MICROBIAL PIGMENT ROBUSTNESS IN MARTIAN AND OCEAN WORLDS BRINES AND EVAPORITES. S. M. Perl[1,2,9], A. J. Celestian[2,4], C. S. Cockell[3], F. A. Corsetti[4], C. Basu[5], B. M. Javier[1,6], M. Garner[7], J. Valera[8], D.M. Nisson[9]. [1]NASA Jet Propulsion Laboratory, California Institute of Technology, JPL Origins and Habitability Lab, 4800 Oak Grove Drive, Pasadena, CA 91109 (scott.m.perl@jpl.nasa.gov), [2]Mineral Sciences, Los Angeles Natural History Museum, Los Angeles, CA 90007, [3]School of Physics and Astronomy, University of Edinburgh, Edinburgh, Scotland, [4]Department of Earth Sciences, University of Southern California, Zumberge Hall of Science, Los Angeles, CA 90089, [5]Department of Biology, California State University, Northridge, 18111 Nordhoff Street, Los Angeles, CA 91330, USA, [6]Department of Microbiology, California Institute of Technology, Pasadena, CA 91125, [7]Chemical & Biological Engineering Department, Montana State University, Bozeman, MT 59717, [8]University of California Santa Barbara, Marine Science Institute, Santa Barbara, CA 93106, [9]Department of Geosciences, Princeton University, Guyot Hall, Princeton NJ 08544, [10]Blue Marble Space Institute of Science, 600 1st Avenue, Seattle, Washington 98104.

Introduction: Terrestrial evaporite minerals like halite and gypsum can preserve biological products over geologic time and can provide micron-scale ecosystem for life to continue post-preservation [1.2]. Halite and gypsum precipitation can incorporate fluid inclusions within the crystalline structures where biological matter can be preserved, potentially offering solar UV protection, desiccation protection from the outside environment, and shielding from potentially damaging enzymatic processes both chemical and physical.

Motivation: While we have studied these fluid inclusions in previous work [1,2] their importance is also noted by others [3-5]. Isolated pockets of brine trapped in halite crystalline structures have been used to study the ancient environments of where fluids originated as well as microorganisms and metabolites from biological processes from ancient brines [1,6-8]. Results reveal that β-carotene has a strong Raman signature that remains robust to UV-C exposure even when entombed in hydrated evaporite minerals. In particular, fluid inclusions within the halite displayed particularly strong β-carotene Raman signatures. Little change was observed even after hundreds of hours of UV-C delivery. Our results reveal that complex organic molecules like β-carotene should be preserved well in halite and respective fluid inclusions generated from the original mineral precipitation and that halite does provide significant protection from organic matter degradation via UV-C. The purpose of this paper is threefold. First we will highlight the optical results of UV-C exposure on β-carotene in Martian surface analog settings. Secondly we will show Raman measurements of biological pigments prior to and after UV-C exposure. Finally we will show the salinity constraints on β-carotene and other carotenoid pigments as a marker for brine salinity and water activity.

Methods: Three lab analogue samples were then distributed into four different solutions of DNA-free water and the salinity was increased to speed up time for NaCl precipitation. After substantial NaCl precipitation at room temperature and separately at 70°C oven temperature, eight Petri dishes of laboratory salts were generated. Raman analyses were performed on all the samples prior to and after UV-C exposure to have the proper non-UV exposed baselines for comparison

Pigment Volumes. β-carotene was mixed in with 500mL of UltraPure DNA-free water in three different volumes (Fig. 1, Tbl. 1).

Table 1: Volumes of β-carotene per brine solution and source of precipitated NaCl evaporites

Relative to In-situ Evaporites	Carotenoid (β-carotene) Pigment Volume		Planetary Analog Field Site
	g	*mM*	
Seasonal	0.02684	0.1	GSL [1]
Moderate	0.07068	0.263	(Laboratory [9])
Desert	0.16995	0.63300	Mojave [9]

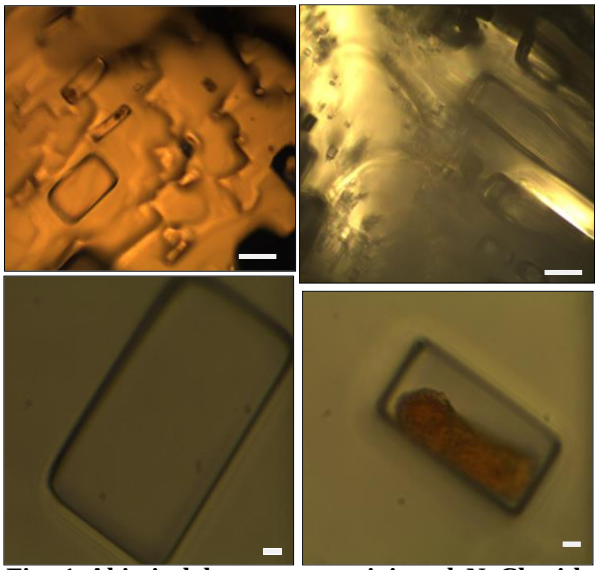

Fig. 1 Abiotic laboratory precipitated NaCl with varying amounts of entombed β-carotene. (Top-L) Fluid inclusions as viewed from the 40x objective showing small dark red sections of β-carotene entombment. (Top-R) Fluid inclusions as viewed from 40x showing almost no β-carotene. (Bottom-L) 100x objective perspective of an inclusion with no visible carotenoid. (Bottom-R) 100x objective from the same crystal as the image on the left but with entombed β-carotene. Scale: Top = 20µm. Bottom = 2µm.

Evaporation Studies of Abiotic Entombed β-carotene. Abiogenic β-carotene was mixed within three brine solutions at different concentrations and the evaporation of the brines was studied optically and with Raman mapping set to the major carotenoid peaks that have been observed.

Post-UV-C Exposure and Validation of Biological Pigments: During the timeframe of the UV-C exposure, pigments can remain in the fluid inclusions formed as the lab-precipitated halite grows or can be concentrated into the inclusions and take up much of the space there. The salts at this point retain trace amounts of pigment now bleached at 0.5-2µm features compared to the host/master mixtures of β-carotene + UltraPure DNA-free water. Once part of the evaporite matrix, these samples were then exposed to the aforementioned UV-C for 24 and ~120 hours (Fig. 2).

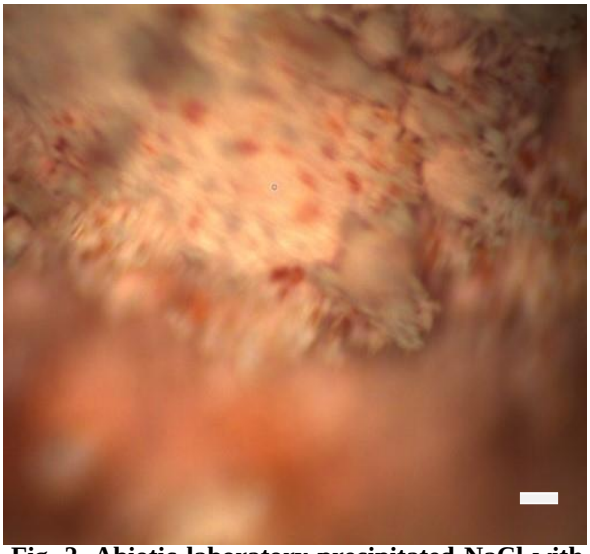

Fig. 2. Abiotic laboratory precipitated NaCl with varying amounts of entombed β-carotene under UV-C conditions. Sections of β-carotene after ~120hrs of UV-C exposure. Scale bars is 2µm. Accompanying fluid inclusions still retained their water volumes with β-carotene either mixed in solution or packed within corners of the inclusion. This spatial arrangement is also typical when halophilic microorganisms are present and preserved [2].

Mars Sample Return (MSR) studies: The future targets for in-situ Martian evaporite and sulfate mineral vein analyses need to focus on signs of fluid both on the vein surface (e.g., vertical sediment features aligned with ancient fluids) as an indication of where within the evaporite or mineral vein that contains evaporitic material. Within those features mm-abrasions of said vein could reveal microtextures that were formed from fluids. It is in these features that fluid inclusions should be the primary target for Mars

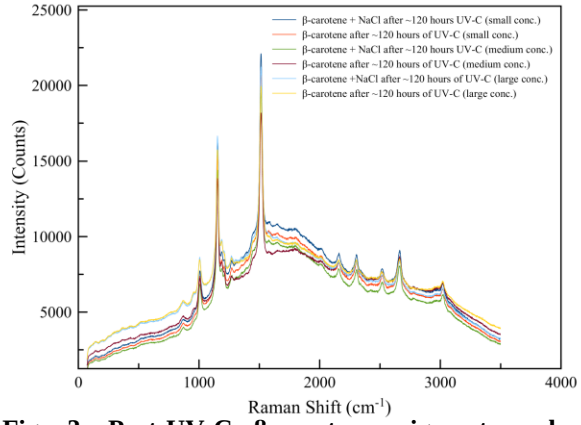

Fig. 3. Post-UV-C β-carotene pigment peaks. While the spatial differences between UV-C exposure and control are vast, pigment robustness after significant UV-C still shows vibrational peaks to that of the control [9] and of halophile preservation [2].

Sample Return (MSR), in-situ analyses, and biogeochemical analysis. As MSR samples are currently being cached by the Perseverance rover with future steps being planned to bring these samples back to Earth for terrestrial laboratory analyses, mm and micron-scale evaporitic features in these samples should be a high priority for extinct/ancient signs of biology. The ability of these fluid inclusions to retain water over geologic time would allow a paleoclimate record to be potentially preserved in irradiated and desiccated Martian surface rocks and minerals. While the MSR campaign is designed to be in the ancient biosignatures category of life detection, possible linkages from surface preservation can aid in understanding future shallow subsurface and deeper subsurface missions [10]. It is these future missions (e.g., an MLE-type of lander with regolith extraction) where extant life campaigns and authigenic biological processes can have a higher probability of being preserved. Moreover extant microbial communities can be maintained if available nutrients and liquid solvents are stable beneath the harsh surface environment and at the start of the Martian cryosphere [11].

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