EVALUATION OF BRINE CONCENTRATION EFFICACIES FOR NUCLEIC ACID PRESERVATION.

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Introduction & Motivation: Brine environments on Earth maintain thriving halophilic microbial communities and offer UV radiation protection and long-term desiccation prevention to halophilic microorganisms through enclosed brine fluids in evaporite minerals or fossilized stromatolites [1] The evaporite salt minerals in these hypersaline environments, created through billions of years of wetting and drying events, is uniquely responsible for life preservation in brine environments in geologically old mineralogy.

Modern Mars and Europa contain signs of evaporites and the characteristic wetting and drying events, signifying that these brine environments may have the same preservation potential to harbor extant life as on Earth.

In-situ experimentation on evaporitic sites on both Mars and Europa for extant life has the highest potential for detecting life in these planetary environments. The standardization and optimization of the scientific methodology utilized for planetary life-detection missions are fundamental to producing accurate measurements that evaluate planetary extant life while mitigating mission logistics. Microbiological life-detection methods, especially in low biomass environments as Mars and Europa are expected to be, traditionally concentrate samples to achieve ample DNA for DNA sequencing and then quantify the DNA present.

The purpose of this paper is to establish the efficacy of concentrating brine for nucleic acid extraction with three planetary analogue field sites: two hypersaline brines (Searles Lake and Boulby Mine) and one alkaline lake (Mono Lake). This research will guide specific parameters to aid the optimization of sample concentration and DNA quantification machinery onboard landed missions for potential microbial sample quantification and identification. Moreover, future onboard sample concentration efficiency will increase for landed missions, thus increasing overall potential yield for extant life in planetary brine environments.

Methodology & Laboratory Processes: After sampling at each of these sites, quantification of DNA before concentration was conducted. Samples will be concentrated and remeasured (post-concentration) for changes in bulk DNA quantification measurements.

Fluorometer Measurements: We utilized the Qubit 4 Fluorometer to quantify the DNA in the samples. Due to the low biomass of the samples, the dsDNA HS (high sensitivity) kit was used in these experiments. DNA quantification was measured in both ng/ml and ng/µl.

Planetary Analogue Field Sites: *Searles Lake:* Searles Lake is a dry, evaporitic saline lake located in the Mojave Desert in Searles Valley, California, USA. Due to the abundance of rare materials and evaporite minerals, Searles Lake has mining operations that extract sodium- and potassium-rich minerals (namely trona, hanksite, and halite) from its subsurface brines for industrial use. This lake experienced at least four extreme drying events throughout its history, creating a potentially hospitable environment for evaporite minerals to form. In these drying events, the salts become pigmented from photobiological responses [2] reminiscent of the haloarchaeal communities that inhabited the lake during each drying event. The oldest geologically dated subsurface brine in our sampling is ~1.1 million years old from the mid-Pleistocene.

Boulby Mine: Located near Loftus, North Yorkshire England, the Boulby Mine is an industrial salt mine charged with mining polyhalite, polysulphate, and potash for use as agricultural fertilizers. It is the second deepest mine in Europe, at depth of 4,600 ft. The deepest part of the mine is geologically dated to be 253 million years (Permian age) and of the Zechstein Formation, derived from the Zechstein Sea [3]. The sites sampled in the Boulby Mine were 327-18X/C, 29X/C, “White Crust”, and Bentham Baths.

Mono Lake: Mono Lake is a terminal saline soda lake in Mono County, California, USA. Situated at the north end of the Mono-Inyo Craters' volcanic chain, it is both geologically active from heat produced by lava flow and microbiologically active with thermophilic organisms. Two thermal sites were sampled at Pahoa Island: near the geologically active site and downstream of the site.

Pre-concentration Analysis: Quantifiable DNA was found in almost all the pre-concentrated samples and was relatively consistent within each site. Reported

below are partial datasets from total observations. In both the Searles Lake and Boulby Mine samples, a trend seems to be absent in terms of depth and DNA quantification measurements (Table 1). This is particularly interesting, as the expectation was that as the depth increased, microbial DNA density would as well. In the Mono Lake samples, only the samples stored in the freezer contained quantifiable DNA, despite bio-visual cues otherwise in the shelf samples (Table 1). This indicates a measurement anomaly within the data set, and investigative measures will be conducted to determine the cause.

A) Boulby Mine Pre-Concentration Bulk DNA Per Site		
Site (All Permian)	DNA (ng/ml)	DNA (ng/µl)
328 18 x/c	85.6	0.0833
	84.5	0.0836
White Crust	71.8	0.0892
	99.4	0.0835
Bentham Baths	61.7	0.077
	75.9	0.0751
29 x/c	68.0	0.0849
	105.0	0.104

B) Searles Lake Brine Pre-Concentration Bulk DNA		
Depth (m)	DNA (ng/ml)	DNA (ng/µl)
Surface	432.0	0.434
149- 149.6	337.0	0.327

C) Searles Lake Resuspended Salts Pre-Concentration Bulk DNA		
Depth (m)	DNA (ng/ml)	DNA (ng/µl)
Surface	86.4	0.0869
29.5	91.9	0.0887
103	109	0.104
144.7	114	0.114

D) Mono Lake Pre-Concentration Bulk DNA (ng/ml)		
Temperature Stored	Green	Red
Shelf (Room temperature)	Too Low	Too Low
Fridge (4 °C)	Too Low	Too Low
Freezer (-20 °C)	6	30

Table 1. Selected Pre-Concentration Reads for Bulk DNA. (a) Boulby Mine partial pre-concentration DNA. (b) Searles Lake partial pre-concentration DNA quantification in the brine and (c) in the resuspend salt minerals from transects in relation to depth of sample. (d) Mono Lake pre-concentration DNA quantification. Samples were incubated for about four years at room temperature, 4 °C, or -20 °C.

Preservation of Nucleic Acids in Brines: As microorganisms may become entombed in evaporitic salt mineral fluid inclusions, the biomolecules associated may be preserved as well. Biomolecules (nucleic acids, carotenoids, etc.) serve as excellent biomarkers in brine environments and as a signifier for halophilic life, extant or extinct, in hypersaline planetary environments [4].

Considerations of Biomass Loss: By design, the side of the pore filters used in the concentration process will dictate the total biomass, nucleic acids, and intact cells, that are contracted. Any biomolecule volume that is concentrated after the pores are filled will be lost to the filtrate. While this is unavoidable due to conservative estimates of planetary analogue brines, future robotic missions could utilize the filtrate for further sample analyses.

Ocean Worlds Sample Extraction: Future landed missions to Europa will likely involve producing ice melt from crustal regions for in-situ and onboard sample analyses [5]. These brines will come from heated ice samples that, if containing any biomass, would need to be heated slowly to reduce the risk of osmotic shock to cellular structures such that onboard geobiological analyses can take place. The decision to concentrate any samples, due to short duration commanding windows and mission timeline, will likely lead to an umbrella sampling procedure that would include brine concentration. While appropriate, determining the efficiency of such cryobrine concentrations would be critical for understanding total biomass per volume.

Summary & Future Work: These samples are from low biomass environments, so the presence of quantifiable DNA challenges our dependency on concentration to quantify DNA and provides an opportunity to witness the efficiency of concentration in these settings. In turn, an evaluation of the efficacy of concentration for DNA quantification will aid future in-situ experimentation and quantification of potential extant life present in planetary brine environments.

For future directions, these samples will be concentrated and measured for their DNA quantities. In this, we will be able to evaluate potential changes in diversity between the pre-and post- concentrated samples and determine if the act of concentrating modifies the diversity present in the samples.

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