

VARIATION AND CORRELATION OF BIOMARKER DETECTION METHODS: FIELD RESULTS FROM ICELANDIC MARS ANALOGUE ENVIRONMENTS.

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Introduction: Robotic planetary exploration is our primary tool for *in situ* investigation of potential habitats and biomarkers in the solar system. For mission planners to ensure that the limited analyses available in a astrobiological robotic exploration context are likely to yield representative results, *a priori* understanding of the spatial and temporal variance of biomarkers (as detected by a particular instrument or assay) is essential [1]. Where such knowledge is imperfect, selection of complementary biomarker assays with known (expected) correlations can compensate.

We conducted a planetary exploration analogue expedition to the recent Icelandic lava fields Fimmvörðuháls and Eldfell [2]. We used three biomarker detection techniques -- an adenine-triphosphate (ATP) luciferin/luciferase assay, nucleic acid staining for fluorescence microscopy (FM), and quantitative polymerase chain reaction (qPCR) with three domain-level primers -- on samples taken at four nested spatial scales (1 m grids, 10 m grids, 100 m grids, and > 1 km grids). Each technique's variability was calculated at each spatial scale. The level and strength of correlation between the detected levels of each biomarker were also determined.

Results: Variability within and between sites (defined at a 1 m, 10 m, 100 m, or > 1 km scale) was characterized for each assay using several statistical techniques, including the *F*- and *H*-tests. For relative ATP content, all spatial scales were highly diverse. For cell counts, as determined by FM, the 10 m and 100 m groupings showed significant variation, but at the > 1 km scale, results were highly consistent. For bacterial 16S, archaeal 16S, and fungal 18S DNA content, as determined by qPCR, the 1 m, 10 m, and 100 m scale groupings showed highly significant differences for all three tested domains; however,

at the > 1 km scale, the mean bacterial and archaeal DNA content differed strongly between sites, but not the fungal DNA content.

The non-parametric Spearman's rank test was used to determine the level (ρ) and significance (p) of correlation between each biomarker assay, with domain-level qPCR results treated separately. Cell counts were not correlated with any other data at any scale. ATP concentration was positively correlated with DNA content from all three domains, though the correlation with archaeal DNA content was by far the weakest. Bacterial DNA content, which accounted for > 99% of total DNA content, was positively correlated with archaeal and fungal DNA, but the correlation between archaeal and fungal DNA was of borderline significance.

Conclusions: The biomarker ATP, as measured with a bioluminescence assay, appears to be by far the most sensitive to small changes in sampling location and environment. Bacterial and archaeal DNA content appear similarly suitable for studying small-scale variation or micro-environments, but not necessarily for drawing conclusions about larger areas. Cell counts and fungal DNA content have significant local variation but appear relatively homogeneous when averaged over scales of > 1 km, making them better suited for wide-ranging but sparse sampling.

The lack of consistent correlation between ATP, cell count, and qPCR results underscores the need for further investigation into their dependence on environmental (physicochemical) variables. Future work to repeat these tests with additional environmental data appears warranted.

References: [1] Conrad P. G. and Nealson K. H. (2001) *Astrobiology*, 1, 15-24. [2] Amador E. S. *et al.* (2014) *Planetary and Space Science*, 106, 1-10.