Muammar MANSOR
Lewis & Clark Fund for Exploration & Field Research in Astrobiology
awarded June 2013

Detecting Biosignatures from Biominerals Formed in Subsurface caves: Analogue to Mars

Research Report

Introduction

Life on Mars may have persisted in the subsurface regions of the red planet. But how can we know without being able to send man to Mars?

One way to achieve this through robotic means is by identifying biosignatures, specifically in minerals that were formed in association with microorganisms (biominerals). Gypsum ($CaSO_4.2H_2O$) minerals are abundant on Mars and on Earth. Thus, we can look at Mars analog site on Earth such as the Frasassi caves (43°24'03"N 12°57'43"E) in Italy.

600 meters below the ground, microorganisms thrive in the form of biofilms. A particularly interesting example is the snottites, acidophilic hanging biofilms found on the cave walls that are surrounded by needle-shaped gypsum crystals (Fig. 1). Besides needle-shaped gypsum, 'toothpaste' gypsum ("microcrystalline mineral aggregates with physical appearances ranging from very soft to cottage cheese to dry talcum"; Northup & Lavoie, 2001) are also present but without clear association with any microorganisms.

In this study, preliminary work was performed to identify biosignatures in gypsum biominerals in Frasassi through crystal morphology. Sample collection and results are discussed in the following sections.

Figure 1: Snottites surrounded by needle-shaped gypsum (Macalady et al., 2007)

Sample Collection

49 gypsum samples were collected from Ramo Sulfureo cavern (Fig. 2; see Appendix for a 3D-model of the cavern) in August 2013. Samples vary by morphology, height from water table, H_2S concentration and distance from cave wall. Aspect ratio (length/width) of each sample was determined via either a dissecting microscope or Scanning Electron Microscopy (SEM). A snottite sample was also collected for SEM imaging.

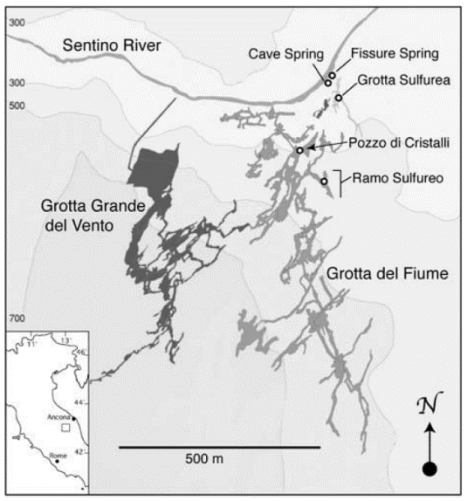

Figure 2: Map of Frasassi caves (Macalady et al., 2008)

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Results

Gypsum distribution in the cavern

Three gypsum morphologies were identified: toothpaste, needle-shaped and tabular. Toothpaste gypsum minerals were prevalent on all corners of the cavern and particularly abundant near the stream. Needles were sparsely found near the stream in pockets or depression on the wall in close association with snottites. Further away from the stream, larger needles were observed in higher quantities, reaching up to 15 cm long. Tabular gypsum minerals were only seen at one location in the cavern.

SEM images

Figure 3 is a collection of representative images for the various gypsum morphologies. Toothpaste are aggregates of fine-grained gypsum minerals, typically <10 µm in length. Needles are thin, long gypsum minerals that can reach up to 15 cm in length. Tabular gypsum minerals resemble thick needles that were less elongated on one axis.

Biofilm-mineral interactions are clearly visible in snottites. Fine-grained gypsum minerals are seen embedded in them, supporting our notion that certain gypsum minerals are biominerals.

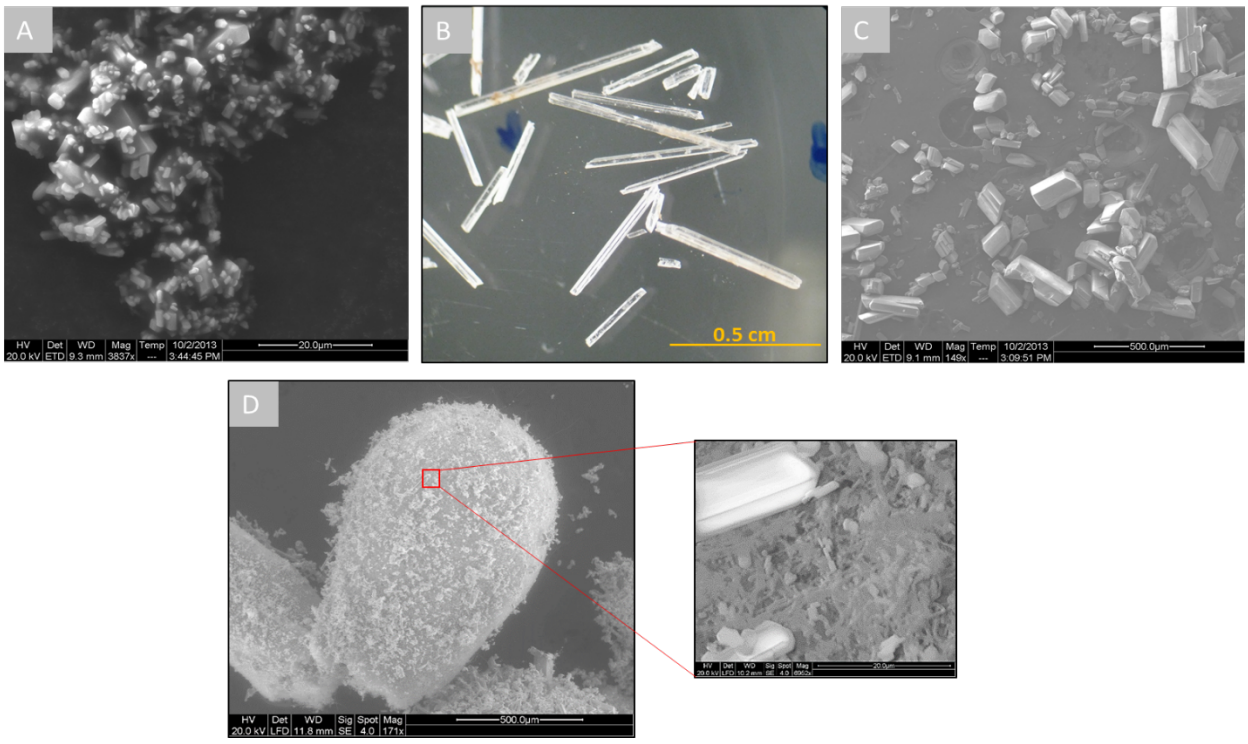

Figure 3: Representative images of various gypsum morphologies and snottite. A) SEM image of an aggregate of fine-grained gypsum minerals in toothpaste. B) Optical image of needle-shaped gypsum minerals. C) SEM image of tabular gypsum minerals. D) SEM image of a snottite head. Inset shows fine-grained gypsum minerals embedded in a filamentous biofilm matrix.

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Gypsum morphology: aspect ratio

Aspect ratio (length/width) of a mineral provides quantitative estimates for its morphology. Table 1 summarizes the aspect ratio for all samples. Snottite's gypsum at sampling site 3 showed the highest aspect ratio (22), which indicates very long and thin needle-shaped gypsum. Aspect ratio of gypsum samples plotted against height above water table and distance from cave wall showed no correlation (not shown).

When plotted against H_2S concentration (Fig. 4A), the data suggested that gypsum minerals with aspect ratio higher than 5 (i.e. needles) were only observed with H_2S concentration of less than 2 ppm, with the exception of snottite's gypsum at site 3. The data were suggestive of a biosignature in the form of anomalously high aspect ratios.

Similarly, when plotting aspect ratio against length, snottite's gypsum (length = 0.8 - 1.4 cm) had anomalously high aspect ratios. Gypsum needles from site 2 (length = 0.2 – 0.3 cm) and site 4 (length = 0.3 – 0.5 & 2 – 3 cm), which were not in the proximity of snottites only reached a maximum aspect ratio of 15. This may represent a threshold value for aspect ratio of gypsum minerals that were not formed in close association with snottites.

Summary & Future Work

Morphological data suggested that gypsum minerals formed in close association with snottites are distinguishable from those that are not. However, the data is not clear cut and must be interpreted carefully. Future work will focus on calcium and sulfur isotopic studies of the gypsum minerals in order to further determine an unambiguous set of biosignatures.

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Sample	Morphology	Height from water table (m)	Distance from cave wall (cm)	H_2S (ppm)	Aspect ratio (length/width)	Length (cm)	Stdev length
Site 1							
1.2m-A	Toothpaste	1.2	-	5	2.27 ± 1.02	1.23E-04	1.21E-04
1.2m-B	Toothpaste	1.2	-	5	2.18 ± 1.1	1.05E-04	1.12E-04
1.2m-C	Toothpaste	1.2	-	5	2.04 ± 0.82	2.11E-04	5.31E-04
1.2m-D	Toothpaste	1.2	-	5	2.37 ± 1.11	1.20E-04	6.35E-05
2m-A	Toothpaste	1.2	-	5	3.07 ± 1.8	3.79E-03	2.10E-03
2m-B	Toothpaste	2	-	5	1.8 ± 0.78	2.38E-04	4.26E-04
2m-C	Toothpaste	2	-	5	2 ± 0.97	2.92E-04	2.54E-04
2m-C2	Toothpaste	2	-	5	1.99 ± 0.89	3.36E-04	3.55E-04
2m-D	Toothpaste	2	-	5	2.54 ± 1.3	8.12E-03	7.32E-03
2.6m-A	Toothpaste	2.6	-	5	1.97 ± 0.89	1.73E-04	7.77E-05
2.6m-B	Toothpaste	2.6	-	5	1.81 ± 0.74	1.73E-04	2.04E-04
2.6m-C	Toothpaste	2.6	-	5	2.08 ± 0.91	2.67E-04	3.30E-04
2.6m-D	Toothpaste	2.6	-	5	2.05 ± 0.83	7.06E-04	6.84E-04
Site 2			-				
1	Toothpaste around needle	1.6	-	1.5	3.37 ± 1.72	5.52E-03	4.65E-03
1N	0.5cm long needles	1.6	-	1.5	12.19 ± 4.74	0.3	0.11
2	Toothpaste around needle	1.6	-	1.5	3.27 ± 1.57	5.22E-03	3.65E-03
2N	0.5cm long needles	1.6	-	1.5	11.02 ± 5.18	0.29	0.16
3	Toothpaste around needle	1.6	-	1.5	3.35 ± 2.73	4.01E-03	3.10E-03
3N	0.5cm long needles	1.6	-	1.5	10.5 ± 5.36	0.21	0.09
4	Toothpaste	1.6	-	1.5	2.93 ± 1.37	3.05E-03	1.75E-03
5	Toothpaste	1.6	-	1.5	1.95 ± 0.76	1.72E-03	1.19E-03
6	Toothpaste	1.6	-	1.5	2.33 ± 1.16	2.02E-03	1.73E-03
7	Toothpaste	1.6	-	1.5	2.22 ± 1.45	9.02E-04	4.96E-04
8	Toothpaste	1.6	-	1.5	2.66 ± 1.59	1.12E-03	7.16E-04
9	Toothpaste	1.6	-	1.5	3.34 ± 1.4	6.84E-03	3.27E-03
Site 3 (close to snottite)			-				
1	1-2cm long needles	2.05	-	4	22.08	1.39	-
3	1-2cm long needles	2.05	-	4	22.67 ± 10.42	0.77	0.36
Site 4			-				
1	2-3cm long needles	4	-	BDL	11.76	2.4	-
2	2-3cm long needles	4	-	BDL	14.23	2.99	-
3A	2-3cm long needles	4	-	BDL	7.98	2.33	-
3B	2-3cm long needles	4	-	BDL	12.50	2	-
4	2-3cm long needles	4	-	BDL	7.33	2.68	-
4B	2-3cm long needles	4	-	BDL	10.86	2.13	-
5	0.5cm needles	4	-	BDL	9.8 ± 5.55	0.32	0.19
6	0.5cm needles	4	-	BDL	12.57 ± 3.56	0.46	0.17
8	0.5cm needles	4	-	BDL	10.82 ± 4.57	0.45	0.2
9	Toothpaste	4	-	BDL	1.6 ± 0.87	1.66E-03	1.90E-03
10	Toothpaste	4	-	BDL	2.17 ± 1.09	1.84E-04	1.19E-04
11	Tabular	4	-	BDL	3.79 ± 1.49	0.22	0.09
12	Tabular	4	-	BDL	2.36 ± 1.61	9.42E-03	5.80E-03
Site 5			-				
1	Toothpaste	1.8	2.5	4.5	2.14 ± 0.97	2.32E-04	2.43E-04
2	Toothpaste	1.8	2.24	4.5	2.18 ± 0.88	3.37E-04	3.13E-04
3	Toothpaste	1.8	1.96	4.5	2.04 ± 0.84	2.08E-04	1.64E-04
4	Toothpaste	1.8	1.68	4.5	2.2 ± 0.91	2.16E-04	2.84E-04
5	Toothpaste	1.8	1.4	4.5	2.14 ± 0.99	2.24E-04	3.00E-04
6	Toothpaste	1.8	1.12	4.5	2.12 ± 0.9	1.82E-04	1.02E-04
7	Toothpaste	1.8	0.84	4.5	1.88 ± 0.68	2.23E-04	2.55E-04
8	Toothpaste	1.8	0.56	4.5	2.08 ± 0.85	2.08E-04	2.92E-04
9	Toothpaste	1.8	0.28	4.5	2.2 ± 1	2.26E-04	1.08E-04

Table 1: Summary of characteristics of the collected samples. BDL: Below Detection Limit

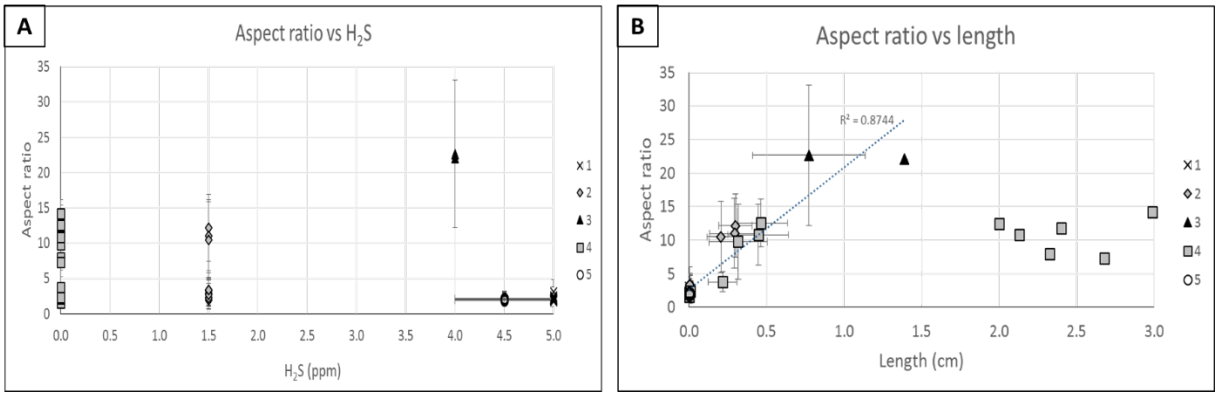

Figure 4: Aspect ratio of 49 gypsum minerals plotted against A) H_2S concentration and B) length. Minerals from different sampling sites are represented with different markers. In B), correlation coefficient (R^2) is calculated by excluding minerals with length >2 cm.

References

1. Northup, D. E. & Lavoie, K. H. (2001). "Geomicrobiology of caves: a review." Geomicrobiology Journal, 18(3), p. 199-222.
2. Macalady, J. L., Jones, D. S. & Lyon, E. H. (2007). "Extremely acidic, pendulous cave wall biofilms from the Frasassi cave system, Italy." Environmental Microbiology, 9(6), p. 1402 – 1414.
3. Macalady, J. L., Dattagupta, S., Schaperdoth, I., Jones, D. S., Druschel, G. K. & Eastman, D. (2008). "Niche-differentiation among sulfur-oxidizing bacterial populations in cave waters." The ISME Journal, 2(6), p. 590-601.

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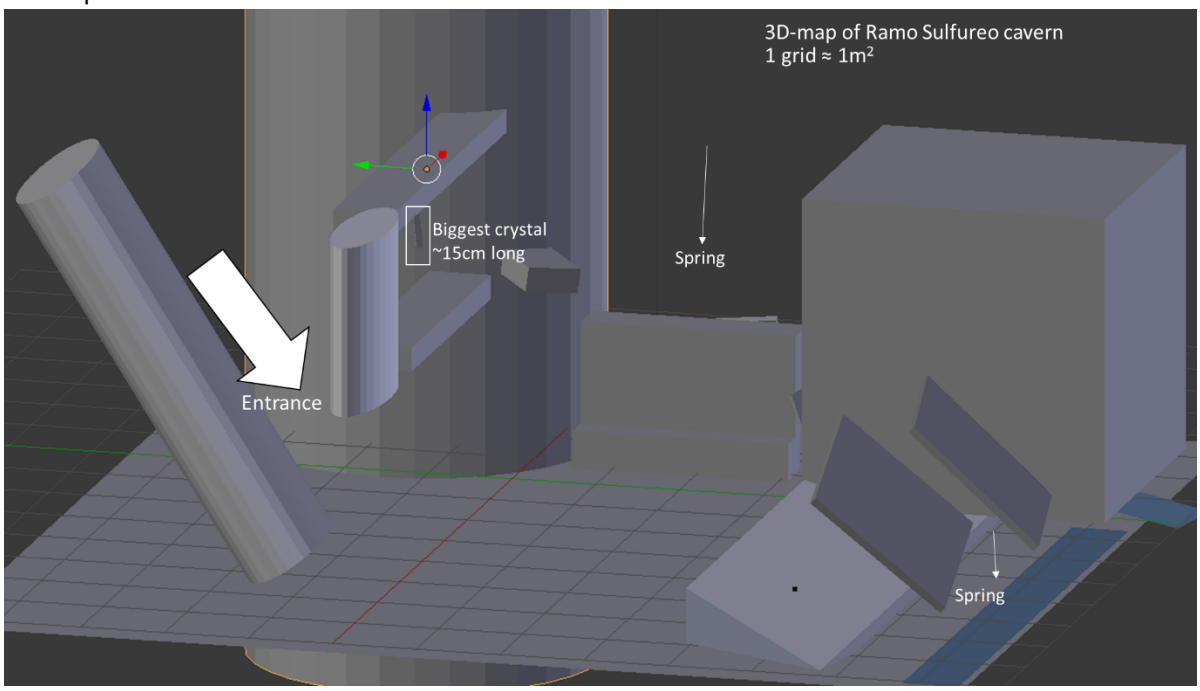
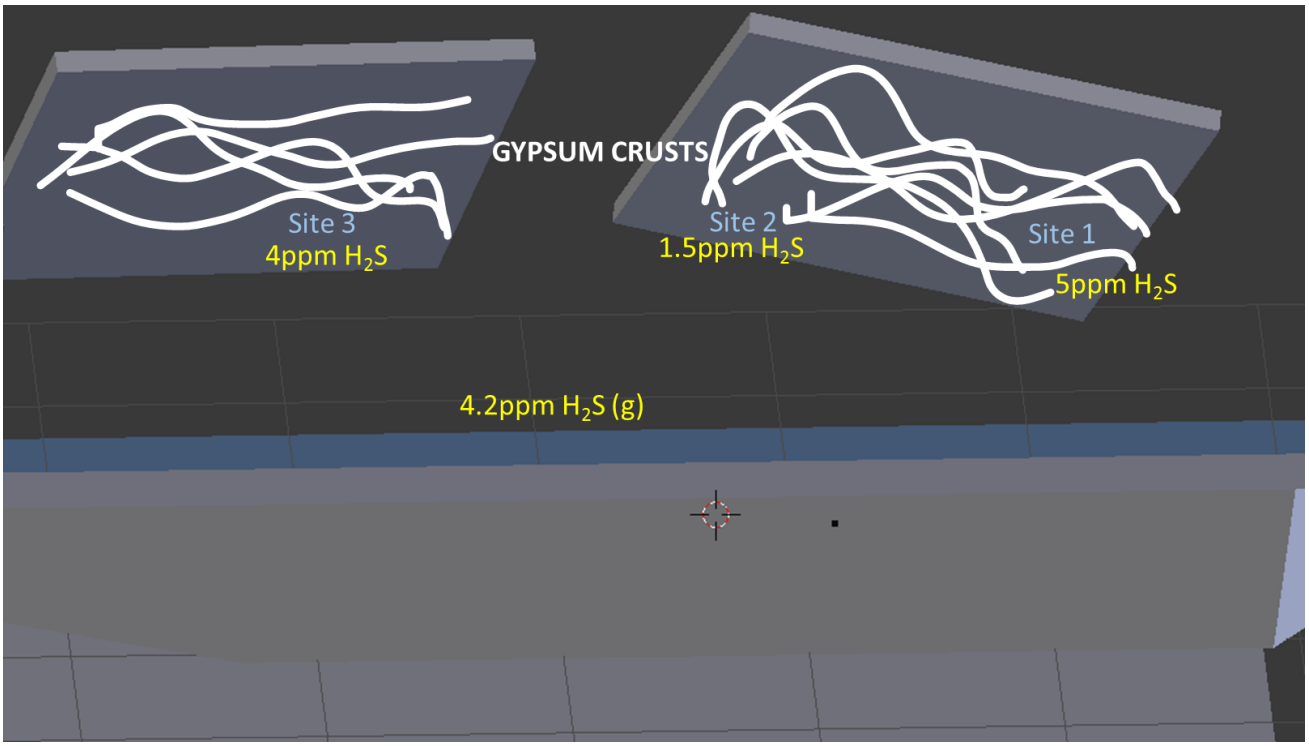
Appendix

A) 3D-map of Ramo Sulfureo cavern



Overall view



Sampling site 1, 2 & 3

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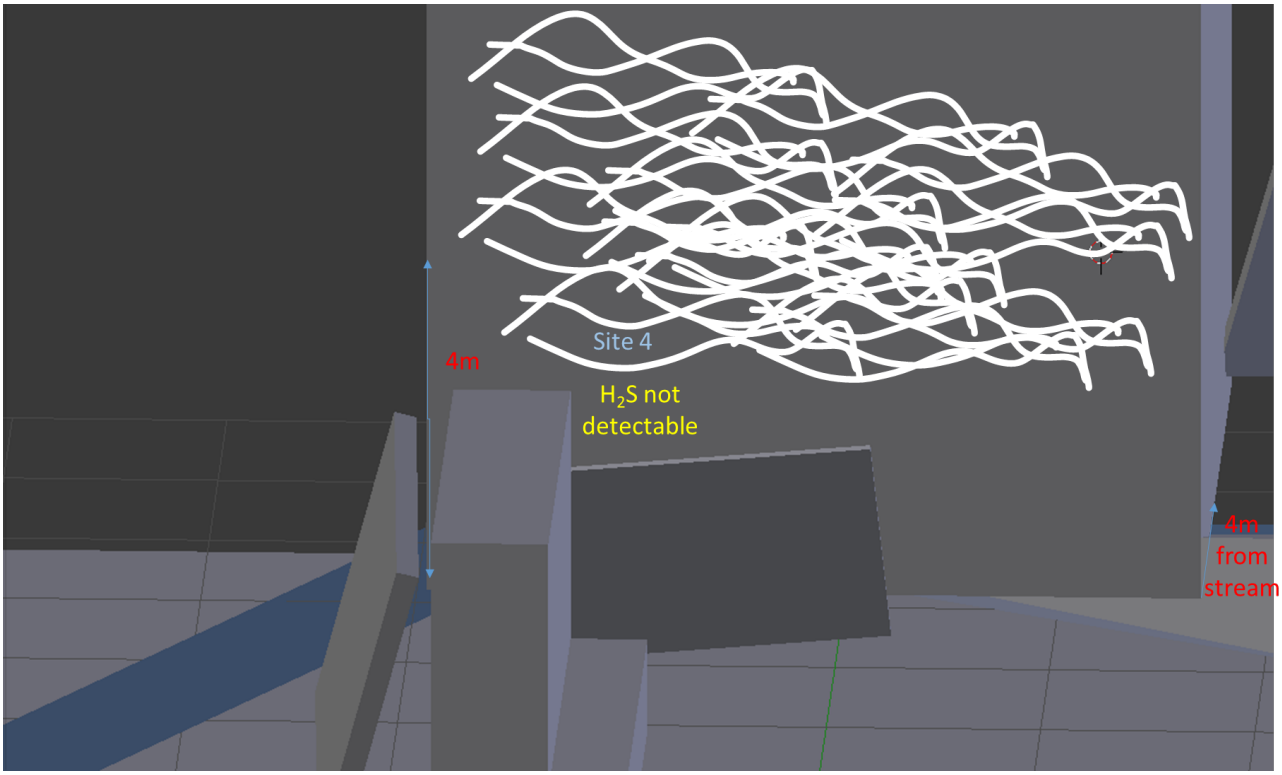
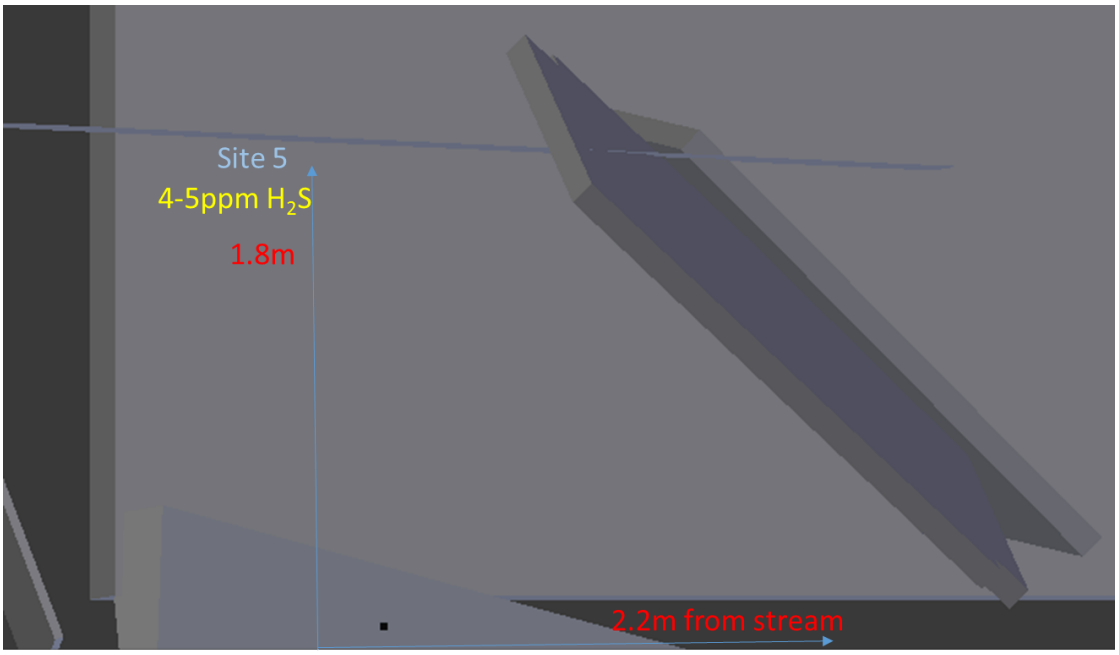


Sampling site 4



Sampling site 5