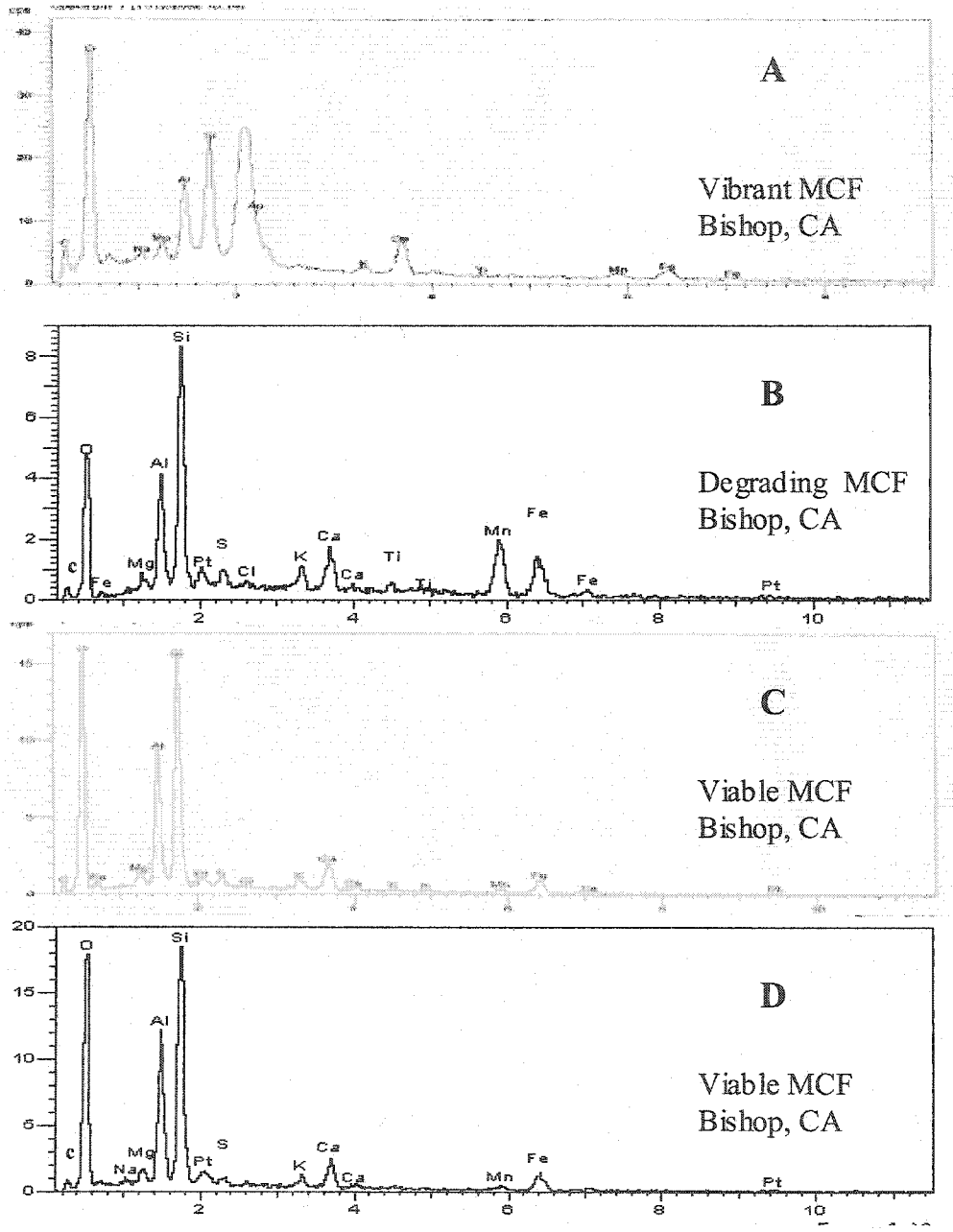


Figure 3-30. EDS comparison of MCF. EDS-A is from viable newer colony (Figure 5-9). The EDS electron beam was placed so that any beam penetration would extend past the rock substrate. EDS-B is for and old degrading MCF colony (Figure 5-11). The bottom two EDS-C and D are from two different spots as from the same MCF as depicted in Figure 5-9. The white arrow in Figure 5-9 shows the analysis point for EDS-C.

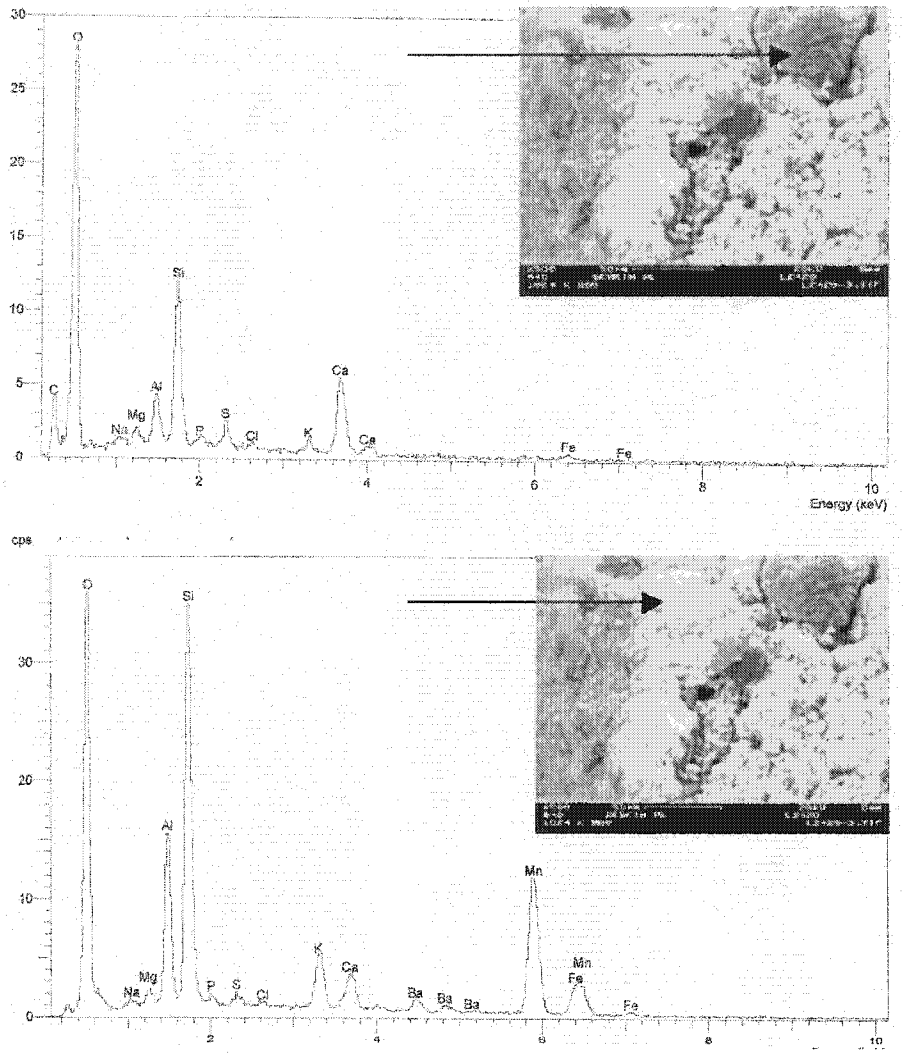


Figure 3-31. EDS of degrading MCF(top) from Bishop, California. Lower image and EDS are of the varnish surface. Elements including S, Cl, P, Na, Mg, K, Ca, Fe are in both coating and MCF, however the ratios and amounts are quite variable. The exceptions are substantial Mn and measurable Ba in the coating with neither detected in MCF.

Elemental composition of varnish topcoats compared to MCF

Figure 3-30 compares the elemental chemistry of MCF to a desert varnish substrate. The chemical composition of the varnish coating, while variable, is comparable to all varnish coatings analyzed. Heterogeneity is normal among coatings but there are threads of consistency. Oxygen is the largest peak present. This is almost always followed by silicon and the aluminum. The Si/Al ratio can vary significantly (Figure 3-31). After O, Si and Al there is no typical sequence of elements. Generally speaking, iron and manganese are the next most conspicuous. While manganese is often the tallest peak (after O, Si, and Al) in EDS tracings, it is not always present (Figure 3-30). Iron, however, is almost always present in all varnish coatings (Figure 3-30). Other elements vary significantly from sample to sample with K, Mg, Ti, Ca nearly always present and C, Cl, S, P, and Ba are frequently detected in varying quantities along with elements mentioned in the previous section. Carbon, Cl, S, and P are are elements frequently found in MCF (Figure 3-30).

All MCF analyzed with SEM-EDS have detectable carbon. Mineralized varnish coating samples also contain substantial carbon in their outer monolayers, as shown using XPS (Figure 3-12). Phosphorous and Chlorine were detected in increased amounts in varnish coatings in electron microprobe analyses (Table 3-2).

Perhaps the most noticeable trend in the ageing of some MCF is increasing manganese concentrations as shown by EDS analyses. Figure 3-30 (A, C, and D) compare EDS of younger colonies and show minimal manganese peaks. Visual inspection suggests that sample A may be younger than C and D (C and D are measurements from two locations on the same MCF as shown in (Figure 3-31). Iron is enhanced in C and D over manganese more than in A. The oldest degrading colony, sample B, shows an increase in iron over younger MCF but manganese is now substantially increased. It should be noted that sample A was coated with gold (Au) for SEM viewing and B-D were coated with Platinum (Pt). The Au peak obscures areas

where sulfur and Chlorine appear.

MCF conclusions

The elemental chemistry of MCF, as detected by EDS, is remarkably similar for all MCF analyzed, but with unique differences. As shown in Figure 3-30, oxygen is not always the most abundant peak detected. Silicon vies for the tallest tracing peak with oxygen. Of particular interest is how the elemental chemistry changes as colonies of fungi age. The age determination at this point is arbitrary and based on visual inspection. It is assumed that degrading colonies are indeed older and that healthy looking, less mineralized fungi are younger (compare Figure 3-28 to Figure 3-31).

In general most elements present in or on the surface of MCF are similar to varnish coatings. Carbon is enhanced and sodium seems more prevalent in the youngest colonies. There are substantial quantities of calcium in all MCF. Potassium seems to increase in older colonies, and as noted previously the iron/manganese ratio is inverted as manganese increases over iron in old colonies.

Summary

Oxide-rich areas are observed on varnish surfaces along with areas of nearly pure silica. The presence of silica is readily observed in XRD spectra in the clay-sized fraction after size separation of clays, silts, and sands. XRD spectra before separation detected the minerals albite, augite, anedosine, diopside, and quartz. Varnish crystalline source minerals from dusts and soil deposits dominate the spectra before size separation, obscuring clays and amorphous hydrated silica (opal) in the clay-sized fraction This may provide an explanation of why silica has not previously been detected. D-spacing of the clay sized fraction is suggestive of a layered hydroxide such as pyroaurite or hydrotalcite, rather than kaolinite or smectite in interstratified layers. Soils separated into similar sized fractions contained kaolinite, smectite and trace chlorite. Varnish samples, however, had only trace amounts of smectite and contained illite and kaolinite.

Outer surface monolayer analysis (XPS and TOF-SIMS) confirms that silicon is

the dominant inorganic element. Substantial quantities of carbon (~30 atomic weight %)were detected. After surface sputter removal of ~10 nm, carbon quantities remained high but were reduced to ~10 atomic weight %.

MCF are frequently associated with desert varnish coatings. Detrital grains cover MCF surfaces and the elemental chemistry of aging MCF resembles that of varnish coatings. Upon death, MCF appear to become incorporated into varnish surfaces along with remaining detrital grains and organic substances.

CHAPTER 4 ORGANIC COMPONENTS

'There is no phenomenon on the surface of the Earth, which is independent of life.'

W. Vernadsky, "La Biosphere"

Presented in this chapter are organic data results, obtained using various techniques, for total organic carbon (TOC), amino acids, DNA, and a diverse group of polymorphic organic compounds. All data obtained are from bulk chemical analyses. SEM was used to determine the presence of microbes on the surface of the varnish coatings. Few bacteria were observed. It cannot be known for certain whether or not detected organic components were from the interior or exterior surfaces. However, the lack of observable surface microbes, combined with the presence of carbon and nitrogen, following surface ablation during XPS and TOF-SIMS analyses, suggests but does not prove that the majority of the organic components found are from within varnish coatings.

Previous work

Merrill (1898 as quoted in Laudermilk, 1931) said that he found a small quantity of organic matter in desert varnish, which he suggests, "to have been deposited by external sources." Since the prevailing hypothesis of the era was deposition by the rise and fall of lakes, he attributes the organic mater to sediments formed by ancient lakes or oceans.

White (1924) proposed that the probable source of coatings was from wind-blown pollen of desert plants such as cacti, which adhered to the surfaces of rocks. The organic residue containing manganese compounds was changed to oxides by the action of heat and desiccation. It is now generally accepted that the source of materials for most varnish coatings is wind-blown matter. White's 1924 suggestion that wind-blown pollen (aeolian bio-compounds) of desert plants adhered to surfaces is supported with TOF-

SIMS surface chemical analyses later in this study, where organic substances such as Eugenol derived from desert plants are detected in the outer surfaces of varnish topcoats.

The first contemporary analyses of organic matter in varnish coatings were made by Dorn and DeNiro (1985). They suggested that, "varnish organic matter is derived from micrometer-sized airborne plant material that accumulates on rock surfaces (Dorn and DeNiro, 1985, p. 1472). They go on to suggest that microorganisms then metabolize some of this plant material. They made the point in their paper, that 13C varies with differing environmental zones.

A reference in a footnote in their paper states that, "varnish contains less than 1 percent organic matter by weight (Dorn and DeNiro, 1985, p. 1474)." It was not stated where or when organic matter was measured, nor how the measurement was determined. The amount of <1% organic matter has nevertheless become the accepted norm when considering varnish organic components.

Table 4-1. Total organic carbon (TOC) of desert varnishes from the Mojave Desert. Analysis was performed at Humble Geochemical Services in Houston, Texas as described in the methods section. All samples were collected, with one exception, on Jan 13 and 14, 2004 and immediately sent for analysis.

Collection site	**TOC**
East Death Valley site X, Site X, Desert Varnish	
Sample A	0.43
Sample B	1.31
West Death Valley- Panamint Springs, Desert Varnish, Springs, Desert Varnish	
Sample A	1.07
Baker, California	
Sample A	1.84
Sample B	0.71
Sample C	0.82
Sample D*	0.15
Soil from surface (Figure)	0.8
Soil from 6cm below surface (Figure)	0.21
* Sample collected Jan 2003 and not processed until October, 2003.	

The only previous measurement of total organic carbon (TOC) for a rock coating, known to me, was made for one sample, by Nagy *et al.* (1991). They reported C_{total} as 1.4% and $C_{organic}$ as 0.35%. Total organic carbon data presented in Table 4-1 show that samples measured from three different areas of the Mojave Desert commonly have TOC greater than then 1%. As noted by Staley *et al.* (1992), most of the bacteria isolated from varnish are Gram-positive organisms. Nagy *et al.* (1991) also claimed the predominant micro-organisms cultured were Gram-positive, spore forming bacteria, and also filamentous streptomycetes (see MCF this chapter). A study by Palmer *et al.* (1986) found that most of the isolates from the Sonoran and Mojave Deserts capable of manganese oxidation were Gram-positive bacteria, including *Micrococcus, Planococcus, Arthrobacter, Geodermatophilus,* and *Bacillus.* Perry and Adams (1978) also isolated a gram-positive *Bacillus* sp. from the surface of desert varnish near Deem Hills. Likewise, of the 79 bacteria isolated from varnish (Hungate *et al.*, 1987), only one was gram-negative. Eight of these were spore forming, gram-positive rods, five of which were motile and identified as *Bacillus* species. Two other gram-positive microbes were slow-growing cocci, belonging to the genus *Geodermatophilus.* Three types exhibited a rod-to-coccus transformation in their development cycles, characteristic of *Arthrobacter sp.* One group contained gram-positive, non-motile cocci that formed tetrads, as well as single cells, and were identified as *Micrococcus* sp. Eppard *et al.* (1996) found only members of the order actinomycetes, including *Geodermatophilus* species, on rocks and monuments with the exception of one *Bacillus* species.

In contrast, most of the aquatic bacteria that oxidize manganese as well as iron are gram-negative and include the sheathed bacterial genus *Leptothrix* and others such as *Pedomicrobium* and *Hyphomonas.* "*Metallogenium*" has been described from aquatic habitats and soils (Perfil'ev *et al.*, 1965; Bolotina, 1976) but there is some doubt that it is a living organism (Klavness, 1977; Gregory *et al.*, 1980). However, Dorn and

Oberlander (1981) identified gram-negative "*Metallogenium*"-like bacteria and *Pedomicrobium*-like organisms from desert varnish in the American Southwest and proposed that these organisms explained the origin of desert varnish in general.

Culture-based techniques tend to underestimate microbial diversity. Dramatically different results are obtained by culture independent 16S rRNA sequencing as presented herein. Evidence for preliminary results of culture independent analyses of desert varnish was first presented in Perry *et al.* (2002) and Perry *et al.* (2004e). Subsequently, microbial DNA was detected in another preliminary study (Kuhlman *et al.*, 2003) from different area of the Mojave Desert.

Total organic carbon (TOC)

Presented here (Table 4-1) are the first analyses known to me for total organic carbon of desert varnish from east and west Death Valley and near Baker California. TOC from the Sonoran desert was previously published by Nagy *et al.*(1991) for one sample with the result of 0.35 %. The samples analyzed were observed to have no visible macroscopic surface organisms such as lichens and MCF. Samples were collected in January and there was rain fourteen days before the collection time. While no macroscopic organisms were observed, the wet conditions provide a viable environment for microorganisms such as Cyanobacteria, Bacteria or Archaea. The samples were preserved in glutaraldehyde, as described in the methods section, for later analysis using scanning electron microscopy.

All of the three collection sites in the Mojave Desert have samples that contain measurable organic carbon (Table 4-1). The lowest amount is for a sample collected and stored for a year before analysis. The stored sample was analyzed in a preliminary analysis. It is noteworthy that the surface soil sample contained similar amounts of TOC to the varnish coatings (far less than some varnish coatings), and that the lowest TOC measured, excluding the stored sample, was for a soil sample ~6 cm below the surface. The presence of measurable total organic carbon represents the first step in asserting that organic compounds are indeed present in or on varnish coatings. The chemical analysis

for TOC suffers from the fact that it is bulk in nature making it impossible to discern whether the measured TOC was from the interior or exterior of the varnishes. It is the nature of the carbon compounds that will be the focus of the rest of this chapter.

Carbon and Nitrogen in varnish coatings

XPS measurements of varnish topcoats were made before sputter removal of the surface (as collected) and after sputter removal of 5-10 nm of surface material. All surfaces are capable of adsorbing aerosols, and carbon compounds are adsorbed within hours of exposure to the air. Varnish coatings, therefore, adsorb these compounds over time. Amino acids are present and nitrogen is part of their chemical structure. Carbon is, of course, a fundamental part of organic compounds and carbon should be present for both amino acids and DNA. Most previous measurements of carbon have been made using electron diffraction x-ray analyses (EDAX). Lighter elements are not easily detected using EDAX, but are readily detected using XPS.. Several figures (e.g. Figure 4-1) are presented showing varnishes, bottom coats, and substrates both before and after sputter removal of the surface from several areas. The results vary but substantial amounts of carbon are present, typically near 30 atomic weight % as collected, and are reduced to typically 10 atomic weight % after sputter removal. Thus, carbon is present in the varnish coats. Red bottom coats have a similar drop in carbon after sputter (~20 weight % dropping to ~8 weight % after sputter). The varnish substrate or rock interior shows typically < 1% carbon and nitrogen both before and after sputter removal. Besides substantial amounts of carbon and nitrogen these data also show substantial amounts of silicon and low amounts of manganese on and in outer nanometer scale layers (Figure 4-1).

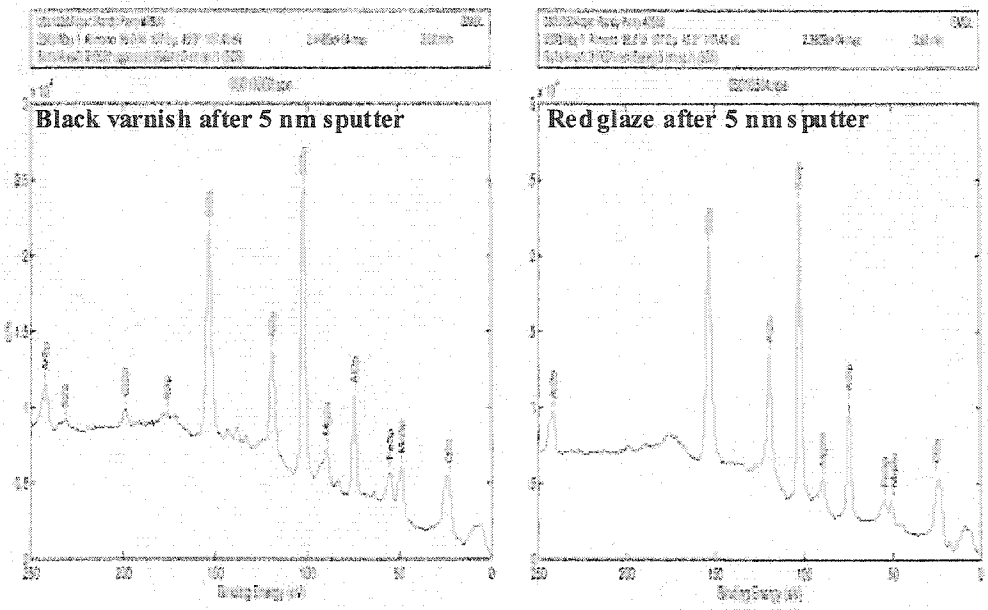
Amino acids

Thirteen protein amino acids were detected in samples from the Sonoran Desert (Table 4-2). While the concentrations varied from sample to sample the relative amounts stayed remarkably similar (Figure 4-2). Two unusual amino acids not associated with

protein, β-alanine and γ-amino butyric acid (γ-ABA) (Table 4-4) were found. They are known to be decarboxylation products of aspartic and glutamic acids, respectively, which were present in high abundance. The decarboxylation reaction is generally attributed to enzymatic activity (Meister, 1965; Schroeder and Bada 1976) and the presence of the decarboxylation products therefore suggests the possibility of microbial activity.

It is noteworthy that the proportion of D-alanine and D-glutamate to their respective L-stereoisomers is considerably greater than that of D-aspartic acid when D-aspartic acid was detected (Table 4-3 and Figure 4-3). Since D-aspartic acid is known to undergo rapid racimization relative to other L-amino acids, this finding is suggestive of a biological origin of the D-alanine and D-glutamic acids. D-alanine and D-glutamic acids are found in the petidoglycan of almost all members of the domain Bacteria (Beveridge,1989). Therefore, the presence of these D-enantiomers can be explained by the occurrence of bacteria or their petidoglycan remnants in or on the varnish as has been reported for bacteria in anoxic marine sediments from the Saanich Inlet in British Columbia (Kvenolden *et al.* 1970).

Diaminopimelic acid was not detected in any sample. This diamino acid is not found in protein, but is commonly found in the bacterial peptidoglycan of many gram-negative bacteria. In contrast, lysine is not only an amino acid in protein but is the common diamino acid of the peptidoglycan of many gram-positive bacteria. Lysine was found in the desert varnish samples analyzed. This finding is consistent with the presence of Gram-positive bacteria or their remains. The source of D-valine is unknown. It has never been reported in protein or in bacterial peptidoglycan. It is possible that D-valine is co-eluting with another substance in the chromatographic columns. GC/MS analyses were not performed to clarify its identity.



C	31.33
N	2.15
O	47.16
Na	0.52
Mg	1.05
Al	4.18
Si	10.21
Mn	1.01
K	0.89
Fe	1.50

Atomic % black varnish top coat "as collected"

C	20.02
N	2.96
O	54.78
Na	0.30
Mg	1.41
Al	5.20
Si	13.28
Mn	0.13
K	0.65
Fe	1.27

Atomic % red bottom glaze "as collected"

Figure 4-1. XPS of desert varnish top coat (left) and bottom glaze, "red" coat (right) from Baker, California, Mojave Desert (sample VR31903). The spectra (top right and left) are after 5 nm removal of surface material by sputtering with Ar+ ions *in situ*. Typically, carbon is reduced the most by sputter and in this sample from 31.33 atomic % before sputtering to 10.76 atomic % for black desert varnish, and from 20.02 to 7.97 atomic % in the red glaze. Carbon is deposited on the surface, over time, by aerosols. Other elemental components are consequently increased to bring the total values to 100%, however the increases as described in the text are not necessarily proportional. The atomic % listed (lower left and right) are "as collected" before Ar+ ion sputter.

Table 4-2. Amino acid data for deem hills and Painted Rocks, Arizona.

	Cation exchange chromotagraphy					HPLC
	Painted Rocks		Deem Hills		Painted Rocks	Deem Hills
Sample #	#1	#2	#3	#4	#5	#6
Amino acid	umol/g	umol/g	umol/g	umol/g	umol/g	umol/g
Alanine	1.69	5.06	0.43	1.43	4.67	0.64
Arginine	0.37	1.51	0.07	0.27	0.08	n.d.
Aspartic acid	1.14	3.96	0.28	0.94	4.87	0.23
Glutamic acid	1.38	4.87	0.33	1.1	5.62	0.32
Glycine	1.21	4.85	0.45	1.33	4.25	0.59
Histidine	0.09	0.3	n.d.	n.d.	0.56	0.06
Isoleucine	0.55	1.81	0.11	0.34	1.26	0.1
Leucine	0.8	3.07	0.15	0.57	2.08	0.52
Lysine	0.57	1.75	0.1	0.35	0.87	0.49
Methionine	n.d.	n.d.	n.d.	n.d.	0.04	n.d.
Phenylaline	0.15	1.04	0.06	0.24	*	0.3
Proline	0.56	1.5	0.15	0.5	n.d.	n.d.
Serine	0.8	3.71	0.25	1.11	3.47	0.32
Threonine	1.25	4.18	0.23	0.76	4.14	0.06
Tyrosine	n.d.	0.17	n.d.	n.d.	*	n.d.
Valine	0.87	3.95	0.18	0.75	2.34	0.6

Note n.d. is no data or below detection limit * Peak interference prevents calculation.

Ion exchange chromatography analyses were run at AAA Laboratory in 1991, High Performance

HPLC analyses were performed at University of Oklahoma in 1991.

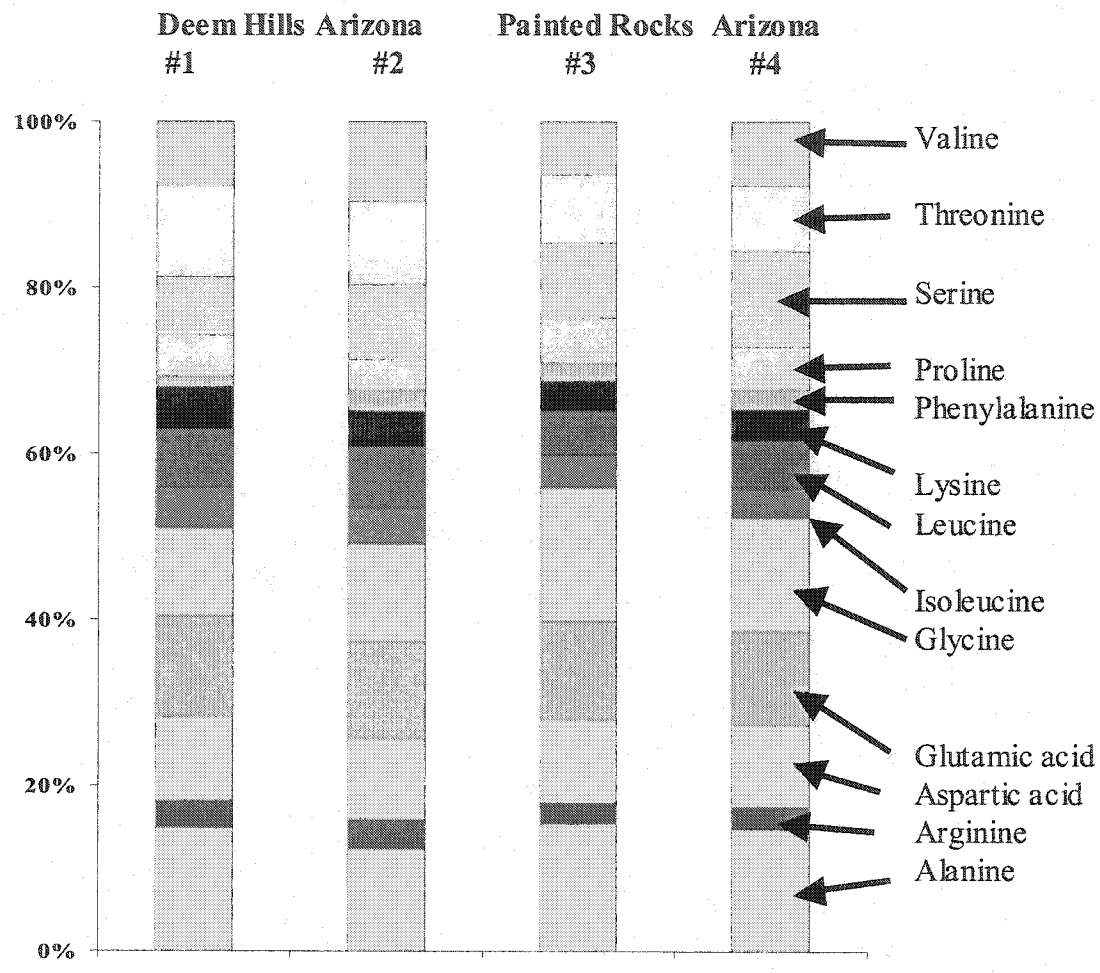


Figure 4-2. Relative abundance of 13 protein amino acids analyzed at AAA Labs, Seattle, Washington, using cation exchange chromatography in 1991.

Table 4-3. D/L enatiomeric ratios

	Painted Rocks #5	Deem Hills #6	Deem Hills*	Death Valley*
D/L-alanine	0.046	0.030	0.237	0.067
D/L-glutamic acid	0.055	n.d.	n.d.	0.180
D/L-valine	0.061	0.100	0.098	0.026
D/L-aspartic acid	n.d.	n.d.	0.071	0.022

Note n.d. is no data or below detection level.

Stereochemistry of the amino acids in #5 and #6 from Arizona were performed in 1990 at the University of Oklahoma using gas chromatography, and were calculated from peak areas.

* Deem Hills and Death Valley, California samples were measured using HPLC at Scripps Institute of Oceanography and the ratios were calculated by height measurements.

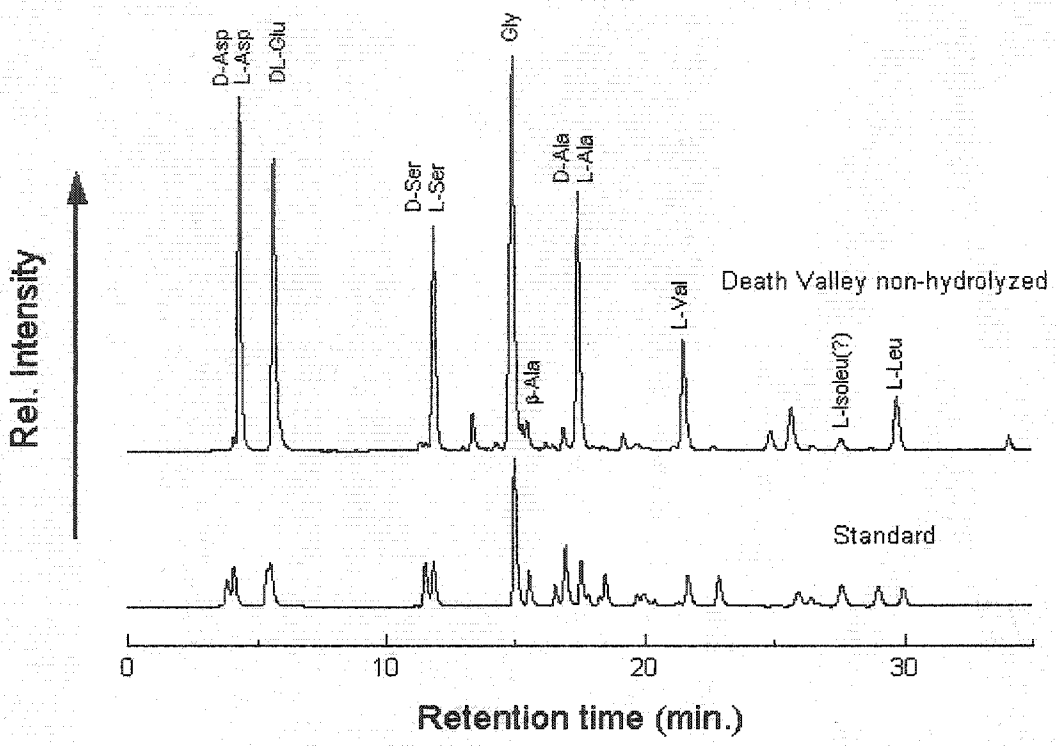


Figure 3 Amino acid tracing using HPLC Chromotagraphy at Scripps Institute of Oceanography in 2001.

Figure 4-3. Amino acid tracing using HPLC, Scripps Institute of Oceanography (from Perry *et al.*, 2003a)

Table 4-4. Non-protein amino acids.

	Painted Rocks Arizona μm/ gram	Deem Hills Arizona μm/ gram
D-alanine	0.21	0.02
D-glutamic acid	0.31	n.d.*
β-alanine	0.49	0.15
γ-ABA	0.14	1.03

Note n.d. is no data or below detection level.

The overall low concentration of amino acids in this sample may account for the inability to detect D-glutamic acid.

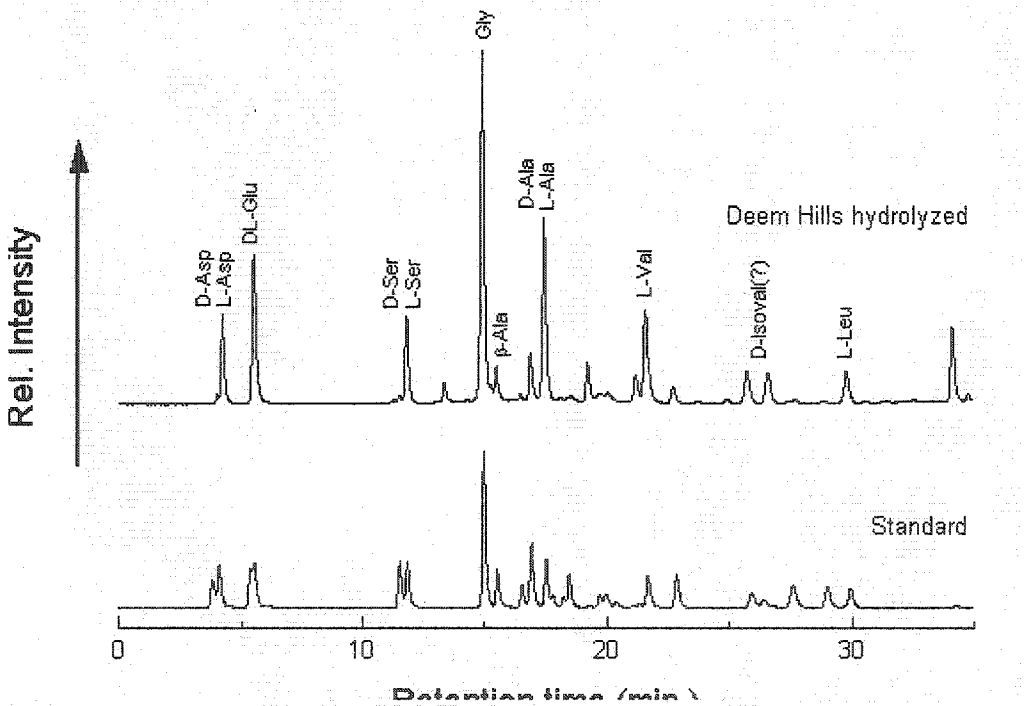


Figure 2. HPLC chromatogram of Deem Hills Arizona desert varnish analyzed at Scripps Institute of Oceanograph

Figure 4-4. HPLC chromatogram of Deem Hills, Arizona hydrolyzed desert varnish analyzed at Scripps Institute of Oceanography. (from Perry *et al.*, 2003a)

DNA Culture independent results

Initial results of culture independent analyses of desert varnish were presented by Perry *et al.*, 2002 and 2004e. DNA in this study was extracted from 500 mg of varnish, and using Polymerase Chain Reaction (PCR), was amplified for bacterial 16S ribosomal RNA (rRNA) genes for DNA from varnish as template with primers as discussed in Chapter 2 (Methods). Clone libraries of resulting PCR product were constructed and screened by amplified ribosomal DNA restriction analysis (Moyer *et al.*, 1994). Clones were divided into groups that shared the same RFLP pattern, and plasmid from selected clones in each group was sequenced with the same primers used for amplification by the UW-Seattle Biochemistry DNA Sequencing Facility. Resulting full-length sequences were checked for chimeras provided by the Ribosomal Database Project-II (RDP-II) (Cole, 2003), which identified that many of the bacterial sequences were chimeric in nature, but none of the Archaea. Sub-sections of these sequences that did not appear to be chimeric in nature aligned by the sequence aligner. These sub- sections of sequences were assigned to a taxonomic group using a sequence match tool provided by RDP-II and by BLAST (Altschul *et al.*, 1997). If this analysis indicated similarity to more than one group, the sequence was designated as "uncertain" taxonomic affiliation as shown in Figure 4-5. Archaeal sequences were not chimeric, and phylogenetic analysis inferred them to be closely related to non-thermophic soil Crenaorchaeota (Figure 4-5).

These DNA analyses indicate that there is a diverse community of microbial species on or in varnish surfaces (Figure 4-7 and Figure 4-6). The presence of 16S rDNA sequences similar to those of photoautotrophic organisms, namely non-sulfur bacteria and algae, suggests that primary producers may be present (Figure 4-5).

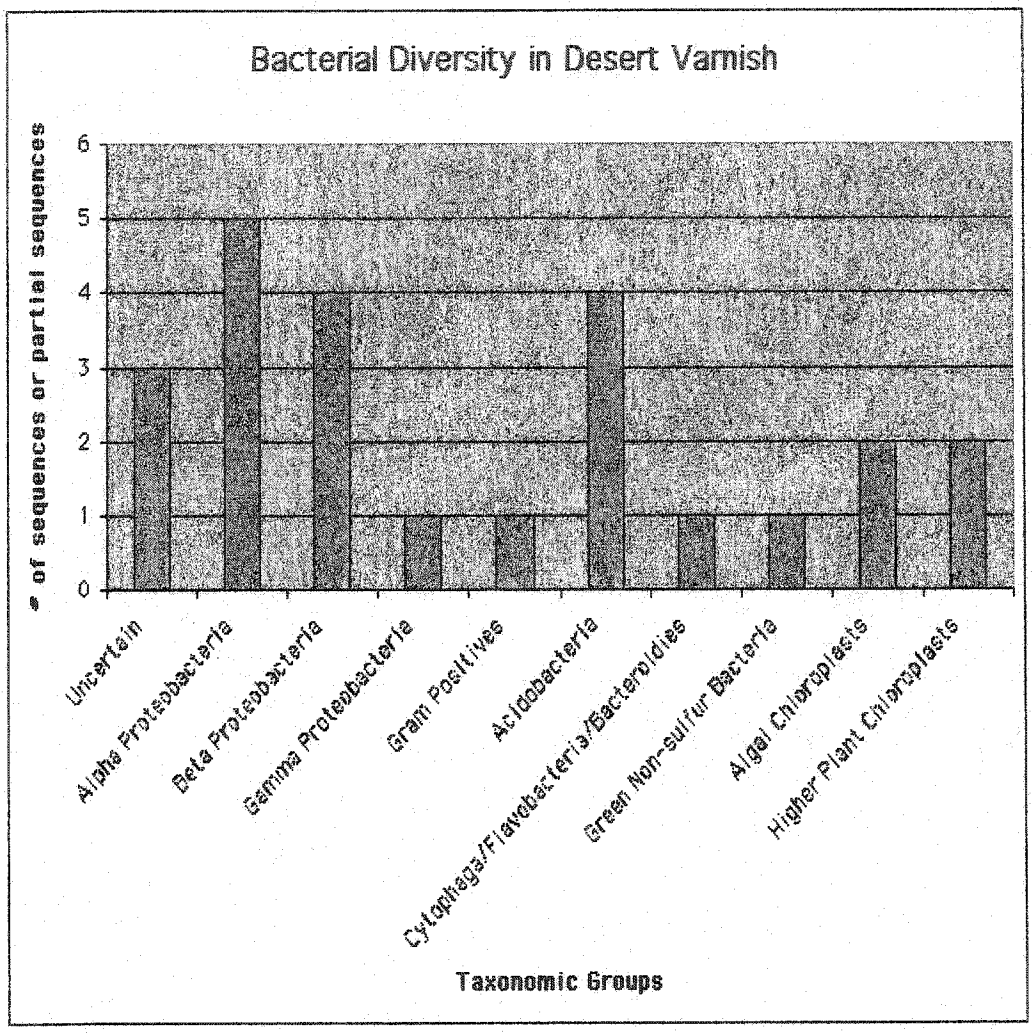


Figure 4-5. Bacterial diversity in desert varnish coating from the western boundary of Death Valley, CA. A sequence match tool, as described in the text and Chapter 2 (Methods), was used. If the analysis indicated similarity to more than one group, the sequence was designated as "uncertain" taxonomic affiliation as shown on the far left of the histogram above.

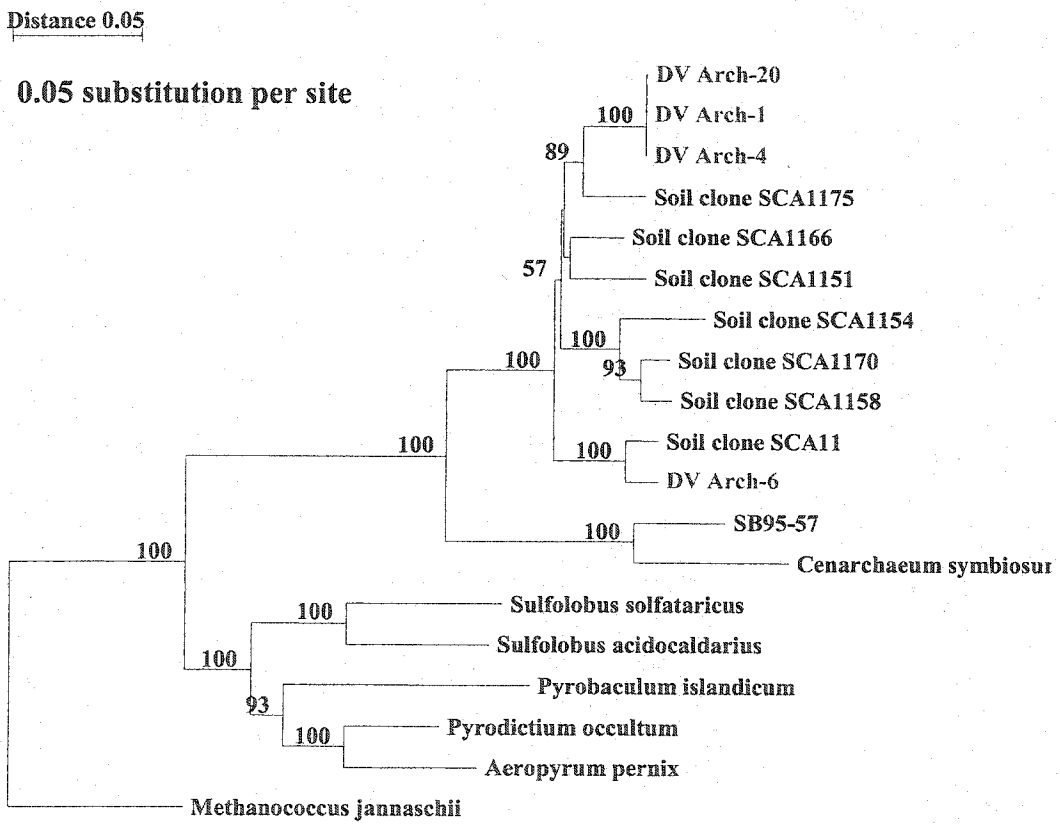


Figure 4-6. Tree of archaeal clones of 16S Rrna genes, PCR amplified from DNA isolated from desert varnish, Death Valley, CA. Desert varnish clones from this study, designated "DV Arch" for Archaea and followed by the clone number, are in red. Bootstrap values below 50 are not shown on the tree. Representatives of the non-thermophilic (upper portion) and thermophilic Crenarchaeota are shown. There are no cultured representatives of the non-thermophilic Crenarchaeota (e.g. SCA clones).

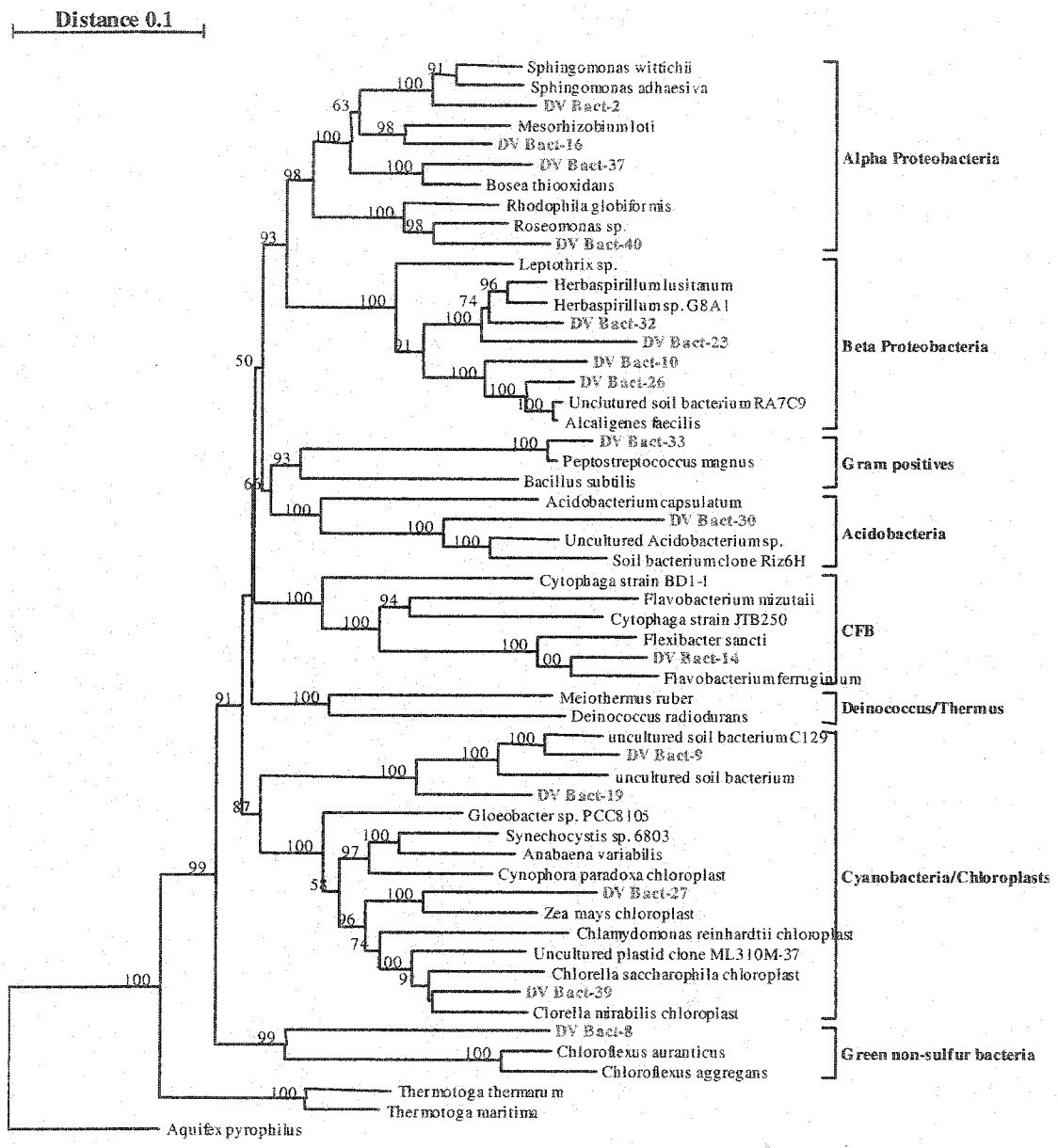


Figure 4-7. Preliminary bacterial tree. Sequences for selected bacteria shown in Figure 9.4.

In contrast to the cultural work, there were several Gram-negative bacterial signatures identified using molecular techniques. Especially noteworthy were a diverse group of alpha and beta proteobacteria. Besides the Gram-negative bacteria groups of Gram–positive bacteria, Acidobacteria, Green non-sulfur bacteria, cyanobacteria, and Cytophaga/Flavobacteria/Bacteroldies were also present. Six Archaea were also identified. All of the Archaea fall within a group of soil clones as yet unidentified in culture (Figure 4-6).

Polymorphic organic chemical signatures

Several polymorphic organic compounds were identified using TOF-SIMS. Figure 4-8 shows the chemical structure of twelve compounds. One of the twelve compounds was not found in the chemical data base (NIST). Of the eleven that were in the NIST data base four were from plants or pigments such as Number 3 (eugenol), below and in Figure 4-8, which is a phenol typically from desert plants such as sagebrush (*Artemesia*). Two of the compounds (Number 7 and 10) are used in pesticides. Number 4 is a naturally occurring plant component of mitochondria. Number 8 is another organic by-product and Number 12 is quinone found in tar-contaminated soils, in other words, a remnant of the oil age. The finding of these compounds makes sense in that all would be expected in the environment not many miles from metropolitan Los Angeles. Pesticides and byproducts of oil industry and desert plant residues are being adsorbed onto varnish surfaces. Lead 208 was found in this study in the Mojave Desert (Figure 3-18), east of the Los Angeles industrial basin, and in a much reduced amount in the Gobi Desert. The absorbtion of aerosols also supported by Bao *et al.,* (2001) suggests that these chemicals may be deposited in the micro-sedimentary layers of varnish coatings and provide a potential measurement of paleo environments. Following is a description of the polyfunctional organic compounds identified from Death Valley desert varnish (Figure 4-8) :

N'-ethyl-N,N-dimethyl-p-phenylenediamine. An amine that forms strongly

colored yellow to red salts, and has been shown to adsorb on nanocyrstalline TiO_2, ZrO_2, and Al_2O_3.

1-[3-methoxy-5-hydroxybenzyl]-1,2,3,4,5,8-hexahydro-Isoquinoline. Possibly an alkaloid.

4-allyl-2-methoxyphenol is a phenol present in many plants such as including sagebrush (*Artemesia spp.*). eugenol (phenyl with hydroxyl, methoxy (meta to OH), and propenyl (para to OH) see Figure 4-8.

2,3,5,6-tetramethyl-p-benzoquinone. Duroquinone. Naturally occurring compound possibly involved in electron transport in plant mitochondria

2,4,5-trimethyl-benzoic acid. Trimethylbenzoic acid present in hardwoods. Possibly flavones and found in plant pigments.

2-methoxy-6-(2-propenyl)-phenol. An aromatic compound isolated from *Populas* spp. tree bark. *Populas tremuloides* (Quacking Aspen), *Populus angusifolia* (Narrowleaf Cottonwood), *Populas trichocarpa* (Black Cottonwood) may contain this oil.

2,3-dihydro-2,2-dimethyl-7-benzofuranol. Carbouranphenol found in pesticide.

4-ethenyl-1,2-dimethoxy-benzene. An organic compound marker in lake sediments.

2,5-dibutyl-3,6-dimethyl-piperazine. Not found in NIST.

4,5-dimethoxy-isobenzofuran-1,3-dione. Possibly found in pesticides.

1,8-Anthracenediamine. Heterocyclic aromatic compound.

9,10-Anthracenedione. Polycyclic aromatic hydrocarbon. Anthra quinine that has been found in tar-contaminated soils.

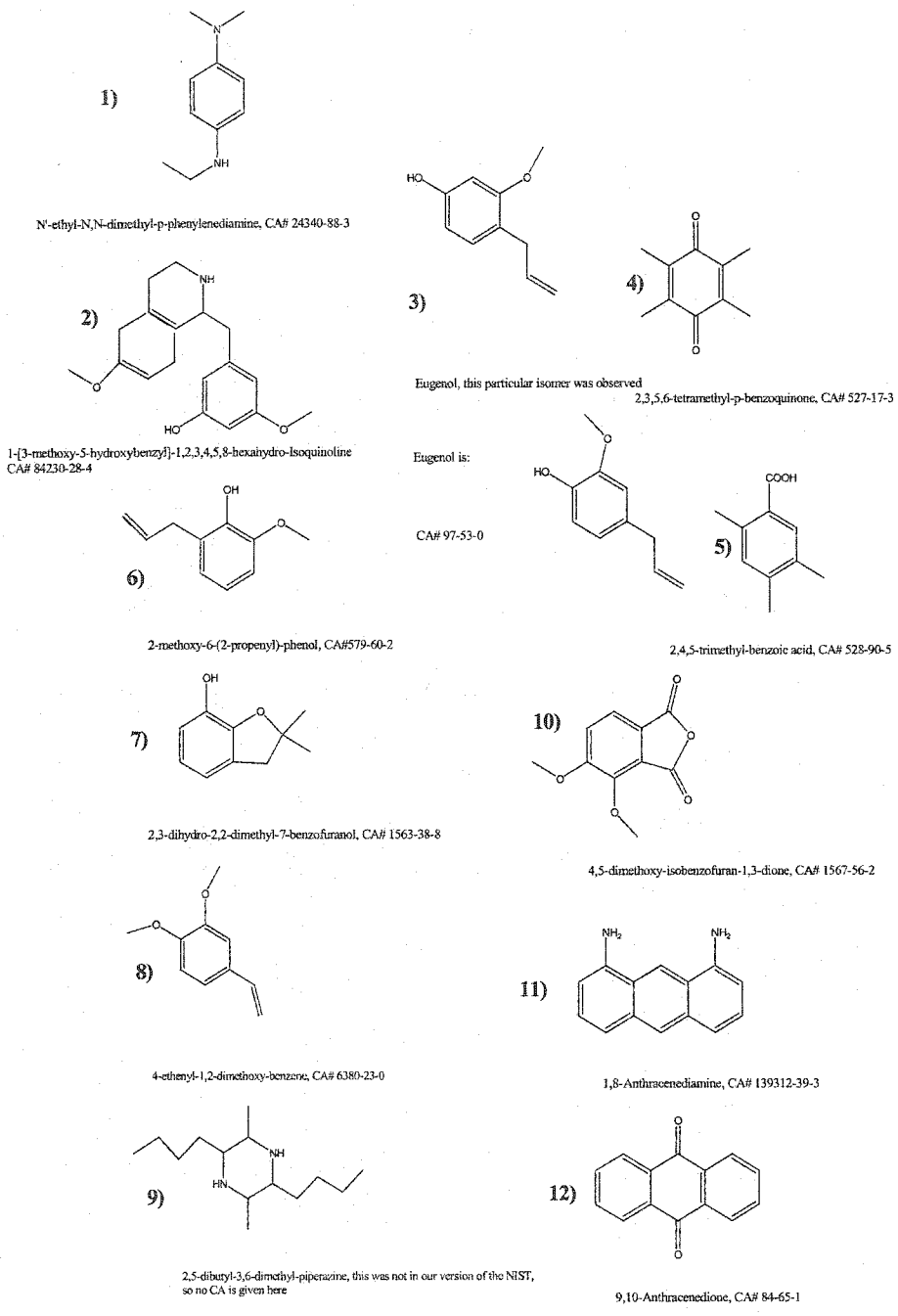


Figure 4-8. Polyfunctional compounds identified in varnish coatings from Death Valley, California, using TOF-SIMS.

Summary

The analyses of varnishes from the US southwest show that they contain complex and measurable amounts of organic compounds, carbon, nitrogen, and TOC. These are the first analyses known that have shown D-enantiomers of alanine and glutamic acid in a rock coating. The presence of D-alanine and D-glutamic acid along with lysine is consistent with the chemical composition of the peptidoglycan in cell walls of Gram-positive bacteria.

Previous culture based studies have also primarily found Gram-positive bacteria. However, culture based studies tend to underestimate microbial diversity. The culture independent results of 16S sDNA from rock coatings, in this study, indicates that there is a diverse population of microbes, including Gram-positive and Gram-negative bacteria, as well as primary producers in varnish bulk powder samples. Many of the sequences of bacterial 16S rDNA were chimeric. Archaeal sequences, however, were not chimeric, and phylogenic analysis inferred them to be closely related to non-thermophilic soil crenorchaeota.

The presence of pesticides, organic components from plants such as eugenol from Joshua trees, support the view that coatings accumulate materials from local environments. Evidence for MCF as subaerial biofilms, becoming part of coatings is consistent with this hypothesis, if they become part of coatings after death. Unstable amino acids, along with nitrogen, carbon, and polyfunctional chemicals, require a preservation mechanism. Unstable amino acids, such as serine and threonine, along with glutamic and aspartic may be preserved when complexed with amorphous silica. It is the ability of these amino acids to form complexes with the hydroxyl groups of silicic acid that may provide an explanation for their presence.

CHAPTER 5 DISCUSSION

Only theory can tell us which experiments are to be meaningful.

...theory cannot be fabricated out of the results of observation, but that it can only be invented.

Albert Einstein.

The discovery of biological chemicals in desert varnish, some of which are unstable, raise the question, how can unstable organics persist? Labile organics such as hydroxylated amino acids and DNA require a mechanism for their preservation, especially in hot deserts. The finding of silica in US southwest deserts provides an explanation for preservation by complexation and/or entombment of unstable organic compounds, which then leads to a possible explanation for how desert varnish forms. The polymerization of hydrous silicic acid ($Si(OH)_4$) or (di)silicic ($(HO)_3Si\text{-}O\text{-}(Si(OH)_3$) into amorphous silica provides explanations not only for the preservation of labile compounds but, also explanations for questions concerning what makes desert varnish and silica glazes hard, have a lustrous patina, and form botryoidal and lamellar morphologies.

It is suggested here that the slow dissolution of silica from both anhydrous and hydrous minerals, its subsequent gelling, polymerization, and hardening, provide a possible underlying mechanism of formation for desert varnish coatings and silica glazes and the incorporation of detrital grains, organics, and aerosols from local environments into coatings. Silicic and (di) silicic acid form various complexes with ions and organic molecules, especially those enriched in hydroxyl amino acids (serine and threonine), and others found in this study such as glycine, aspartic and glutamic acids. Although, organo-silica complexes were not directly observed, previous work has shown that

amorphous hydrated silica forms both Si-O-C and Si-O-metal complexes (Sullivan, 1986).

This mechanism for cementing coatings can be applied generally to most subaerial coatings, including silica glazes (Curtiss et al., 1985; Farr and Adams, 1984; Farr, 1981), and possibly other iron (red) and manganese (rich dark) coatings from arid andsemi-arid worldwide locations. Silica glazes from Peru and Hawaii (Farr and Adams, 1981) resemble desert varnish in their luster and hardness. They are thin and often exhibit growth patterns similar to varnishes, but otherwise do not resemble dark varnish coatings that have additional mineral, detrital and organic components from their local environments.

The silicic acid process then, although involving organic compounds, falls on the continuum line just on the side of a non-biological process. However, rock coatings occur in diverse environments, and the diversity exemplified by their variable compositions is quite different. While polymerized silica may provide the glue that adheres various components together, the addition of organic compounds can perturb the solution chemical processes. As has been shown microorganisms (MCF) or their decaying components (amino acids, DNA, and polymorphic organic compounds), can become incorporated into coatings. Living bacteria or fungi, their saccharide and glycoprotein residues, will have an effect on altering the chemistry of coating formation.

A proposed model for the role of silicic acid in rock coating formation

The role of silicic acid in desert varnish formation has not been previously explored. The presence of silicic acid as a primary component is a shift from the established belief that varnish topcoats are mostly composed of clays cemented by oxides. Black oxide-rich topcoats in this study have little clay, and no birnessite manganese minerals, which supports the findings of Raymond *et al.*, (1992). The clays that are present are different from soils and dusts (Figure 3-24 and Figure 3-25), suggesting they may be altered before or during incorporation into coatings. The opposite is true for red bottom glazes. They are composed primarily of layered clays

(Figure 3-7 and Figure 3-21), which supports the analyses of Potter and Rossman (1977). Topcoats, as shown in this study and in previous studies, are derived from materials that land on surfaces. Evidence for red bottom-coat formation, in this study, is by the dissolution of adjacent soils and their subsequent deposition onto rock undersides. The solution deposition process for both topcoats and under-coats removes silicon from silicates, promotes the formation of silicic acid, which polymerizes, and serves as the glue that binds together components present from the local environment.

Silicic acid, $Si(OH)_4$, is the principal product of dissolution of silica, SiO_2, (quartz or amorphous silica) and also from the dissolution of silicates. The solubility of SiO_2 is very low at pH values that are less than ~ 9 (Figure 5-1). At high pH the, concentration of silicate ion is increased. The solubility of silicic acid is enhanced by the presence of amino acids and other organic molecules including carbohydrates (Coradin and Livage, 2001). Silicic acid is prone to polymerization via condensation of the silanol groups, SiOH, a process in which water is eliminated (Figure 5-2 and Figure 5-3), to form silica gel.

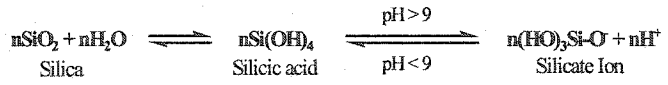


Figure 5-1. Formation of silicic acid, silicate ions.

The production of silicic acid under desert varnish conditions is dependent on the presence of water, organic materials, and pH. Dusts are composed of various materials including silicates, alumina silicates, oxides and oxyhydroxides, pollen, diatoms

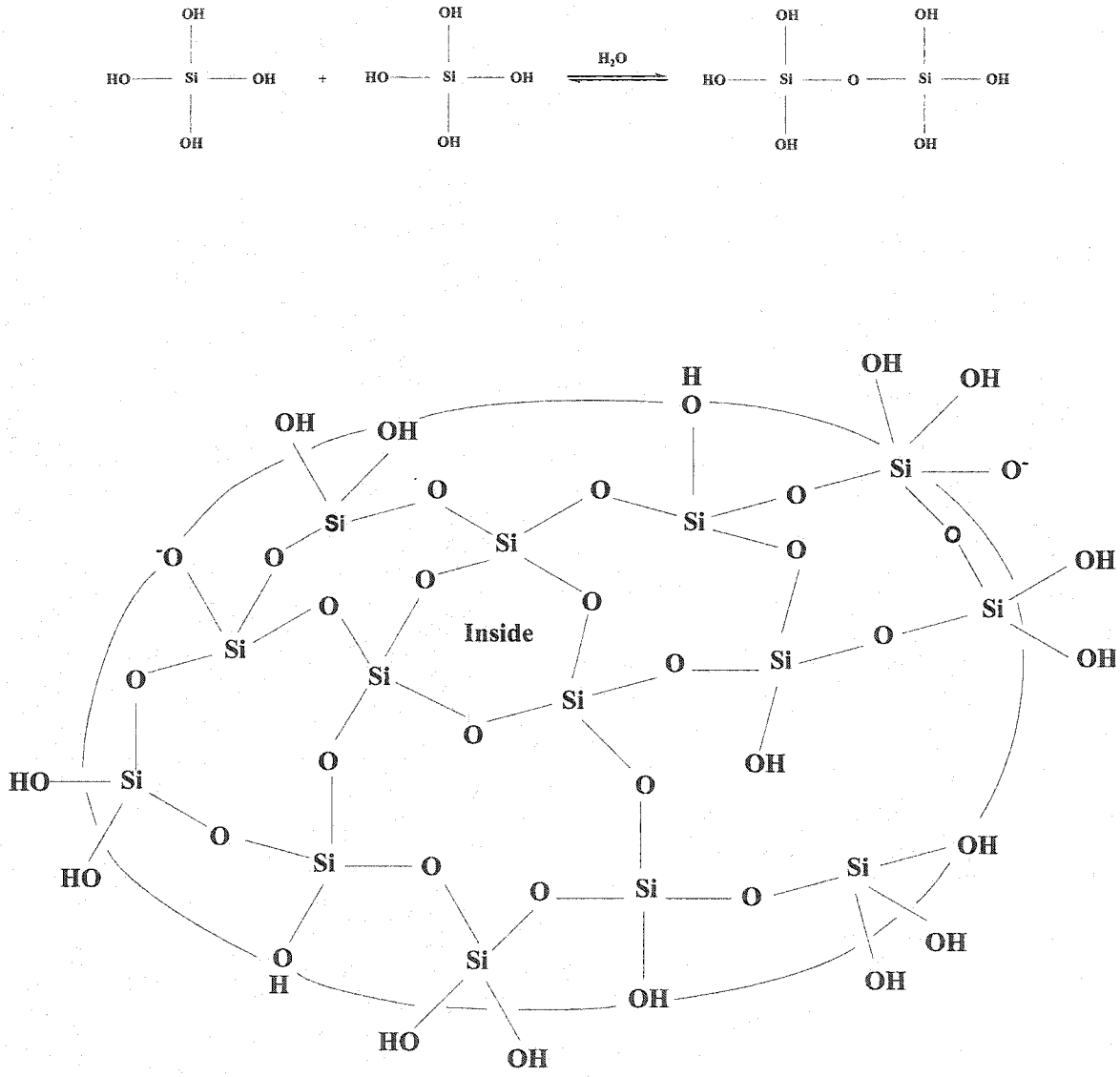


Figure 5-2. The formation of silicic acid, silicate ions, and disilicic acid are depicted in the drawing above. During the process, water is eliminated as silicic acid condenses. Amorphous hydrated silica (lower image), after S. Mann (2001) p. 15.

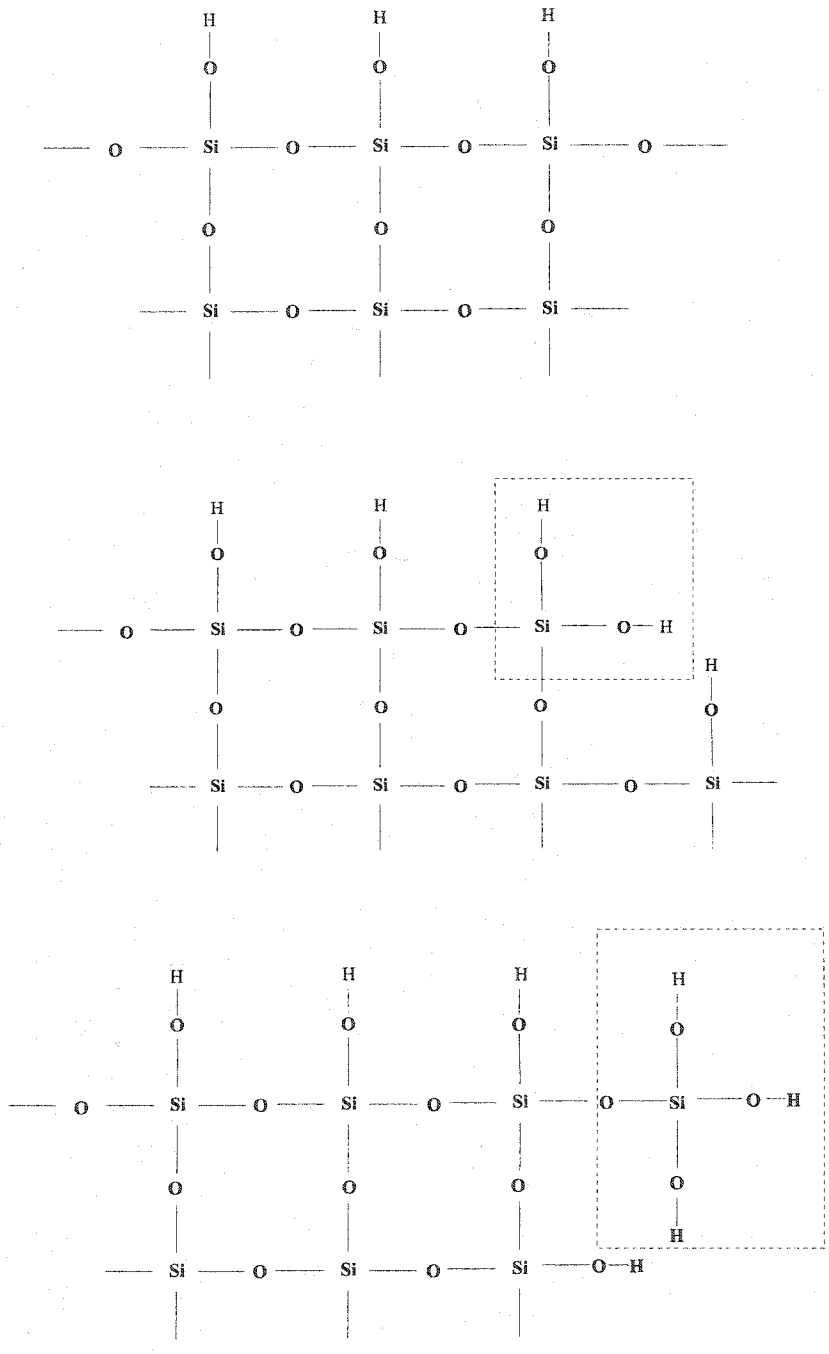


Figure 5-3. Silicic acid polymerizes, eliminating water and leaving different hydroxyl configurations.

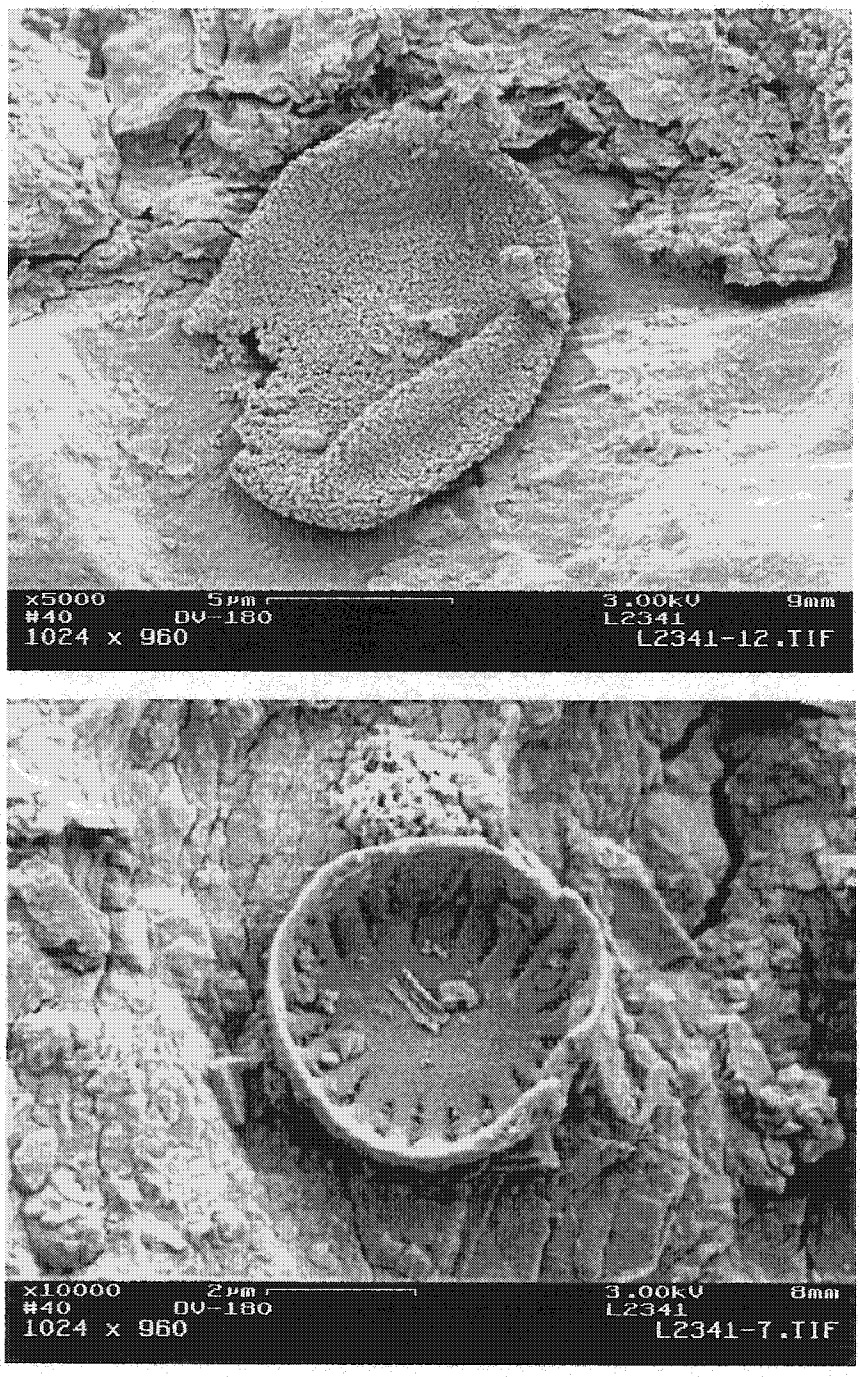


Figure 5-4. SEM images of biological detritus. Apparently pollen in the upper image and a diatom, lower image.

(Figure 5-4), humic substances, proteins, amino acids, carbohydrates and other biomolecules. Detritus, microbes, and microbial products are deposited onto rocks and concentrated in depressions (Figure 5-5 and Figure 5-6). Moisture and other components are added in many forms including rain, dew, fog, snow or aerosols. Rain adds sufficient moisture to fill depressions on rock surfaces and is usually slightly acidic on rock surfaces at ~6 pH (Figure 5-5 and Figure 5-6). Water can remain on surfaces for many days in cool overcast weather (Figure 5-6) or evaporate rapidly on hot sunny days or in low humidity conditions. If a significant amount of water relative to dusts is present, then the variable but usually small amount of aeolian deposits probably does not significantly alter the pH over hours to days. If new water is not added then evaporation begins and pH levels can be raised when appropriate salts and detritus are in solution (Figure 5-5). Many chemical processes occur in water-filled depressions, including those of complexation and photochemistry (see later section this chapter). Small amounts of silicic and disilicic acid, ($(HO)_3Si\text{-}O\text{-}Si(OH)_3$), could be formed. When water evaporates, the pH rises, the solubility of silica increases and more (di)silicic acid is formed (Knight and Kinrade, 2001). Condensation processes then form silica gels. The ability of these acids to remain in solution is further enhanced by the presence of amino acids and other organic molecules including carbohydrates (Coradin and Livage,2001). Eventually in deserts, the gels, especially at the surface and outer layers, are dried and dehydrated in low humidity and are exposed to surface rock temperatures often rising to ~ 60°C or more. During baking, progressively more hydroxyls are driven from the gels resulting in hard coatings. The shrinking and drying process causes local curvatures and partially sintered assemblies of spheres and multilayers (Van Damme, 2000), which provides a plausible mechanism for botryoidal growth forms in varnish. The difference amongst coatings depends on what materials are present during this process. When bacteria or fungi are present, they may contribute to the weathering process by bringing minerals into solution with organic acids adding to the pool of dissolved silicates. Bacteria (Flood *et al.*, 2003) and fungi (as shown here and by Taylor-George *et al.*,

1983) may eventually become silicified (Figure 5-12 and Figure 5-13) and incorporated into the coatings. Flood *et al.* (2003) and Toporski *et al.* (2002) tested mechanisms for silicification of bacteria with silicic acid. One might expect a diverse genetic imprint in coatings. The molecular analyses, presented in Chapter 4, of 16S rDNA fragments in varnish powders suggests bacterial diversity is present in coatings from Death Valley, California (Perry *et al.*, 2004 e; Perry *et al.*, 2002). The incorporation and complexation of other organics such as amino acids (Figure 5-8) with silicic acid favors serine, threonine, glutamic and aspartic acid (Sahai and Tossell, 2001), but may include other amino acids (Coradin and Livage, 2001). As shown in Chapter 4, thirteen amino acids were found, including glutamic and aspartic along with the labile amino acids serine and threonine, in varnishes from the Southwestern U.S (Perry *et al.,* 2003 a).

The surface zone, where minerals, dusts, aerosols, water and microbes interact, is complex. Contributions of myriad other processes, such as chelation processes of bacteria (via catechols) and fungi (via hydroxamates), photochemical processes, oxidation and reduction of oxides by microbes, and other inorganic mechanisms, may contribute to the dynamics of the chemical process. Although the natural environment where varnish forms is dynamic and complex, silicic acid processes probably provide the cementing agent that allows the incorporation of inorganic elements that are frequently found in varnish coatings, as well as organic molecules, detritus, clays, and microbes when they are present. A limiting factors in coating formations is the amounts of water, since too much water probably washes solutions from the surface and removes silicic acid that is the product of the slow process of dissolution of silica. The small amounts of silicic acid brought into solution support previous observations that varnish forms slowly.

Proposed model for the role of silica in complexing organic molecules

Silicic acid can form a variety of complexes with ions and organic molecules. Examples are: mucopolysaccharides, glycoproteins that are enriched in hydroxyl amino acids (serine and threonine), glycine, aspartic and glutamic acid (Sahai and Tossell,

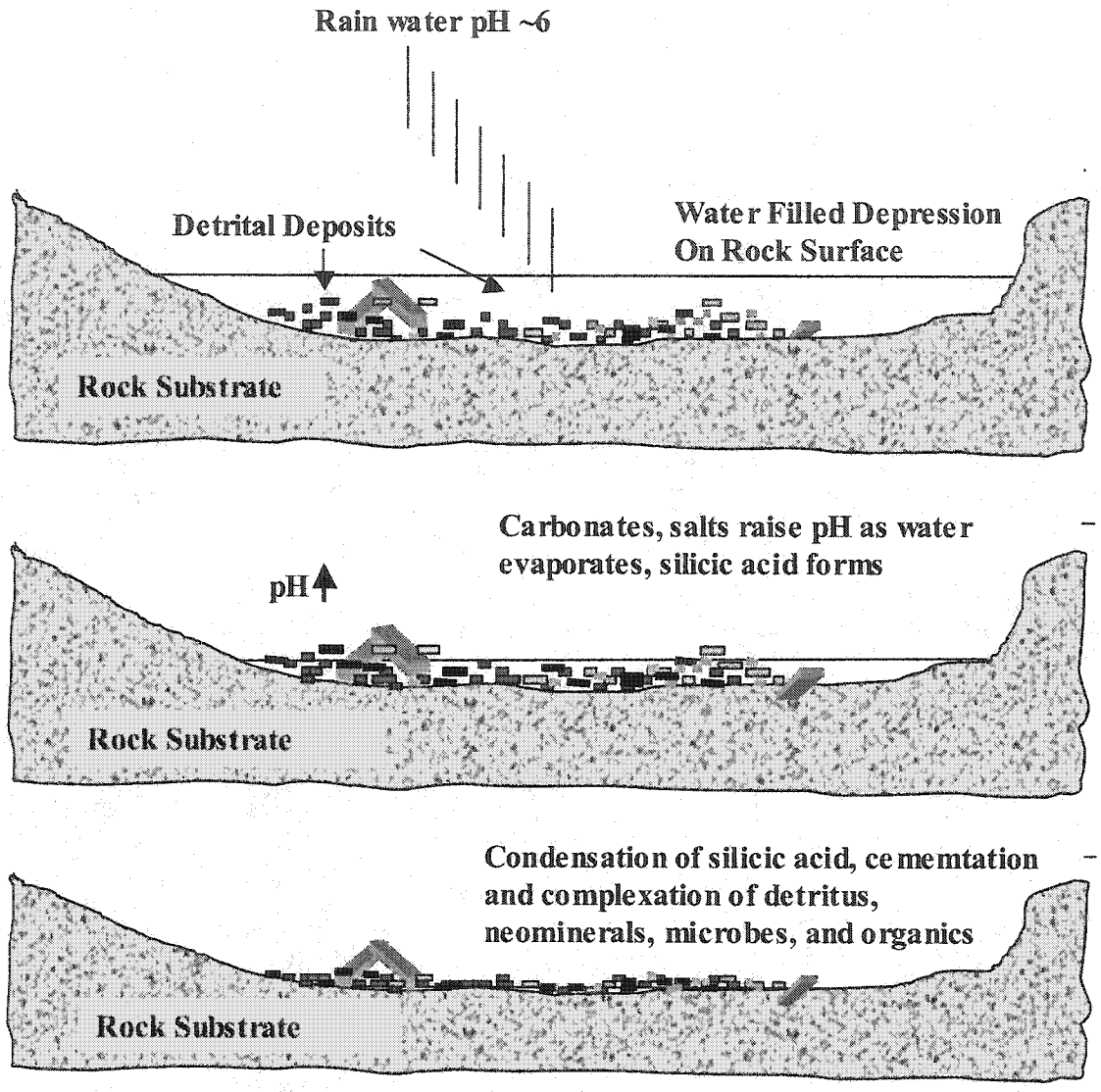


Figure 5-5. Schematic of rain filled depression, undergoing evaporation, forming silicic acid, leaving complexed and cemented micro-sedimentary deposits, eventually resulting in a hydrous silica rock coating.

Figure 5-6. Desert varnish coated rocks in the Mojave Desert east of Baker, California. Rainwater coats the rock and is pooled in depressions along with detrital grains. Larger depressions are miniature sedimentary basins. In cool winter months with low sun angles, surface moisture can be retained for days and for substantially longer time periods in depressions. Shaded locations and more moderate temperatures at higher elevations also contribute to the presence of surface moisture on varnish surfaces.

2001). In addition, silicic acid is expected to form organic silicate complexes Si-O-C with oxyanion centers derived from cis-1,2-diols that are fixed at ca. 0.26nm. Candidates include some oxyanions of sugars, unsaturated polyhydroxy compounds, catechols (1,2-diphenols), and other compounds with rigid structures and a correct "bite" that matches the O-Si-O angle and thereby makes a stable complex (Sullivan, 1986). Flexible sugar-related substances, such as polyols and sugar acids, also make Si-O-C complexes with silicic acid if they possess at least four hydroxyl groups in a particular stereochemical blown to the varnish surface, or from the bacteria and fungi present, or their remains, can make Si-O-C complexes with silicic acid and contribute to the crosslinking and hardening of the silicate polymers that are formed. Significantly, silicic acid also makes Si-O-metal complexes, such as the Si-O-Fe complex with ferrihydrate (Vempati and Loeppert, 1989). Detrital clays are incorporated into varnish coatings (Perry and Kolb 2004 a; Potter and Rossman, 1977). However, typical clay components of Al, Mg, Na, and associated organic compounds also can complex readily with silicic acid (Mann *et al.*, 1983). This means that not only organic substances, but metals as well, can participate in polymerizing, crosslinking and hardening (by elimination of water) of the silicic acid.

Complexation with organics and chemical pathways

Alcohols, amino acids, sugars, and amines are commonly present in soil, and may be transported to the rock surface via wind. The reactions of these compounds with silicic acid, cement some organic compounds into varnish as organosilicates. Silicic acid (pKa 9.5) and its mono silicate ion, Si(OH)3O-, are present in equal concentrations at pH 9.5. In the pH range of 8-9, substantial concentration of silicate ion is present. Silicic acid can condense in a process in which the silicate oxyanion acts as a nucleophile, Si from silicic acid acts as an electrophile, and one of its OH groups acts as a leaving group (Zubay, 2000). Other oxyanions may react in principle, for example an alkoxide, which is derived from an alcohol. In such cases an organosilicate should be formed as a product. Figure 5-8 shows a chemical equation for the condensation of silicic acid and

for

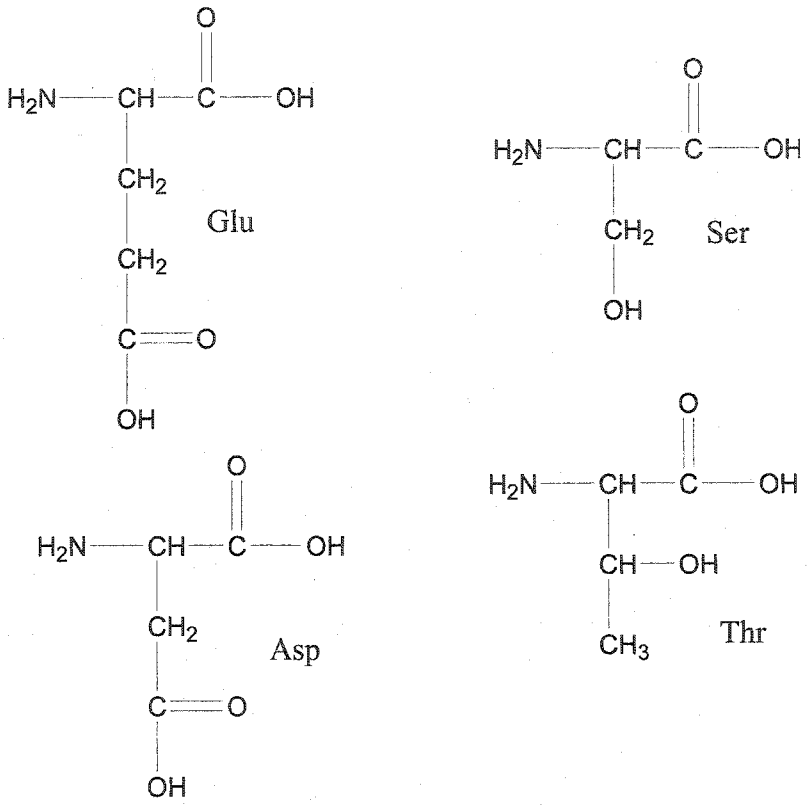


Figure 5-7. Glutamic (Glu), Aspartic (Asp),Serine (Ser), and Threonine (Thr). All are hydrophilic with the exception of threonine. Serine and threonine are found in substantially increased amounts in proteins of diatom silica over cellular levels. Silicic acid may undergo condensation reactions with serine.

organosilicate formation.

The amount of the organosilicate formed will depend on the position of the equilibrium. In the case of alcohols, it was found that they can form organosilicates at high pH (Kinrade *et al.,* 1999). The alcohols used were aliphatic polyhydroxy compounds, "polyols", derived from sugars. Specifically, sorbitol, xylitol, and threitol reacted. The polyols that reacted satisfy the following requirements: they contain four or more hydroxy groups, two of which must be in a *threo* configuration. Silicon makes bonds with the hydroxy oxygens from two carbons that are located at either side of the *threo* hydroxy pair. The organosilicates that form probably have five- and six-coordinated silicon (Figure 5-2).

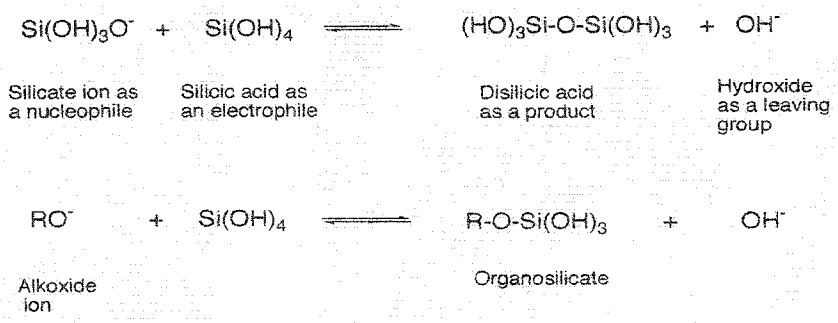


Figure 5-8 Formation of disilicic acid and an organosilicate by a nucleophilic attack of oxyanion from silicic acid and alcohol, respectively.

Sugar acids, such as gluconic, glucoheptonic, and saccharic, also give organosilicates in the alkaline solution (Kinrade *et al.,* 2001 a). The silicon in these compounds is five- and six-valent. Gluconic acid in neutral solution gives a five-coordinated organosilicate (Kinrade *et al.,* 2001 b). It is interesting that the carboxylic acid remains free, and the polyol part becomes bound to silicon, obeying the same requirements for stereochemistry as for the simple polyols.

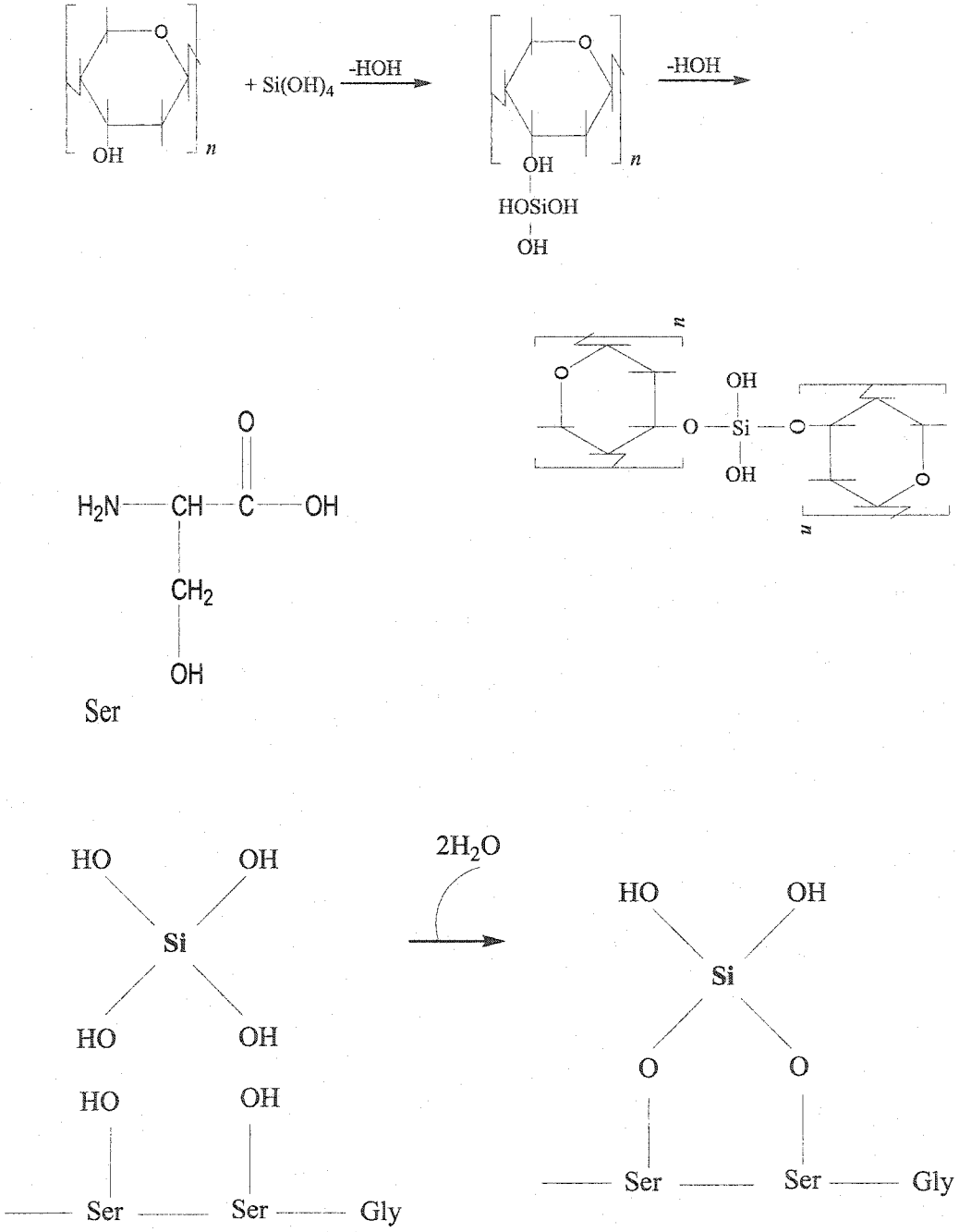


Figure 5-8. Possible covalent structure formed between carbohydrates and silicic acid (upper). The structure of serine (middle). Possible condensation reactions of silicic acid with hydroxyl groups of serine (lower). From *Zubay, G. Origins of life on the Earth and in the Cosmso*, 2000, page 391-392.

Sullivan (1986) stated that silicic acid, is expected to form complexes with organic hydroxy compounds that have *cis* diols, which form anion centers whose separation is ~0.26 nm. This distance matches the oxygen-silicon-oxygen separation in silicic acid and stable organosilicates should result. He listed various organic molecules that fulfill these conditions: some sugars, 1,2-cathechols, serine, tyrosine, and others. Zubay (2000) pointed out that serine and threonine (Figure 5-7) would create a *cis* alcohol pair, if they are adjacent to each other in a peptide (Figure 5-8). He also suggested that hydroxyl groups of sugars may be cross-linked via silicic acid to make silicate bridges (O-Si-O), which could explain high levels of tightly bound silicon in pectin and alginic acids, which are plant polysaccharides.

It appears then that various alcohols are able to make organosilicates. It should be noted that these organosilicates will still have additional silanol groups (Si-OH) available, and will be able to condense further with additional molecules of silicic acid or other hydroxyls, some of which may be of inorganic origins (such as Fe-OH or Mn-OH). Alcohol complexes thus may be embedded in varnish coatings. Some amino acids and peptides catalyze silicic acid polymerization. Examples are: serine, lysine, proline and aspartic acid, poly-lysine and poly-proline (Coradin and Livage, 2001). Amines and polyamines also catalyze polymerization of silicic acid. Polypeptides such as poly-lysine and poly-arginine promote the spontaneous gelation of silicic acid solutions. The compounds that promote silicic acid polymerization may self-entomb by this process. It was shown that polyamines from the diatom cell walls induce rapid precipitation of silica from the silicic acid solutions, and are simultaneously incorporated into the formed mineral. A mechanism for this amine-mediated silicic acid condensation was proposed. (Pohnert, 2002).

Much work remains to be done on elucidation of the structures of organosilicates and on formulation of a theory that will be able to predict reactivity of various hydroxyls, amines, amino acids, sugars and more complicated carbohydrates, and other classes of compounds. However, the examples shown above illustrate that it is

reasonable to expect that among a myriad of organic compounds that are found in desert dust, many may react to form organosilicates, and subsequently become embedded in the varnish.

Experimental evidence supporting silica in varnish coatings

Energy dispersive x-ray analyses (EDS) of a desert varnish coating (Figure 3-1 and Figure 3-3), indicates silicon-rich areas, providing the first supporting evidence that coatings contain discrete amounts of non-detrital silicon. Figure 3-1 shows areas on a varnish surface of nearly pure silicon. The Fe and Mn rich areas that have been traditionally studied are nearly equally mixed with micron-sized areas of Si. Amorphous hydrated silica was found in samples from several locations, including the Mojave, and the Gobi deserts (Figure 3-8, Figure 3-19, Figure 3-24), using transmission electron microscopy (TEM-EDS and XRD). Using TOF-SIMS (Figure 3-2), outer monolayers show high concentrations of silicon and less manganese. Additional supporting evidence for Si and an organic surface monolayer were found (Table 3-1 and Figure 3-13) also using X-ray photoelectron spectroscopy (XPS). X-ray diffraction analyses (XRD) confirms the presence of amorphous silica-mixed phase Opal A and Opal CT (Figure 3-23 and Figure 3-24).

Red bottom-coats

Red bottom-coats have little chemical relation to their substrate rocks. This suggests that they derive their material from contact-soils and are not a weathering product of substrate rocks. Silicic acid probably plays the same role as in topcoats; however, bottom coats are composed primarily of clays (Figure 3-7 and Figure 3-21), formed and deposited by dissolution of, in the case of Mojave Desert samples,, granular (granitic) soils, and probably cemented with polymerized amorphous hydrated silica.

Mojave Desert soils, in this study that are in contact with redcoats are potassium-rich feldspars, which contain little manganese. This would provide an explanation for why K is enriched in redcoats but Mn is not, even though both are enriched in the

substrate rock analyzed (Figure 3-5 and Table 3-2). Red undercoats are composed primarily of clay (Figure 3-21). Enhancement of Mn apparently does not take place, since there is no new supply of ingredients via the dust "conveyor belt" (note manganese is higher in the substrate rock and enhanced in black topcoats, Table 3-2). Other microbial components, aerosols, and detrital deposits cannot be easily incorporated into bottom coats as they are on topcoats. Elements, including Mn, can only be provided to bottom coats by dissolution of adjacent minerals or groundwater solutions, or by the actions of microbes. Apparently groundwater, containing enhanced manganese, does not reach the bottoms of the playa stones by transpiration in the collection sites analyzed. Iron is relatively immobile in ground water solutions, however it may be complexed or chelated. Mn, on the other hand, should be extremely mobile. This provides evidence that red bottom glazes are formed from dissolution of adjacent minerals in soils.

Although the experimental data produced to date support the hypothesis that silicic acid is a dominant process in forming desert varnish, it is probably a complex natural process that develops where microorganisms are present to varying degrees in different environments. Accordingly, further testable hypotheses and experiments are needed to explore the many possible complexation processes, especially those of organic compounds. Additionally, the photochemical process may play a role by reducing organic acids and adding Co_2 to surface solutions.

Biological components of desert varnishes

Culture independent microbial anlayses

This study indicates that there is a diverse community of microbial biochemical signatures on or in varnish coatings. Notable groups sequenced are Proteobacteria and Acidobacteria. Algal chloroplasts and higher plant cholorplasts are also present. Algae is frequently observed under translucent rocks such as quartz, and along the edges of varnish rocks in contact with soils (Figure 1-2). The presence of 16S rDNA sequences

similar to those of photoautotrophic organisms, suggests that primary producers may also be present (Figure 4-5). Since few bacteria are observed on surfaces, DNA is probably derived from within the coating, not precluding that it imight be a result of enhancing bacteria from outer surfaces.

Other culture-based studies, as summarized in Chapter 1 and 4 have found primarily Gram-positive bacteria (eg. Hungate *et al.*, 1987; Perry and Adams, 1978).. The predominant genera were Bacillus, Geodermatophilus, Arthrobacter, and Micrococcus. Amino acid analyses supports the possibility of an association with Gram-positive bacterial peptidoglycan (Perry *et al.*, 2003 a). However, Gram-negative bacteria also contain peptidoglycan in their cell walls, but it composes a minor part when compared to typical Gram positive bacteria. Consequently, is it possible that either Gram-positive bacteria are present, that peptidoglycans are preferentially preserved, or that the cell walls of Gram-positive bacteria withstand desiccation better than the cell walls of Gram-negative bacteria. The advent of sequencing, using molecular techniques has proven that culture-based techniques significantly underestimate microbial diversity.

All SEM images, e.g. Figure 1-6, confirm the general lack of bacteria present on surfaces. Other investigators also noted that bacteria are not frequently observed on surfaces (Perry *et al.*, 2003, Adams *et al.*,1992, Taylor-George *et al.*,1983). Jones (1991) also noted a lack of bacteria using microscopy but was able to stain surfaces, suggesting that microorganism were present. The results presented here support the contention that the amino acids and DNA found in coatings are possibly from non-living microbes and are preserved in the interior of the varnish coatings. While bacteria are infrequently seen on the surface, this is not the case for MCF (Figure 3-28 and Figure 3-29). They are frequently observed in association with varnish coated rocks (Staley *et al.*,, 1982). This may be an adaptation to a preferred environment conducive to growth of both varnish coatings and MCF.Evidence is presented here, and previously (Talyor-George *et al.*, 1983) for the inclusion of degrading MCF into coatings, but whether they are a primarily causative agent in varnish formation remains an open question.

In addition to the possible importance of manganese-oxidizing bacteria, Adams *et al.* (1992) suggests the importance of iron mobilization by bacteria in desert varnish formation. They suggest siderophores produced by bacteria on rock surfaces might reduce ferric iron from dust. The iron could then be concentrated as iron oxide or oxhydroxide on the cell walls of the bacteria. They investigated the presence of siderophores and their ability to fix iron in rock coatings from US southwest deserts, Hawaii, Antarctica, and Australia. Many of these samples had MCF on their surfaces; even so, the samples tested positively, more frequently for catechols (bacterial siderophores) than for fungal hydroxamates. In addition, chelators produced by microbes have an impact on the chemistry of the chemical process and have been shown to complex with silica (Chenu, 1989). The presence of siderophores was not investigated in this study.

DNA has not been previously extracted from rock coatings (Perry *et al.*,2002, 2004e). The preservation of DNA in the mineral matrix has wide-ranging applications: forensic sciences, medical and archeological studies, historical microbiology and pathology, and evolutionary history. While DNA may also be entombed in silica or complexed by Si-O-C bonds, it can also complex with oxides of iron (Higuchi and Wilson, 1984) and/or manganese (Kasyaneko and Plotnikova, 2001).

This diversity is striking compared to the 78 out of 79 Gram-positive bacteria found by Hungate *et al.* (1987). All of these studies differ from the finding of Dorn and Oberlander (1981) where they found Gram-negative bacteria as the causative agent for desert varnish formation. While there remains controversy over their suggestion of "Metallogenium"-like microorganisms, and no studies have been able to replicate their results, culture-independent studies support that both Gram-positive and Gram-negative bacteria are present in varnish coatings from US southwest deserts.

Proteobacteria are ubiquitous in most environments including marine, soil, and fresh water. They are well adapted to saline environments and would be expected to be tolerant of alkaline deserts. Alpha- Proteobacteria are known to flourish in sandy soils

and deserts, dunes, and on plant roots as nitrogen fixers. These plants include beach grass and some cacti.

There were only slightly fewer types of Beta-Proteobacteria detected than Alpha-Proteobacteria. Some of the Beta-Proteobacteria are known to be prodigious oxidizers of both manganese and iron and also have been shown to dissolve silicates with their acid by-products. Whether or not they are growing on or in varnish surfaces, or are transported to the surfaces, their presence in the varnish environment provides for a mechanism to oxidize manganese and other metals at neutral pH's.

Green non-sulfur bacteria found in this study are found in hot springs. These bacteria, such as Cholorflexus sp. which exist at moderate temperatures ~60-8-°C.Green non-sulfur bacteria contain pigments (Sterflinger *et al.*, (1999) that contribute to orange patination on rock surfaces.

All six of the Archaea identified from varnish coatings (Figure 4-6) have not been previously cultured but have been sequenced from DNA from soils. Most species of Archaea are found in environments such as acid waters or hydrothermal vents but survive in a wide range of environments including high salinity. They also provide another analogy for rock mineral coatings when considering other planets. They adapt to extreme environments (that is environments differing from anthropocentric ones), can be chemoautotrophs, grow in caves (Northrup *et al.*, 2003) and in rock crevices, and as this study suggests, in arid hot deserts.

Amino acids

The amino acids found are consistent with Gram-positive bacteria or their byproducts. The interpretation that fits with the "sticky paper" hypothesis is that the peptidoglycan in the cell walls of Gram-positive bacteria are less prone to desiccation in soils, and deposited onto surfaces of the rocks where they become complexed or entombed in coatings. The chimeric nature of the Bacterial DNA sequences supports this explanation. Bacterial DNA in coatings is derived from fragments of DNA probably attached to dusts. The non-chinmeric nature of previously uncultured Archaeal

sequences suggests that Archaea are not added as fragments as are Bacteria. Archaea are either growing on or in coating surfaces, associated with MCF, or are surviving aeolian (alien) transport to coating surfaces.

While it is reasonably certain that the amino acids present are derived from microbes, it is less certain how they are preserved, even if they are of recent origin (ca. 200-400 y rather than thousands of years old). The harsh conditions present on desert surfaces, intense radiation, oxidative, alkaline, and high temperatures are not conducive to the preservation of labile amino acids, particularly serine and threonine. While it is possible that these amino acids are present either because the coatings are young, as suggested above, or because they were only deposited in the outer-most recent layers and subsequently mixed in the homogenized powder, it is possible that they are preserved in minerals.

Racemization of amino acids under the harsh desert conditions is not understood. However, it is one possibility that should be considered besides sequestration or complexation. Following this line of thought, the occurrence of only small quantities of D-aspartic acid could suggest a fairly recent origin of the varnish. The relatively high abundance of the labile amino acids, serine and threonine, along with the lack of D-alloisoleucine, might be explained if the coatings were young. L-isoleucine epimerizes very slowly, and even if the samples were several thousand years old, only small quantities of D-alloisoleucine would be expected. To continue this admittedly speculative assertion to a conclusion: assuming that the activation energy of isoleucine epimerization in the bacterial protein is equal to that in carbonate systems and the limit of detection is 0.002 D/L, the maximum time since the bacteria died can be estimated. If the effective temperature was 20°C, than the amino acids are no older than a few centuries; if 30°C, than the bacteria would be no older than a few decades (personal communication with Darrel Kaufman, Northern Arizona University). The relatively high abundance of labile amino acids serine and threonine, along with the lack of D-alloisoleucine, suggests that the amino acids in desert varnish are possibly less than 200

y old if the amino acids are evenly spread throughout the varnish rather than on or near more recent surface deposits. While racemization should be considered, a more plausible explanation for the presence of the labile hydroxalated amino acids is probably sequestration or complexation with minerals, silicic acid or clays.

Serine and threonine, along with glutamic and aspartic may also form complexes with amorphous silica. It is the ability of these amino acids to form complexes with the hydroxyl groups of silicic acid that may provide a better explanation for their presence. This is true particularly for serine and threonine, since they are generally unstable in the natural environment, and under arid UV intense conditions. Thus, their detection in desert rock coatings suggests there is a preservation mechanism.

Other chemical pathways by which amino acids and organic compounds, including alcohols, amino acids, sugars, and amines, might be embedded in the varnish matrix need to be considered. These compounds are commonly present in soil, and may be transported to the rock surface via wind or rain splatter, possibly attached to clay particles. The reactions of these compounds with silicic acid (Figure 5-8), might cement organic compounds into varnish as organosilicates.

It is intriguing to speculate whether or not the amino acids comprise a peptide that plays a role in the stabilization of rock coatings. In this regard it is noteworthy that the carboxylated amino acids, glutamic and aspartic acids, along with the hydroxyl amino acids, serine and threonine, may comprise part of a peptide that forms stable complexes with oxide/hyroxide and/or clay minerals in varnish coatings.

Other considerations

Morphology of coatings

Boulders, pebbles, and talus blocks coated with desert varnish are shiny… "and glisten in the desert sun as if they had been polished or coated with shellac" (White, 1924). The name desert "varnish," implies the surfaces are glossy as if coated with shellac or "varnish", or "are polished so as to give the rock the appearance of

'*esienkiesel*' (Ball, 1903).

Patina has been employed as a descriptive tool, more so in Europe than in the US. Sterflinger *et al.* (1999) describes the term 'patina' as one in which, "we normally associate the inimitable and sometimes nostalgic traces of the aging processes of art objects." The term has been broadly applied to bronzes and the 'patina' of well-worn leather. Sterflinger goes on to say, "that patina is used with reference to two types of aesthetic changes to rock surfaces: first, the development of brightly pigmented crusts covering the rock surfaces and, second, a pigmentation that is directly incorporated into the fabric of the uppermost layer of rock crystals."

Morphology, as suggested in the general sense by Wolfgang Krumbien (pers. comm.., 2003), is an important and often overlooked consideration. The morphology of coatings or, more specifically the micro-morphology, may suggest clues as to how coatings form. The beauty of gem-quality opals is a result of the refraction of light from their silica composition and morphology. Their vibrant colors are generated by the refraction of light by spheres of silica. Amorphous and hydrated silica have two characteristic forms: that found in opal, tiny spheres, and lamellar, found in both inorganic and bio-deposits.

Another morphological feature of varnishes and glazes is their frequent micro-stromatolitic growth form (Perry and Adams, 1978, Farr and Adams, 1984). As an external growth feature, these botyriodal structures are unique. Their likeness to living and ancient stromatolites has been used to suggest a biological origin (Raymond *et al.*, 1992).

In thin section and ultra-thin section ($\sim< 10\mu m$) all desert varnishes examined (Figure 1-4) are composed of micro-laminations a few micrometers thick. Sections cut normal to the surface show alternating dark and light layers. Copious quantities of detrital grains are accumulated within varnish as shown in thin sections (Figure 1-4) and in between botryoidal structures (Figure 1-5 and Figure 1-6) . These grains make up a significant portion of varnishes. One and a half grams of desert varnish powder ground

from rocks produced nearly one and a third gram of detrital material. The balance is composed of oxides, silt, clay-sized particles (which includes opal), organics, and water.

Rock type and morphology may have an important impact on coating formation. As shown in Figure 1-5, botyriodal structures and, hence, manganese concentrations, may frequently be in low depressions or dimples. Low depressions on rock surfaces can hold water (Figure 5-6) for periods of time and in some deep depressions for considerably longer, especially during cool periods. Spaces between growth columns would also retain moisture. Therefore, the micro-morphology may have an impact on the chemistry by preferentially concentrating elements in depressions or on the tops or sides of growth structures. Oxides are mobile in solution and may settle or adhere on structures as solutions evaporate.

A conclusion from this work and previous work is that varnishes reflect their local environments, are composed primarily of oxygen, silica, and literally whatever else blows in on the wind, is in the atmosphere, or grows on the surface. Lakin et al. (1963) found correlation with local ore provinces supporting this hypothesis. Varnishes collected from sites in Death Valley contained boron, which reflects the high boron in the Death Valley area. Varnishes from Nevada, in a region where arsenic, antimony, and vanadium are present in nearby rocks, contained anomalously large amounts of these elements. However, varnish-coated pebbles from an alluvial fan three miles away in a similarly mineralized area, did not contain larger amounts of these elements. Varnishes from Eureka, Nevada, and downstream from a mine, contained silver, lead, zinc, and copper, similar to the ore mined. Varnish from another area of Nevada contained high copper from a copper mining district.

Concentrations of manganese

The question is: what concentrates Mn orders of magnitude over Mn present in soil materials? Iron is also concentrated but not to the same extent, thus the ratio of Mn/Fe can be very high in some varnishes. Experiments performed by Jones (1991) which showed Mn concentrations increasing exponentially over 213 hours when Co_2

was percolated through columns containing desert soil. Iron generally remained stable. Elevation of iron at two times during the experiment was attributed to contamination.

Krauskopf (1957) performed similar experiments where finely powdered basalt was placed in distilled water and stirred for 1-7 days. The temperature was maintained at 55°C and the pH was increased from acidic values (4.4) to alkaline (8.4). Dissolved iron was measured only at pH 4.4 and not above 5.4. Dissolved Mn increased with increasing alkalinity until the pH reached 8.0, where it decreased and was precipitated. In what Krauskopf said in, "…a less elaborate experiment," vertical glass tubes were filled with coarsely ground basalt and Ca_2CO_3 was placed in the lower half of the tube. A solution of 0.1N H_2SO_4 was allowed to percolate downward. A brown precipitate was formed as the solution passed through the Ca_2CO_3. The precipitate was analyzed and the Mn/Fe ratio was ¼ (at a measured pH of 5.5), in contrast to 1/60 in the basalt. There are several examples of Fe and Mn separation in nature by the gradual neutralization of acidic solutions in oxidizing conditions given by Vogt (1906) and Hanson (1932). Ljunggren (1953) found a similar separation with a high Mn/Fe ratio in acid surface waters percolating through soils where iron was precipitated as ferric in soils, and the solutions became enhanced in Mn.

The above experiments and observations show that Mn can be greatly enhanced under appropriate pH and *Eh* conditions. When concentrations are low, solutions must be above pH 8 in order for Mn precipitation to occur. Krauskopf suggests the following:

"One can readily imagine local conditions, however, that might lead to precipitation even from such very dilute solutions. The manganese might become more concentrated by evaporation in an arid region; the solution might become gradually alkaline, so that all iron would be eliminated, and then might ultimately reach an alkalinity at which very small concentrations of manganese would precipitate; the precipitation might be accelerated by bacteria, by catalytic action of MnO_2, or by unusual concentrations of silicate or carbonate."

Water

Several investigators have noted the hydrous nature of varnishes. It was shown by Perry (1979) that one varnish sample contained ca. 9% water while a surface soil sample was ca. 4%. *Eh*-pH diagrams for both Mn and Fe show that these elements would be present in desert conditions as $Mn(OH)_3$ and $Fe(OH)_3$ or $FeSiO_3$ (Krauskopf, 1957). Besides hydroxides and oxyhydroxides, water can be held in clays, particularly mixed-layer expandable clays such as smectites (not found in large quantity in coatings in this study). Desert conditions, though, are by nature dry, low moisture environments. Temperatures measured on rock surfaces during summer months often exceed 60°C (Perry and Kolb, 2004 d). These conditions then do not appear to be ones that would be conducive to retaining water. Mixed layer clays would be "baked" and shrinking layers would possibly expel water during summer months. Other non-expanding clays, such as illite that are identified both in this study and by Potter and Rossman (1977), contain small amounts of water and would not be materially effected by dry desert conditions. But, even hot southwest deserts are not always dry. Precipitation for Death Valley at –58 feet is 46.2 mm average annual rainfall and increases to 119.2 mm at Victorville, California (Turner, 1982). Even with low annual precipitation conditions, winter months can have extended wet periods and temperatures ca. 0°C. Observations in the course of this study in Death Valley, Bishop and near Baker California, have shown that rocks on playas have stayed wet in sunny weather on their north sides for several days after rain.

Bacterial spores

Spore forming bacteria on exposed rock surfaces exhaust nutrients and sporolate as water becomes scarce in summer. Cellular products from the mother cells eventually lyse and contribute organic polymers to the rock surface. The remaining spores concentrate and become encrusted in minerals, particularly those of Ca^{2+} and Mn^{4+}. The mineral encrustations provide protection from harsh conditions and impart heat resistance. Spores are generally heat resistant to ~100°C. However, as temperatures exceed 50°C, some spores such as *B. subtilus* and *B. megaterium* begin to demineralize.

Germination of spores is caused by multiple factors including rising temperatures and exposure to organic compounds such as alanine (Sogin *et al.,* 1972). The demineralized products rich in Ca^{2+}, Mn^{4+}, other oxides and organic compounds, might then be deposited on the rock surfaces. Surviving spores might also deposit byproducts to rock surfaces during germination. The spore demineralization products then combine with extracellular polysaccharides, detrital clays, or oxides to become part of varnish coats. A unique component of spore coats is dipicolinic acid, the finding of which in varnish coats would support this hypothesis. Utilizing TOF-SIMS, we failed to find this acid in samples from the Mojave Desert.

Bacteria and interactions of their saccharides with mineral surfaces

The most likely part of bacteria to interact with rocks is the outer portion of the cell wall that contains oligosaccharides from peptidoglycans, but also other saccharides, such as teichoic acids and related sugar-lipids. The bacteria, upon attachment to the surface, also produce slimy adhesive substances that are predominantly exopolysaccharides (EPS) (Banfield and Nealson, 1997). It is likely that bacterial polysaccharides will interact with the natural surfaces in a process that is probably facilitated by their initial complexation with metals that are found on these surfaces. The complexation may be followed in some cases by a redox-type reaction. It is known that peptidoglycans are highly interactive with dissolved metal ions (Banfiled and Nealson, 1997). This may be the beginning of a sequence of reactions that leads to the cementing of bacteria to the surface. An additional pathway would be via complexation with silicates, of a type described by Kinrade *et al.* (1999, 2001 a, 2001 b) for polyols and sugar acids.

Peptidoglycans are complex polysaccharides found in bacterial cell walls. They contain linear polymers of two alternating sugars, N-acetylglucosamine (NAG) and N-acetylmuramic acid (NAM), that are cross-linked with the short peptides. These peptides are composed of some common amino acids, as well as some unusual ones, such as D-glutamic acid, diaminopimelic acid (DAP), and D-alanine (Madigan, 2003). Another

possible candidate for a biosignature within peptidoglycans would be the peptide inter-bridge composed of five glycines, which is found in some gram-positive bacteria. The peptide cross-links in the peptidoglycans may protect the sugars from decomposition, and thus enabling them to serve as biomarkers.

One can reasonably ask if a simultaneous finding of these unusual amino acids, pentapeptide bridge, and the NAG and NAM sugars or their transforms, could indicate the remnants of the cell walls of bacteria. This would be important in identification of bacterial fossils, and for understanding of the processes by which various biofilms, coatings, and rock varnishes form on the natural surfaces, and undergo mineralization over time.

In general, sugars have not been studied as a biosignature. There is ample reason for this. Common sugars, such as glucose, ribose, arabinose, or fructose, contain aldehyde or keto groups in conjunction with the hydroxyl groups that make them very chemically sensitive. Such sugars are rapidly destroyed under basic conditions. They isomerize under both acidic and basic conditions. Isomerization causes racemization of the optically active centers, and an eventual destruction of the molecules (Collins and Ferrier, 1995), thus preventing their use as biomarkers.

However, some sugar derivatives that are devoid of the aldehyde and keto groups, notably sugar-related acids and alcohols, are more stable and have been isolated from the Murchison meteorite (Cooper *et al.,* 2001). Sugars may be more stable under arid and semi-arid conditions under which most common rock varnishes are formed. Sugars in general, such as carbohydrates and polyols, sugar acids, carbohydrates with nitrogen or other electron-donor atoms, oligomeric- and macromolecular carbohydrates, make a variety of stable complexes with metals, such as calcium, aluminum, iron, manganese and others (Gyuresik and Nagy, 2000), that are commonly found on natural surfaces. The possibility of sugars complexing with components of desert varnish is currently being performed in laboratory simulations.

Photochemical processes

How light might influence chemical processes of varnish formation is one aspect of the natural environment that has not been systematically studied in the laboratory setting. Reductive dissolution of metal oxides from attached soil particles would probably alternate with re-oxidation and precipitation of the dissolved metals in a cyclic fashion, driven by the daily wet-dry regime of the desert climate. At dawn during much of the year, a thin film of water forms on rocks due to condensation of dew, wetting aeolian soil particles. Photochemistry begins as soon as sunlight hits the damp rock surface, reducing iron and manganese oxides by oxidizing organic compounds, such as α-carboxylic acids. Oxalic acids are readily available from fungi and lichens. On the inorganic side, these reactions would lead to the solubilization of metal elements, yielding ferrous iron and manganese (II) in example. The reduced metals could stay in solution via organic chelating agents. At the same time, the presence of organic acids, including amino acids also, causes dissolution of silicates, providing a source of dissolved Si. On the organic side, the photochemistry oxidizes the α-carboxylic acids to CO_2, reducing the amount of organic content on the rock surface. In the case of glutamic acid, this photo degradation leaves a diagnostic byproduct, γ-amino butyric acid (GABA), which was identified in desert varnish in this study. As the dew evaporates and the rock dries out, the dissolved species could begin to oxidize and precipitate, a process likely to result in new mineral phases.

On making varnish *in vitro*

The experiment of Palmer *et al.* (1992, pers. comm.., 2000) was originally undertaken to see if coatings similar to desert varnish could be produced in the laboratory using cultures of bacteria isolated from desert varnish. The coatings produced did not, however, texturally resemble natural varnish coatings and were not as hard. The original deposits were soft, and weakly adhering and could be scraped with fingernails. Metals were concentrated in varying amounts as shown in their experiment. Microbes

may be implicated in many aspects of these processes by concentrating iron and manganese from soil additives or by transforming clay precursors to clays, and by producing polymers which may enhance packing of tiny particulates (Martin, 1971; Robert and Chenu, 1992) thereby aiding adhesion to rock surfaces (Chenu and Guerif, 1991). While coatings were produced, simulating "natural" conditions more closely might have resulted in better adhesion of coatings.

Similar soft-coatings to those produced in the experiments have been reported from nutrient-limited caves (Northrup and Lavoie, 2001). Sulfur, iron, and manganese-oxidizing bacteria were found to generate considerable acidity, dissolving cave walls. Microbial induced mineralization included silicate, clays, iron, and manganese oxides.

Future lab studies could be designed to account for both heat, light and the removal of unincorporated silicates. Varnish forms in the presence of heat and light and its formation requires water and is thus a solution deposit. The source could be dew, rain, or snow. Presumably dew would be available in varying degrees nightly, while rains and/or extended wet periods would be intermittent. Abiological mineral reactions may be essential in producing varnishes. For instance, the effects of light and heat were not part of this experiment, and certainly varnishes in nature are exposed to photochemical process and temperature variations. Adding to the problem are the consortia of species possibly involved, each species having a small role or possibly a symbiosis between two microbes, such as a fungus and a bacteria. Alternatively, it may be entirely unimportant as to which microbes are present, as the extracellular products and decaying cells react with metals, clays, water, light and heat to form coatings.

The results of the ^{13}C stable isotopes show that the greatest fractionation was obtained ($\delta^{13}C$ –25.45) where no bacteria were present. This suggests that the *in vitro* coatings produced by Palmer *et al.*, (pers. comm.. 1992) were probably not of biological origin.

While the process may be more complex, some studies have emphasized microbial properties, which could be important relative to this problem. The sorption of

metals by bacterial cells (Beveridge, 1989; Adams *et al.,* 1992) and the production of high affinity iron chelators and siderophores produced by bacteria and fungi, may be of special relevance (Neilands, 1984).These activities suggest additional mechanisms for mobilizing iron from dust, and fixing iron and manganese to surfaces. In unpublished research, we have found that when FeIII, MnIV, montmorillonite and siderophores (2,3-dihydroxybenzoic acid and desferoxamine mesylate) are added to surfaces, with and without montmorillonite, coatings resembling varnish in color and texture are produced.

There are unanswered questions as to whether the precipitates formed were made by periodically washing with 0.01 N $NaHCO_3$. During drying, the residual bicarbonate solution will lose CO_2 and become progressively more alkaline, and consequently, could cause metal oxide deposits. The pH of the solutions applied during the experiment started at near normal but resultant surface pH did become progressively more alkaline. Dorn (1989) recorded varnish having a pH near 9.0 in a series of varnish samples, while soil in the vicinity had a pH of 9.5. The highest pH, in that experiment, of 9.4 was recorded at the end of the experiment, but is not out of line with natural conditions. Oxide precipitation for iron is favored above pH 5.5, but biological iron oxidation can occur at circumneutral pH (Emerson and Moyer, 1997). Manganese can remain in solution and begins to oxidize at a pH above ~8.0. Microbes may, however, facilitate the oxidation process at lower pHs.

Analyses of $^{13}C/^{12}C$ isotopes might have answered the question as to whether the washing by bicarbonate caused the precipitates. If the controls without bacteria would have shown a non-biological fractionation, then it might be possible to conclude that the precipitates were biologically mediated. It does seem possible then that the bicarbonate solution was at a higher pH and eventually raised the pH of the coatings so that an inorganic autocatalitic oxidation took place, rather than a microbially induced precipitation.

Chemical signatures of Eukaryotic organisms

The surface environments on rock coatings are some of the most hostile on Earth.

High temperatures, low humidity, high incidence of UV light and low nutrients require organisms that have evolved special survival skills. On shaded sides of rocks, or a cooler elevation, lichens and occasionally mosses grow on rock surfaces. In the previous section, it was noted that several researchers have observed the lack of bacteria on desert rock surfaces (Perry and Kolb, 2004 a; Nagy *et al.*, 1991; Jones, 1991; Taylor-George *et al.*, 1983). Green algae and cyanobacteria are frequently seen along the sides and undersides of rocks (Figure 1-2), particularly under small (~15-25 cm) playa stones that are translucent (e.g. quartz). In this study none were observed on rock surfaces.

Gorbushina *et al.*, (2001) suggested a direct biological control of the mineral deposition of fosterite and opal by the fungal phase of stomatolitic lichens. In another study (Gorbushina *et al.*, 1999) stated that subaerial biofilms on exposed rocks are accumulations of cell material and EPS maintaining life in the presence of minimum water, often less than 1%. They summarized that the EPS probably provides a protective layer for cells and prevents cell desiccation with EPS layers and photoprotective pigments.

In order to collect and analyze powdered rock coatings, contamination from ubiquitous surface fungi is a problem. It is impossible to discern whether organic chemical signatures are from coatings or surface microbes. The collection site near Baker California is anomalous in that no MCF are present in the immediate area. The reason for this is unknown. With the help of Marc Volkmann, University of Oldenburg the varnish coatings were analyzed for mycosporins which have been detected on rock surfaces (Volkmann personal communication, 2003). No mycosporins were found in the one sample analyzed from Baker. California. It is impossible to draw a conclusion from this one measurement; future experiments may be able to detect both past and present mycosporins. In the sample measured it may be that no MCF have ever been present or that mycosporins did not survive. Further tests may prove to be useful in providing information about the past presence of MCF.

Mineralization of MCF

Taylor- George *et al.* (1983) and *Perry et al.* (2004f, 2003b) suggested that the colonies of MCF and black yeast microcolonies (Gorbushina *et al.*, 2002) degrade and become part of rock coatings. Figure 5-11and Figure 5-12 show SEM images of older degrading fungi that become integrated in varnish coatings. If the colonies are becoming part of rock coatings or, as Taylor-George *et al.* (1983) suggested, that the "layers of modified remains of microcolonial fungi and precipitated solutes, along with some trapped detrital (inorganic) grains, accumulate to form desert varnish. While the colonies are slow growing they eventually die and subsequently degrade. Younger colonies have less mineral concentrations (Figure 5-9). The principal elements in MCF from the Mojave Desert (Figure 3-28) are similar to those detected by Taylor-George *et al.* (1983). Young colonies usually contain O > Si > Al > Ca and lesser amounts of Mg >K > Na > Fe > Mn (Figure 3-30). Comparison (Figure 3-28) with a Mn rich varnish substrate shows that Ca and C are more highly concentrated in the MCF, while Mn and Si are relatively more concentrated on the substrate. Figure 3-31 shows a degrading MCF that has two elements not present in the varnish coating: S, and Cl. Carbon is enhanced in the degrading fungi over the varnish coating. Other oxides differ in proportions but are similar in decaying MCF and the varnish topcoat. It should be noted that there appears to be no enhancing or lessening of elements surrounding the colonies supporting analyses of MCF from Australia (Staley *et al.*, 1992).

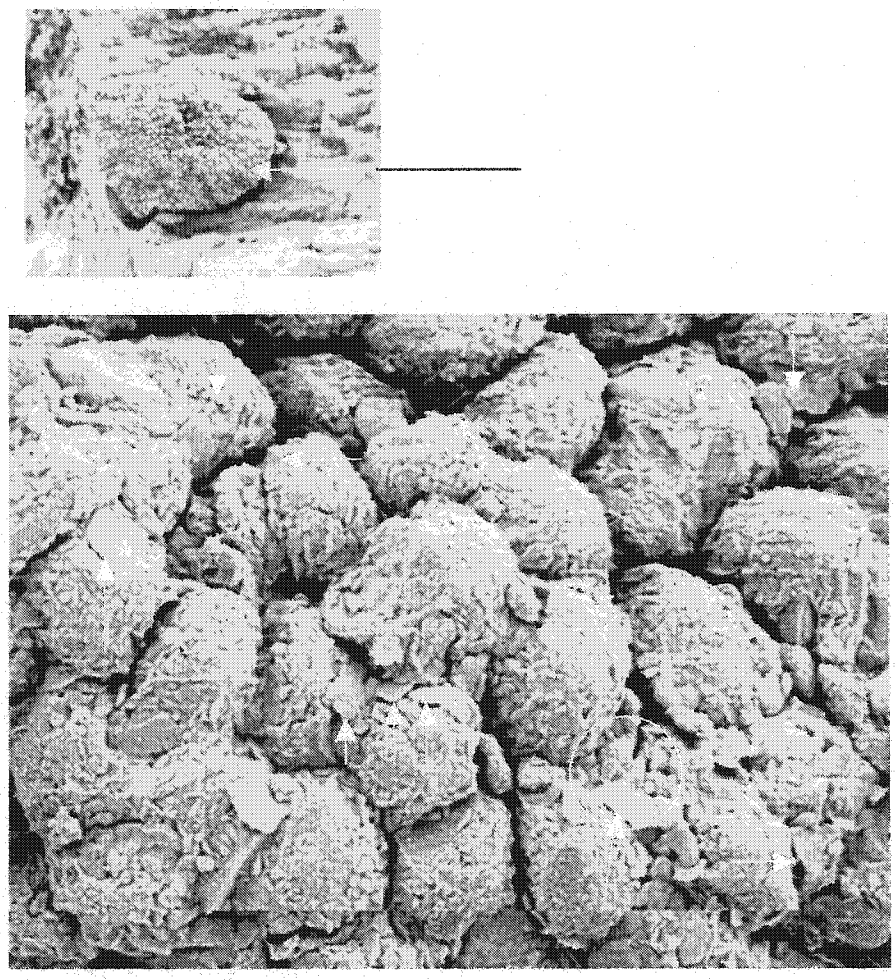


Figure 5-9. SEM image of MCF on sample #180 Bishop, California. Lower image enlarged from area depicted by white arrow (top image). Note mineral grains adhering to MCF (white arrows, lower image).

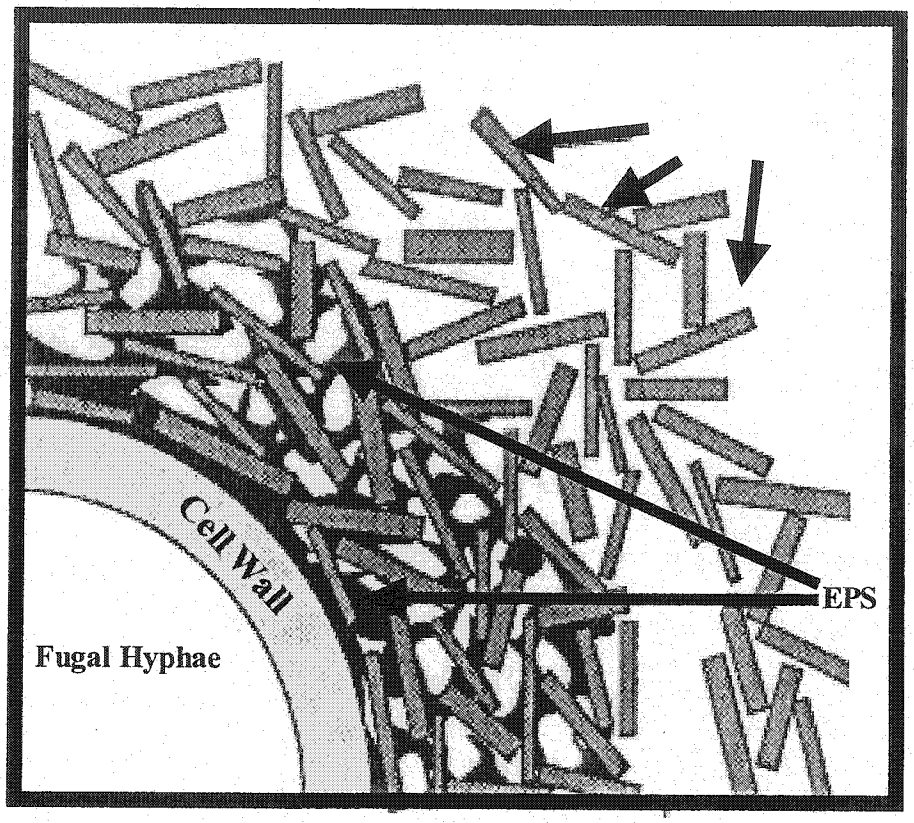


Figure 5-10. EPS composed of sugars which can orient small clay platelets (≺ 0.2μm) on the surface of the hyphae (Adapted from Chenu, 1989).

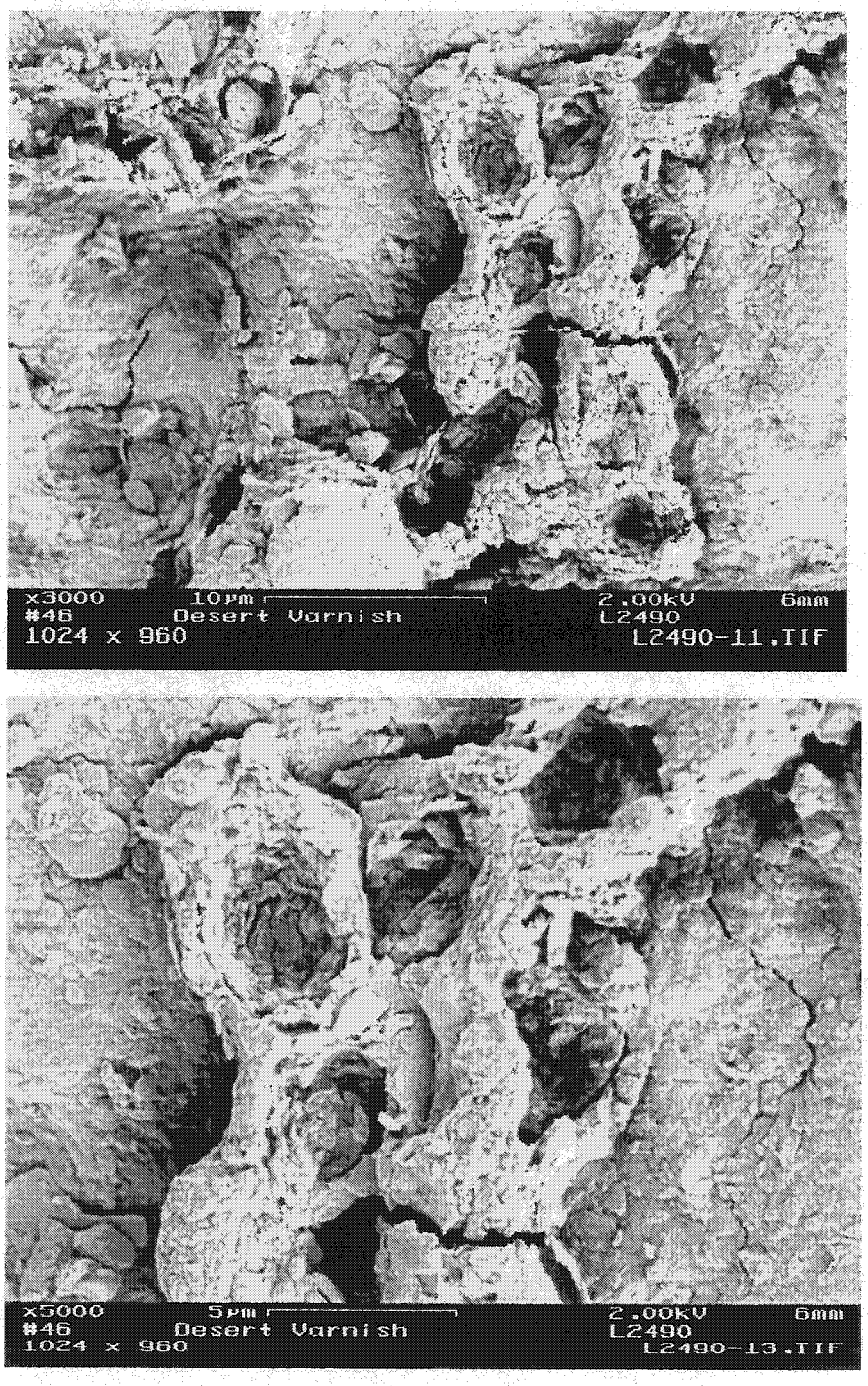


Figure 5-11. MCF degrading on desert varnish coated rock from Bishop, California

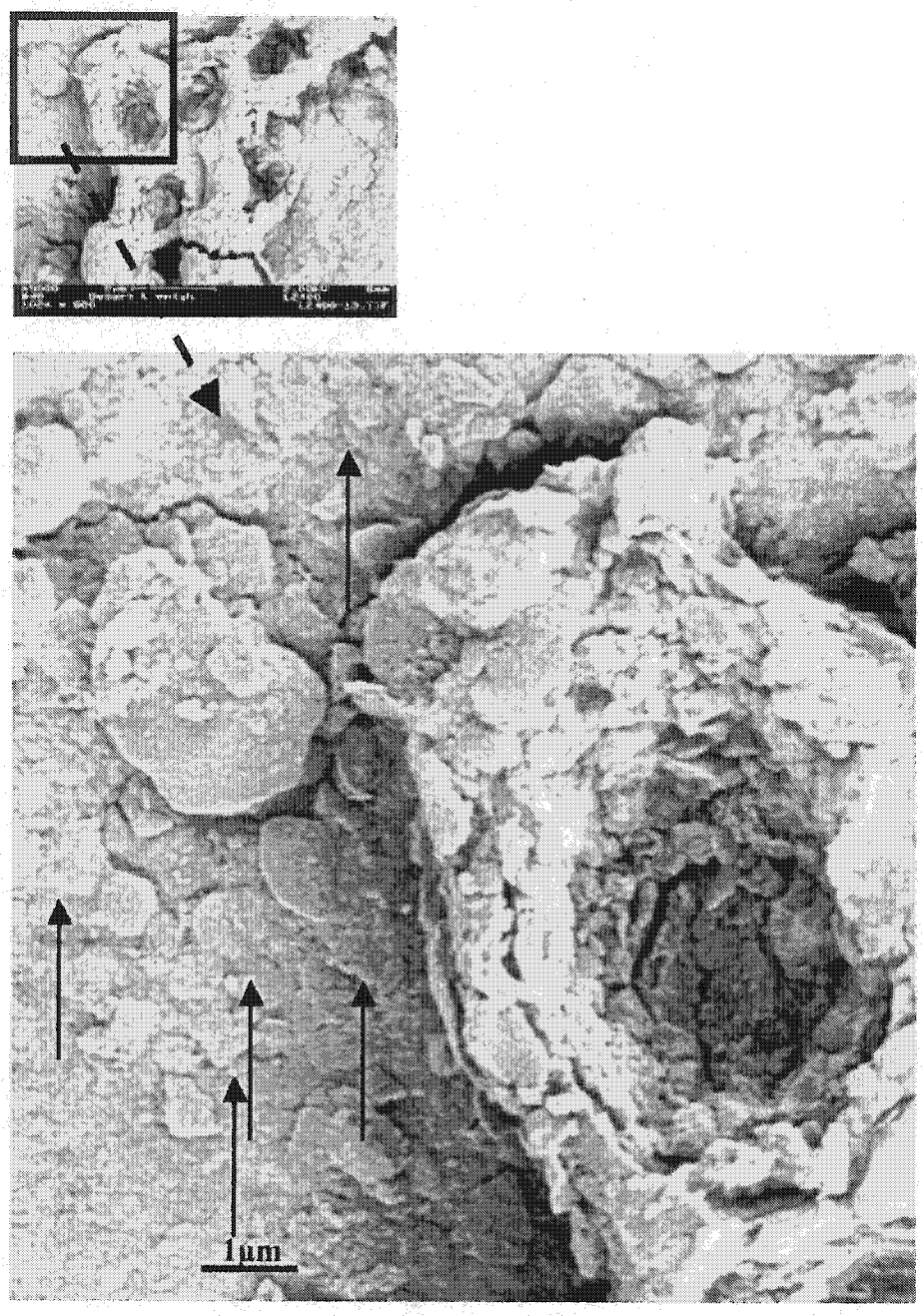


Figure 5-12. MCF degrading on desert varnish coated rock from Bishop, California sample # 11502. Mineral platelets on and being incorporated into varnish coating (arrows).

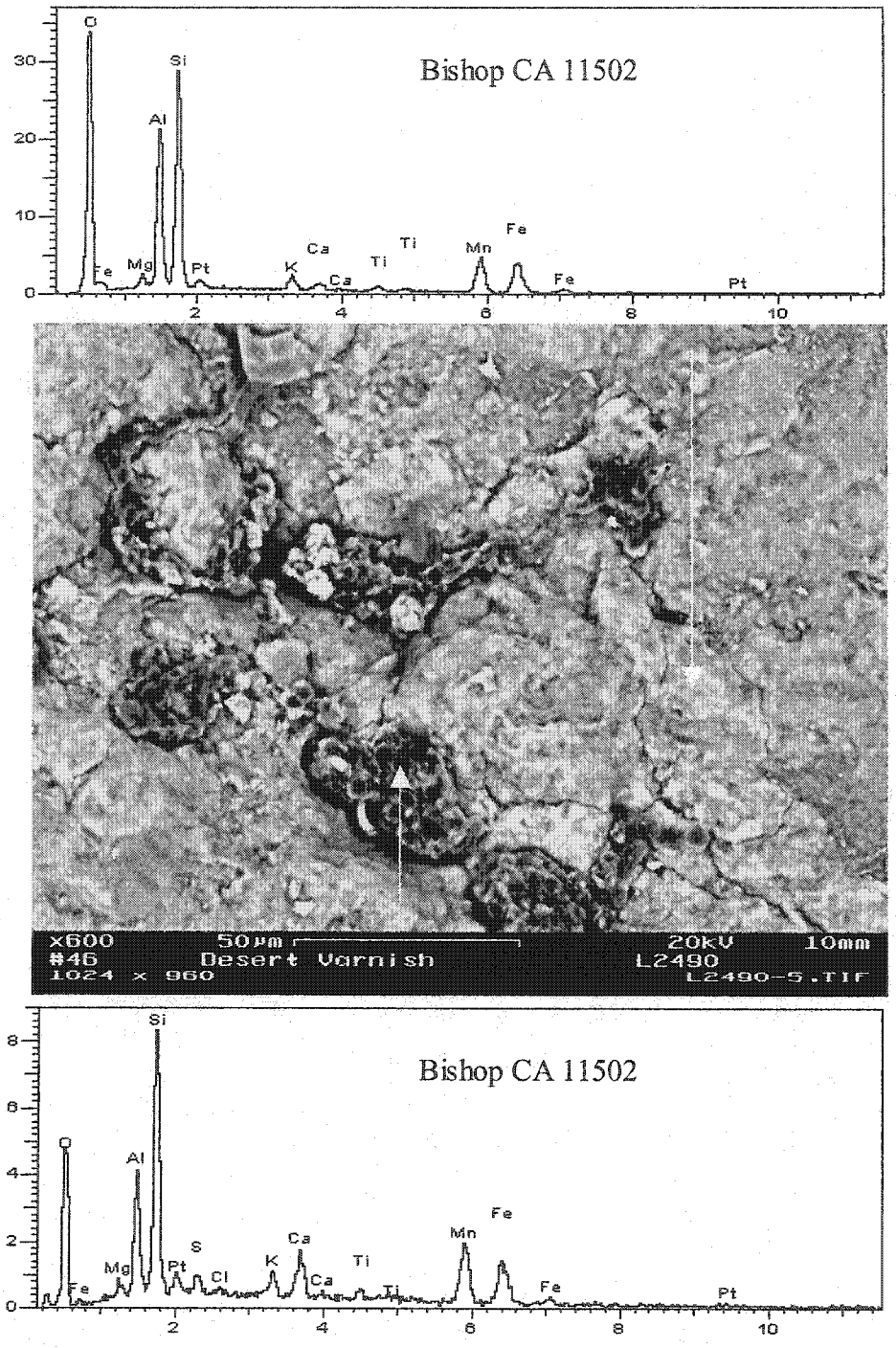


Figure 5-13. SEM of varnish coated rock and EDS of degrading MCF from Bishop, California. Oxides including, Fe, Mn, Ti, K, Si, and Mg. MCF have increased Ca, decreased Al and O. MCF have S, Cl and C that are not present in the coating.

Weathering from mineral grains release Fe (II) which is oxidized to Fe (III) in desert conditions. The Fe (III) is immediately hydrolyzed to form Fe (III) oxides and oxyhydroxides. These reactions break the Fe (II) O-Si bond and form FeOH and SiOH groups, for example goethite (α-FeOOH) formed from olivine fayalite:

$Fe_2SiO_4 + ½O_2 + 3H_2O$

fayalite

$\rightarrow 2_\alpha$--FeOOH + $Si(OH)_4$

goethite

Microbes may play a role in the oxidation of Fe (II) to Fe (III), but this is unlikely when oxygen is available as the electron acceptor, but they may be more important in the reduction (Adams *et al.*, 1992) of Fe (III) in desert Eh-pH conditions. This mechanism involves chelators that are readily present such as hydroxamates from MCF.

Summary

Rock coatings (desert varnish and glazes) derive their components from materials present in their environment. Components of coatings vary from locale to locale. Pollen, organic compounds, and soil components are all added to the surfaces of rocks. DNA, polymorphic compounds, amino acids that are present in soils and in surface microbes (such as MCF), aerosols, along with inorganic and organic compounds such as lead and pesticides when downwind from urban areas, are also added to the surface of rocks. The presence of labile organic compounds requires a mechanism for their preservation. The discovery of silica in desert varnish suggests that preservation may be facilitated by silicic acid ($Si(OH)_4$) or (di)silicic ($(HO)_3Si\text{-}O\text{-}Si(OH)_3$) through the formation of complexes with ions and organic molecules. Although organo-silica complexes were not directly observed, previous work has shown that amorphous hydrated silica forms both Si-O-C and Si-O-metal complexes. Coatings form as moisture from rain, fog, dew, and snow interact with detrital materials on rock surfaces. Too much rain such as that at

higher elevations or in temperate zones, may remove mineral/organic-rich solutions before they can become saturated and deposited through evaporation. However, under favorable conditions, deposits of silica slowly bind the surface material, complex metals and organic compounds, and entomb inorganic and biological substances. Polymerization of silicic acid provides a simple underlying mechanism for the formation of silica glazes and desert varnish.

CHAPTER 6 IMPLICATIONS AND APPLICATIONS

I want to know things I don't even know what they are...

Julia Alvarez, In the Time of Butterflies

Recognizing the early stages of life's evolution on Earth and other planets requires access to geological model systems that mimic early biotic conditions. Can the signature of life (biogenicity) be recognized in rocks from early Earth and Mars? This question will not only drive NASA's Martian rover and sample-return missions of the coming decade, but it is at the crux of a controversy over claims that fossil microbial life is present in a meteorite undoubtedly derived from Mars (Farmer *et al.*, 2001; McKay *et al.*, 1996). It also guides the search for evidence of the earliest life on Earth (Brasier *et al.*, 2002; Schopf *et al.*, 2002). These geological scenarios all share the possibility for the potential to identify microbial biospheres from billions of years ago. Rock coatings are relatively young, however, the processes that preserve labile organic compounds in silica have applications to hotspring sinter (~<20mya) and possibly deep time Earth fossils.

Implications to paleo-environments

Hot springs cogeneric with rock coatings

New Zealand's modern and extinct geothermal regions provide an environment to explore organosilica processes up to ~20 m y). They are both natural model systems for life elsewhere. These hot-spring deposits (sinters) and silica glazes serve as analogues for extreme conditions that prevailed on Earth, and possibly Mars, billions of years ago. Hydrothermal systems were the likely crucibles for the origin and evolution of early life, and are populated by Archaea and Bacteria adapted to life at high temperatures. Rock glazes derive their inorganic and organic materials from dust deposits, aerosols, and subaerial biofilms. Source materials in hot-spring sinters are supplied through continuous exposure to warm organo-mineral solutions.

Although New Zealand's sinters have been geologically well characterized and dated, the historic record of their abundant entombed microbes is still untapped. Recent investigations (Campbell *et al.,* 2001) have shown a unique silica mineralogy in New Zealand's hot springs. Since desert glazes are relatively young and New Zealand's unique sinters span a 20 million-year geological window, this provides a contrast in biochemical-signatures through all the key states of preservation in two seemingly diverse environments, but with a similar mineral matrix. Tracing these processes is likely to help resolve the current controversies surrounding the identification of biogenic signals preserved in old earth rocks with future applications to interpreting past extraterrestrial information preserved in stone.

The organosilica processes involved in contemporary subaerial sedimentary silicification and in hot-spring sinters, also provide recent analogues to Pre-Cambrian siliceous fossils. Understanding the preservation and formation mechanisms for organosilica may eventually lead to answers for putative deep time fossils and their implications to Earth's oldest paleoenvironments (~3,465-million-year-old Apex Chert).

Sugars as potential biomarkers

MCF and bacteria produce exopolymeric substances (EPS). They are composed of saccharides and/or polysaccharides. They have not been considered as chemical biosignatures since they are normally not stable in the environment after cell lysis. The sugars from bacterial peptidoglycans are complex polysaccharides found in the bacterial cell walls. Peptidoglycans contain linear polymers of two alternating sugars, N-acetylglucosamine (NAG) and N-acetylmuramic acid (NAM), that are cross-linked with the short peptides. These peptides are composed of some common amino acids, as well as some unusual ones, such as D-glutamic acid, diaminopimelic acid (DAP), and D-alanine (Madigan *et al.*, 2003), found in desert varnish (Perry *et al.* 2003 a). Another possible candidate for a biosignature within peptidoglycans is the the peptide inter-bridge composed of five glycines, which is found in some Gram-positive bacteria. The peptide cross-links in the peptidoglycans may protect the sugars from decomposition,

and thus enable them to serve as biomarkers.

One can reasonably ask if a simultaneous finding of these unusual amino acids, pentapeptide bridge, and the NAG and NAM sugars or their transforms, could indicate the remnants of the cell walls of bacteria. This would be of importance in the identification of bacterial fossils, and for the understanding of the processes by which various biofilms, coatings, and rock varnishes form on the natural surfaces and undergo mineralization over time. Substantial evidence links the presence of bacteria (and fungi) with the desert varnish formation (Staley *et al.*, 1992).

It is likely that bacterial polysaccharides will interact with the natural surfaces in a process that is probably facilitated by their initial complexation with metals that are found on these surfaces. The complexation may be followed in some cases by a redox-type reaction. It is known that peptidoglycans are highly interactive with dissolved metal ions (Banfield and Nealson, 1997).This may be the beginning of a sequence of reactions that leads to the cementing of bacteria to the surface. An additional pathway would be via complexation with silicates, of a type described by Kinrade *et al.* (1999, 2001) for polyols and sugar acids.

The prebiotic presence of sugars, on the other hand, has been largely disputed until recently. There is little problem with the prebiotic synthesis of sugars, as they can be formed from formaldehyde in an alkaline solution by the formaose reaction. One problem does exist in that the biologically important sugars, specifically ribose, are not favored in the formose reaction. Other problems lie in the fragility of sugar molecules. The prebiotic reactions of sugars haven't been explored much, as it is believed that sugars are not robust enough to survive prebiotic conditions (DNA and/or RNA were not expected to be preserved in desert rock coatings). This belief was put in doubt when sugar-related organic compounds were isolated from carbonaceous meteorites (Cooper *et al.*, 2001). In 2004, Ricardo showed that ribose, one of the key sugars to biotic systems, can complex out of formose mixture by borates (Ricardo *et al.* 2004). Kinrade *et al.* (2003) showed that ribose also makes stable silicate complexes. From these a picture

begins to emerge suggesting that ribose can be preserved in alkaline desert conditions and preserved by complexation with metal and/or silica.

Paleobacteriology

The unique finding in this study that DNA is preserved in the mineral matrix under conditions, requires a mechanism that sequesters and protects fragile DNA. The double stranded DNA molecule is more resistant to damage than the single-stranded RNA molecule (Matheson and Brian, 2003). The sugars of DNA are sensitive to oxidation as are the pyrimidine bases, especially thymine. The phosphate ester bond is susceptible to hydrolytic cleavage. Hydrolytic deamination converts guanine to xanthine, and cytosine to uracil. Free radical oxidation is detrimental to nitrogen bases and photochemical reactions case the formation of superoxide radicals.

Mineralization processes generally have been shown to be detrimental to preserving reproducible DNA, but this depends on the mineral (Eglinton, 1998). The crystallization of DNA into a structure that makes it more stable, acting like an inorganic crystal. According to Matheson and Brian (2003), the major factor in protection of DNA after cell lysis, is the use of small acid-soluble proteins (SAPs). SASPs binding to DNA, alter its chemistry and making it more resistant to UV damage, depurination, and oxidizing agents. Thermophilic bacteria employ the same protective mechanism. Condensing silicic acid, that entombs or complexes DNA may protect DNA from destruction. Sporulation also is a protective mechanism. The cells walls of spores contain oxides including calcium and manganese along with peptidoglycans and dipicolinic acid. As discussed in Chapter 4, evidence was found for peptidoglycans but dipicolinc acid was not detected.

Chirality

The interaction of amino acids and clays and their possible role in chemical evolution and prebiotic chemistry has long been considered (Bernal, 1951). More recently Cairns-Smith (1996) suggested that the interaction of clay surfaces with organic

compounds played a role in organizing organic chemicals that lead to life. Of particular interest is the proposal by Cooks (2003) that homochirality of life might be explained by serine's overwhelming predisposition to form stable homochiral clusters. It is beyond the scope of this research to address the possibility of other amino acids being preferentially polymerized by clays in their L over D form, as suggested by Degens et al. (1970) and Jackson (1971). Nevertheless, the possibility that clay minerals (Jackson, 1971), and the interaction with amino acids (serine) might be selective for enantiomers has implications to prebiotic chemistry. A primary question is why the twenty-two protein amino acids in living organisms on Earth are all L or left-handed? In contrast, DNA, a treble helix of collagen, and α-spirals of globular proteins all are D or right-handed. Recent investigations Takats *et al.* (2001) have suggested that serine has a propensity to form in chiroselective clusters and not form racemic mixtures. It has now been suggested that serine is unique in its ability to tie to glyceraldehydes, glucose, phosphoric acid and transition-metal ions. This observation suggests that serine may provide a key part of the answer to homchirality in living systems (Takats *et al.* 2003). Serine, then, may be unique in that it may have provided a transmission to other biomolecules. It is interesting that serine may be important in the chiral orgins of life and is also the one amino acid that bests complexes with silica. Considering that it is potentially unique in binding to amorphous silica, finding this amino acid preserved in ancient systems, possibly along with other amino acids, might yield important clues to the origin of life, especially if found on Mars.

Applications to Mars

NASA and other space agencies are interested in the search for life on Mars. The early Viking biology experiments are generally accepted to be unsupportive of life, (McKay, 1998; Biemann *et al.*, 1977), although some differences of opinion exist (DiGregorio, 1997). Viking and Mars Pathfinder missions provided images of many Martian rocks showing their dark surfaces. These resemble oxide-rich coatings, reminiscent of rock varnish (DiGregorio, 2002). DiGregorio suggested that rock varnish

might be a habitat for extant life on Mars (DiGregorio, 2002).

Martian meteorite ALH84001 is reported to have four types of biosignatures: bacterial microfossils, organics, carbonate minerals, and magnetite grains (McKay *et al.*, 1996). The evidence is still being examined, Barber and Scott, 2002; Becker *et al.*, 1999; Goldsmith, 1997), and it is uncertain at this time if the signatures observed in this meteorite are indeed biological or are due to non-biogenic processes.

The search for life on Mars continues. If novel forms of life exist on Mars, one needs to anticipate what chemical signatures to look for (Shapiro, 1995). Some valuable proposals have been put forth for the biochemistry of novel life, such as life based on alternative genetic systems (Shapiro, 1995; Kolb *et al.*, 1994; Benner and Hutter, 2002).

Silica coatings dating back to early Mars, may well be preserved in several other environments. Silica is a major portion of earth soils. Cracks and deep crevices in rocks, perhaps deep below the surface where water once penetrated, could serve to protect minerals harboring past environmental information. Boston *et al.*, (2004) and Northrup *et al.* (2003) suggest that manganese and iron oxides in caves resemble those of varnish coatings. They suggest (Boston *et al.*, 2004) a connection between biogenic minerals of caves and, as they postulate, those of desert varnish. They believe that surface varnish coatings on Mars could be used as indicators of previous biological activity and those of subsurface caves as a still-extant biosphere.

Analogues for the early synthesis of organic matter on Mars

On Earth, organic matter is found in association with sediments and sedimentary rocks. Sedimentary rocks that have undergone slight to moderate metamorphism may also contain remnants of organic matter incorporated prior to deformation. Igneous rocks, however, may only contain organic matter if it is introduced via fluid migration into fractures subsequent to crystallization. The Martian meteorites collected to date are all igneous rocks. Thus, the chances that they contain indigenous organic matter, biotic or abiotic, are remote. Whether the organic material in fractures of ALH84001 is indigenous or is contamination resulting from an extended residence time on Earth prior

to collection, remains unresolved. What is clear, however, is that the current collection of Martian meteorites is far from ideal, with respect to determining the types of organic compounds that may have been incorporated into sediments on the Martian surface. Carbonaceous meteorites (Cronin and Chang, 1993) consist of material derived from the solar nebula 4.5 billion years ago. They also exhibit varying degrees of aqueous processing thought to have occurred on a parent body(s) in the region of the asteroid belt during the early stages of formation of the solar system. Life appears to have existed on Earth for as far back in time as the rock record extends (~3.8 Ga). Thus, the only record for the solar system organic inventory that preceded life's origin on Earth or Mars are carbonaceous meteorites. In particular, the CI and CM carbonaceous meteorites contain many of the building blocks for life as we know it. It is interesting to note, however, that of the twenty protein amino acids common to all organisms, only eight have been observed in carbonaceous meteorites. Carbonaceous meteorites, collected at the time of or shortly after impact, provide the most reliable record of the solar system's organic inventory during the early stages of its formation. They provide the best analogue for what organic synthesis and aqueous processing may have been like on planetary surfaces prior to life's origin (Engel and Perry, 2003).

Evidence of life

One of the most universal features of life must be its inherent movement away from the predictable equilibrium chemistry (i.e., its negative entropy). This might be manifested in many ways, including complex structures, complex chemistry, and a variety of unpredicted chemical products that accumulate as a result of life's metabolism (Perry and Kolb, 2004 e in press; Nealson and Conrad, 1999). In searching for ways to detect life, we have thus focused many of our efforts on so-called non-Earthcentric approaches that look for such disequilibria. These might take the form of structures, chemical complexities such as macromolecules, or gradients of nutrients or products.

The first step in the search for life on Mars is still the search for organic molecules, since no positive (undisputed) evidence for them was found by any of the

previous space missions. Recent advances in analytical techniques and instrumentation, miniaturization of computers, and robotics make the technological component for the search for life easier to achieve than in previous missions. However, the conceptual component for the search for life is getting more complicated as exotic forms of life on Earth are being discovered, such as extremophiles that are living in previously unimagined harsh conditions, and species that reproduce extremely slowly such as endolithic microorganisms in Antarctica. It appears that we have only touched the tip of the iceberg for out-of-this-world Earthly life. Looking for life in all the obvious places using standard methods should not prevent us from considering that Martian life, at least theoretically, could be alien, and could be based on radically different biochemistry (Perry and Kolb, 2004e). While we might imagine such life to have self-replicating molecules based on templating and molecular recognition the same as the Earthly algorithm, the chemical description could be quite different. How should we look for alien life signatures and how do they differ from Earth's biosignatures?

Among the many possible approaches, one that starts with a search for amino acids and, if found, their relative abundance might be a starting point, especially considering the ability of serine to complex with silica. As part of a broader search for organic polymers that may have potential for carrying bio-information, amino acid based polymers should be looked for.

Why amino acids? First, they can be obtained easily during prebiotic synthesis as in the well-known Miller-Urey experiment (Miller and Orgel, 1974). The outcome of the experiment is sensitive to the atmosphere chosen, and it works best under reducing conditions (Miller, 1998). Additionally, amino acids have been found (along with other organic matter) in carbonacious chondritic meteroites , such as Murchison (Cronin and Chang, 1993). There are indications of enatiomeric excesses in some meteorite amino acids, which implies stereoselective extraterrestrial synthesis. Hypotheses that address the origin of enantiomeric excesses are reviewed by Pizzarelo (2001). They are typically based on the asymmetric properties of circularly polarized light generated in space by a

variety of means such as neutron stars or magnetically aligned spherical interstellar grains (MIE) scattering. If confirmed, the excess chirality found in some amino acids on Murchison would be of abiotic origin. By extrapolation of this conclusion to the hypothetical situation where amino acids would be found on Mars, any enantiomeric excess needs to be compared to that found in meteorites, before biogenic origin is claimed(Engel and Perry, 2003.

Future direction

The complex nature and diversity of rock coatings provides never-ending paths for future investigations, whether looking at paleo environments, or the chemistry of serine and silica, or understanding ancient DNA, deep time fossils, or the geochemistry and microbiology of hot spring sinter. Desert varnish acts like sticky-paper in accumulating bio-historical and inorganic information from the environment. If silica minerals are preserved from early environments on planets, such as Mars, Venus, or Europa, they may provide a ready source for capturing and holding information.

Summary

Proposed bioorganic reactions with rock coatings and older sinters suggest these concepts are intimately related to deep-time fossils and origin of life issues.
If novel forms of life exist on other planetary bodies, one needs to anticipate what chemical signatures to look for. Proposals have been put forth for the biochemistry of novel life, such as life based on alternative genetic systems (Perry and Kolb, 2004 a), and by the formose reaction described above, whereby ribose is preserved when mixed with borates (Ricardo *et al.*, 2004). If life is not found on Mars, the Martian chemistry still may prove very informative. Complex organic compounds may exist as a testament for an advanced chemical evolution that did not lead to life (for whatever reason). These chemicals may still exist.

CHAPTER 7 SUMMARY AND CONCLUSIONS

'The known is finite, the unknown infinite; intellectually we stand on an islet in the midst of an illimitable ocean of inexplicability. Our business in every generation is to reclaim a little more land.'

T. H. Huxley

Stable and unstable biological chemicals were detected in desert varnish from the Mojave and Sonoran deserts, including amino acids, rDNA and polymorphoic organics compounds. Carbon and nitrogen were detected in outer monolayers and after sputter removal, suggesting that they are present both within the coatings and in increased amounts on outer monolayers. This study presents experimental evidence that shows the presence of biochemicals in varnish coatings and suggests a simple underlying explanation of desert varnish formation, specifically, the role of silicic acid. The data and proposed explanatory mechanism presented account for the heretofore-unexplained features of desert varnish, including hardness, growth morphology and slowness of formation. Other factors that are important in varnish formation, namely incorporation of iron and manganese, as well as the preservation and incorporation of bacteria and fungal components, are also accounted for, as each of these interacts with silicic acid.

Findings

Analyses in this study show that there are variable amounts of TOC up to ca. 1.8%. The presence of amino acids and D-enantiomers suggests bacterial peptidoglycans are present in coatings, but does not prove a causative relationship of bacteria in coating formation. Finding of amino acids suggested that an attempt to extract DNA from varnish coatings should be tried, and a successful protocol was found. A new culture-independent analysis was used to measure bacterial diversity in rock desert varnish coating from Death Valley, California. DNA was extracted from varnish coatings, and

using PCR to amplify 16S rDNA, both Archaea and Bacteria rDNA were amplified from coatings and surrounding soils. This study indicates that there are a wide variety of prokaryotic microorganisms in varnish coatings, including non-thermophilic crenorchaeota, and possibly photoautotrophic primary producers. DNA cloned from coatings and amino acids may be sequestered in silica, clay minerals, or by complexation with divalent and trivalent ions, as it is known that Fe ions interact with phosphate groups and Mn interacts with nitrogen bases of DNA. The addition of Fe ions to a DNA-Mn complex may lead to stability and Mn binding may occur irrespective of Fe-DNA interlinking. This might provide a mechanism for preserving DNA found in silica-rich rock coatings. Not only organic substances then, but metals also might participate in polymerizing, crosslinking and hardening, as small quantities of silicic acids condense and fuse by gelling.

TOF-SIMS and XPS showed that there is an abundance (>10%) of carbon and nitrogen in the outer monolayers of coatings. TOF-SIMS indicated several polymorphic organic compounds including plant compounds from Joshua trees and pesticides. Inorganic analysis showed increased amounts of lead in areas east of metropolitan Los Angeles California but substantially less in the Mojave Desert, suggesting that varnish coatings act like a sponge adsorbing aerosols and organics from dust.

After the finding of several organic compounds, the question became how are they preserved on hot surfaces (~ up to 60°C), with minimal water, and high solar radiation? This question lead to the hypothesis that amorphous hydrated silica (opal), formed by the polymerization and condensation of silicic acid, complexes amino acids, specifically serine and threonine. The preservation of these labile amino acids was not expected under the harsh desert conditions. The discovery of amorphous silica provided at least a partial answer to how labile organics are complexed and stabilized. Serine is also found in siliceous diatoms. The formation of desert varnish appears to be detrital matter and aerosols landing on surfaces, undergoing redox chemical reactions where oxides are leached, complexed and deposited along with organic compounds.

Amorphous silica was identified in varnish coatings and on surfaces by a variety of techniques, and not only provides a possible trap for organics, but it also suggests a mechanism for cementing all of the detritus that comprise desert varnish. This probably is a complex process that adds materials to coatings reflecting the local environmental conditions.

Separating clay, sand, and silt-sized particles from varnish showed that little clay was present in coatings, however, XRD spectra showed that opal A and CT are present. Oxide-rich areas are observed on varnish surfaces along with areas of nearly pure silica. The presence of silica is readily observed in XRD spectra in the clay-sized fraction after size separation of clays, silts, and sands. XRD spectra before separation detected the minerals albite, augite, anedosine, diopside, and quartz. Varnish crystalline source minerals from dusts and soil deposits dominate the spectra before size separation, obscuring clays and amorphous hydrated silica (opal) in the lay sized fraction This may provide an explanation for why silica has not previously been detected. D-spacing of the clay-sized fraction is suggestive of a layered hydroxide such as pyroaurite or hydrotalcite rather than kaolinite or smectite in interstratified layers. Soils separated into similar sized fractions contained kaolinite, smectite and trace chlorite. Varnish samples, however, had only trace amounts of smectite and contained illite and kaolinite.

Outer surface monolayer analyses (XPS and TOF-SIMS) confirms that silicon is the dominant inorganic element. Substantial quantities of carbon (~30 atomic weight %) were detected. After surface sputter removal of ~10 nm, carbon quantities remained high but were reduced to ~10 atomic weight %.

Proposed Model

Rock coatings derive their components from materials present in their environment. Components of coatings vary from locale to locale. Pollen, organic compounds, and soil components are all added to the surfaces of rocks. DNA, polymorphic compounds, amino acids that are present in soils and aerosols, along with inorganic and organic compounds, such as lead and pesticides downwind from urban

areas are also added to the surface of rocks. Upon death, MCF degrade and their components become part of coatings, as was shown by SEM imaging and EDS. Coatings form as moisture from rain, fog, dew, and snow interact with detrital materials on rock surfaces. Too much rain, such as that found at higher elevations or in temperate zones, may remove mineral/organic-rich solutions before they can become saturated and deposited through evaporation. However, under favorable conditions, deposits of silica slowly bind the surface material, complex metals and organic compounds, and entomb inorganic and biological substances.

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Vita

AREA OF INTEREST: Astrobiology/Exobiology. Microbial and mineral interface. Biomineralization, and dissolution and redepositon of minerals. Silicic acid, DNA, amino acids, and sugar interactions. Amino acids in meteorites. Molecular biology of rock coatings. Preservation of microbes and prebiotic chemicals and their detection in ancient Earth environments and on other planets. Understanding the mechanisms of formation of microstromatilitic deposits and subaerial biofilms. Medical geology. Paleobiology and early Earth microfossils. Life in extreme environments. Prebiotic and biotic chemical evolution. Evolution and chemical composition of planets and planetary bodies. Organic signatures of mass extinctions. Biology and knowledge. Philosophy, art, music, education, freedom and peaceful co-existence.

EDUCATION

2000- 2004	PhD	Earth and Space Sciences with Astrobiology certification, co-advisors, Professor Alan R. Gillespie (ESS) and Professor James T. Staley (School of Medicine, Microbiology)
1977- 1980	M.S.	Geology, University of Washington, advisor, Professor John B. Adams
1974- 1976	B.S.	General Interdisciplinary Studies with advisors in Astronomy, Geology and Microbiology, major title"Exobiology"; minor, Art, University of Washington

SELECTED AWARDS

- National Science foundation Internship to Carl Von Ossietzky University of Oldenburg Germany, INSTITUT FÜR CHEMIE UND BIOLOGIE DES MEERES (ICBM), with Wolfgang Elizabeth Krumbein and Anna Gorbushina, Fall 2003
- President's Advisory Committee for White Mountain Research Station, University of California San Diego 2001 – 2002
- Oregon State University, Woods Hole Oceanographic, Atlantis and Alvin Summer 2002
- Stipend NATO/NASA Advanced Studies Institute Astrobiology– Chania, Crete Astrobiology 2001-2003
- Fellowship NSF IGERT for Early Evolution and Astrobiology, University of Washington 2000 - 2003

- Research Assistantship, Geology Graduate Department, 1978-1979
- Scholarship, Interlochen International Music Camp, University of Michigan Summer 1962

OTHER TRAINING

- Pilot Certification with ratings in fixed wing single and multiengine, helicopter, instrument rated
- Scuba certified
- Sky Diving certified
- Lieutenant U.S. Army, Armor, 1966 - 1969, South Vietnam 168-1969, honorable discharge