Examining the Chemical Environments of Underground Microbial Life

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INTRODUCTION

- Microbial life has been discovered by scientists in the **Sanford Underground Research Facility** (**SURF**), located in Lead, SD.
- We have investigated the bacteria's chemical environments through **water sampling**, **biofilm extraction**, **rock**, and **solid samples**.
- The chemical composition of water samples was continued. **ICP-MS** and **Atomic Absorption** were emphasized towards rock samples. This year, the **4850**, **800**, and **300** levels were sampled and explored.

Figure 1: 800 level entry

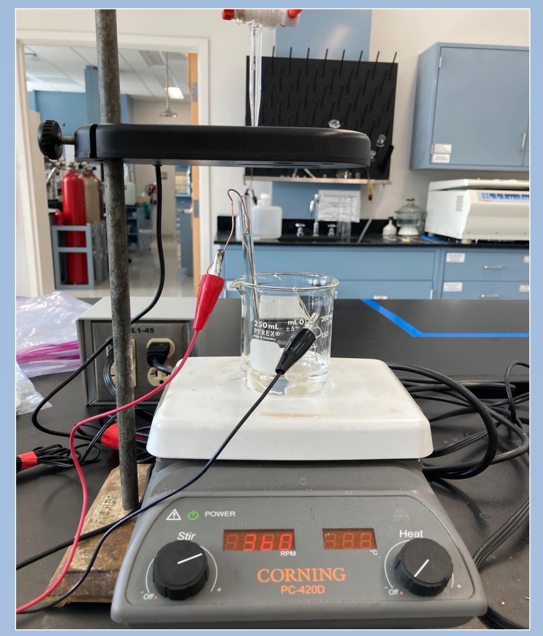

Figure 2: Potentiometric Titration for water sample Cl^- ion concentration

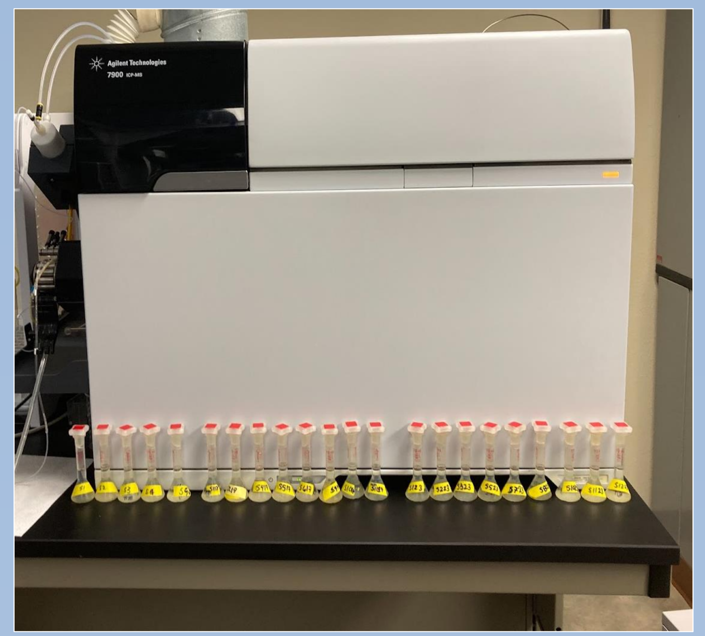

Figure 3: ICP-MS. 22 samples in front of instrument, ready to run.

Inductively Coupled Plasma Mass Spectrometry (ICP-MS) Methods:

- A blank, a method blank, then samples were analyzed with 5% HNO3 wash in between each sample. After the last sample was taken a calibration curve was initiated ranging from 0.01ppb until 100ppb of solution. The ICP-MS was then cleaned thoroughly with HNO3 then air was let in to complete the cycle.

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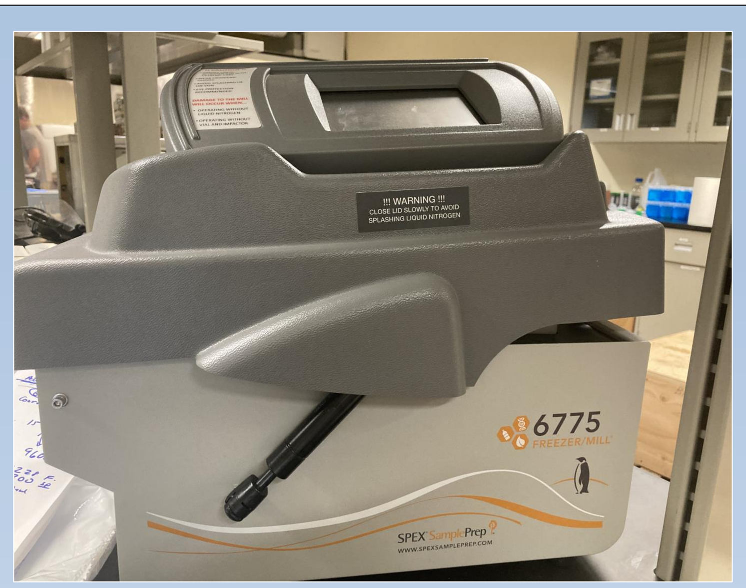


Figure 4: 6775 Freezer/Mill® Cryogenic Grinder froM the SpexSamplePrep Co.

UNDERGROUND METHODS

- Testing using specific Vernier probes and measurements were executed at each location.
- If time was limited, the same measurements were made aboveground in the BHSU laboratory using re-calibrated probes. Measurements include: Conductivity, ammonia concentration, pH, temperature (both air and water), dissolved oxygen, and pressure.
- Samples were collected in a 250 mL - 1L plastic bottles to when necessary. A 50 mL plastic syringe was used to extract water from shallow pockets of waters in the mines and caves.

LABORATORY METHODS

- Chemical analysis on the water samples were done via atomic absorption spectroscopy (AAS – Perkin Elmer AAnalyst 300), titrations, Vernier Model probing measurements, and inductively coupled plasma mass spectrometry (ICP-MS – Agilent 7900) at Black Hills State University.
- Solid samples were also collected and placed in a -90 C refrigerator until ICP-MS and further analysis was needed.

Chloride ion determination Methods (Potentiometric) titration for the precipitation of AgCl via electrochemical method. Electrodes were used and attached to a Cu wire and Ag wire. Both wires were thoroughly sanded before each different run. The salt bridge that was used was a string of $NaHSO_4$. ~50 mL of sample was used and combined with 3 mL of $NaHSO_4$. The Cu wire was contained in a glass tube in $CuSO_4$ solution. $AgNO_3$ was used as the titrant. **(See figure 2)**

Reaction that occured

$Ag^+_{(aq)} + Cl^-_{(aq)} \rightarrow AgCl_{(s)}$

$Cu_{(s)} \mid Cu^{+2}_{(aq)} \mid\mid Ag^{+2}_{(aq)} \mid Ag_{(s)}$

Measurement of alkalinity of the water samples Methods 0.01 M Standarized HCl was. Alkalinity was measured by titrating HCl into ~25mL of sample, initial pH was recorded and titration stopped at a ~2.5 pH electronic reading.

Rock Sampling and Analysis Methods The rock samples were grinded up using 6775 Freezer/Mill® Cryogenic Grinder from the SpexSamplePrep Co. and then digested using a variation of acids. The acids used were nitric (HNO3), perchloric (HClO4), hydrofluoric (HF), and hydrochloric (HCl) in order to draw out any chemical elements from solids for ICP-MS and further analysis. **(Figure 4)**

Figure 5: 300 level sampling

SAMPLING LOCATIONS

Designation	Location	Sample Type
4850-1	End of Ledge (left)	Water
4850-2	End of Ledge (right)	Water
4850-3	Across Smaller sample (left)	Water
4850-4	Smaller sample (right)	Water
4850-5	Thyothrx Falls outflow	Biofilm
4850-6	Thyothrx Falls outflow	Biofilm
4850-7	Thyothrx Falls outflow	Rock
4850-8	Thyothrx Falls end	Rock
4850-9	17 ledge (by locomotive travel area; beginning of trip)	Rock
4850-10	17 ledge (by locomotive travel area; beginning of trip)	Solid Sample collected by old Fe construction

Designation	Location	Sample Type
800-1	Cage Area	Water
800-2	Ditch by Ross sign; by magazine	Water
800-3	50-100ft from Site #2	Water
800-4	Large biologist collection sample site	Water
800-5	Collected by desk in 2nd NASA site	Water
800-6	Around 2nd NASA site	Rock
800-7	25 ft from site 6: Next to railing	Water
800-8	Collected by site #4	Water
800-9	Towards entry of cave	Rock Sample containing Cave Silver
800-10	Close to scavenged Fungi Lab	Water
800-11	50 ft from site #10	Water
800-12	7 paces from site #11	Water

Designation	Location	Sample Type
300-1	Towards Pump room: Edge of cave	Water
300-2	Towards Ross	Water
300-3	Pump room stagnant water: Edge of cave	Water
300-4	Edge of Cave	Water
300-5	In front of scavaged lab (Known as underground Pioneer)	Water
300-6	50 ft. from site 5	Water
300-7	Pipe condensation from walking back towards entry of cave	Condensation from Pipe
300-8	Collected close to samples #1 &#3	Rock
300-9	Collected towards middle of walkway close to suspected clear H2O, black remnants were observed.	Water
300-10	Wall drippings towards entry of cave	Water
300-11	Wall drippings 50-100ft from site #10	Water

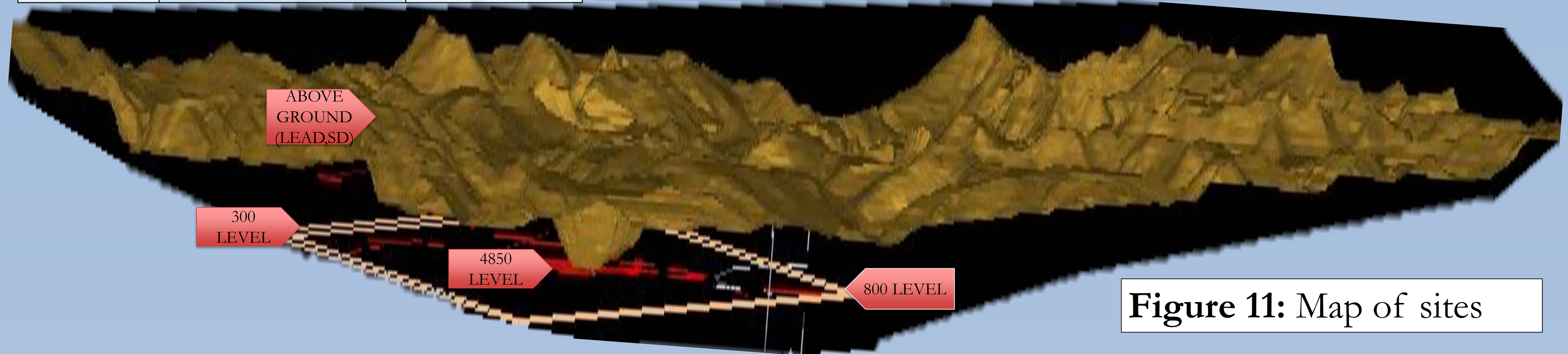


Figure 11: Map of sites

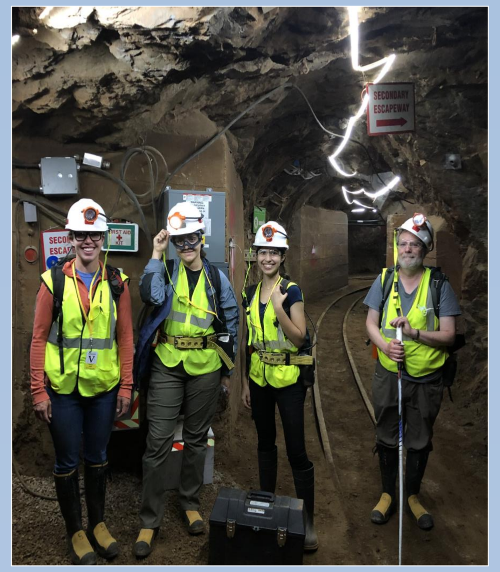


Figure 6: Group photo in front of 4850

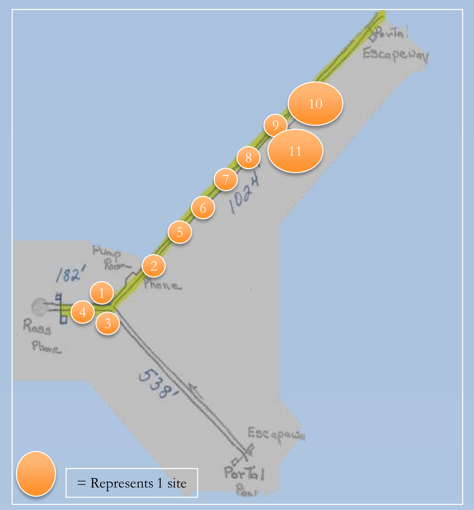


Figure 7: Map of 300

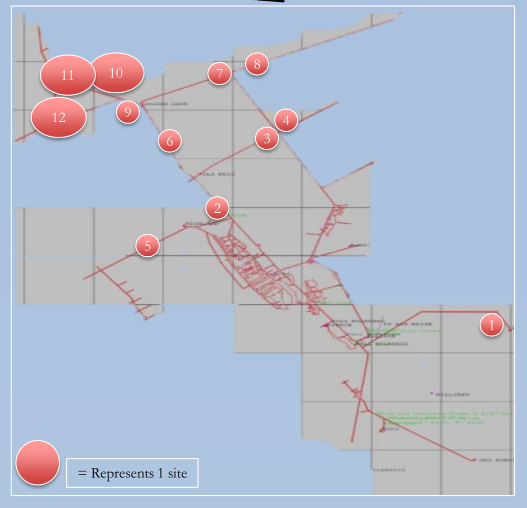


Figure 8: Map of 800

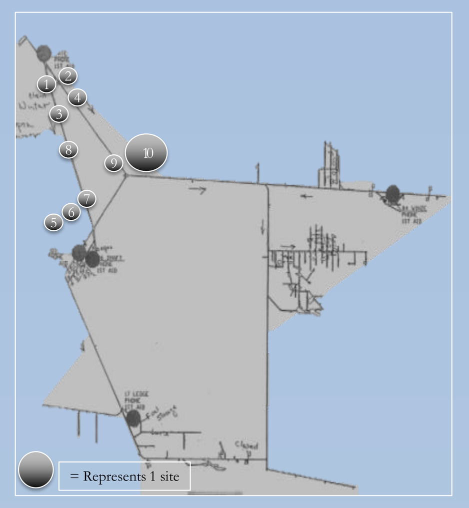
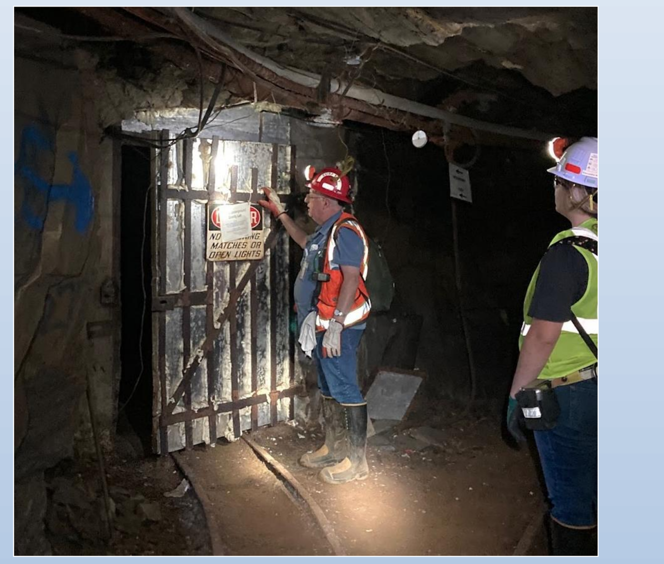


Figure 9: Map of 4850

Figure 10 : 4850 sampling

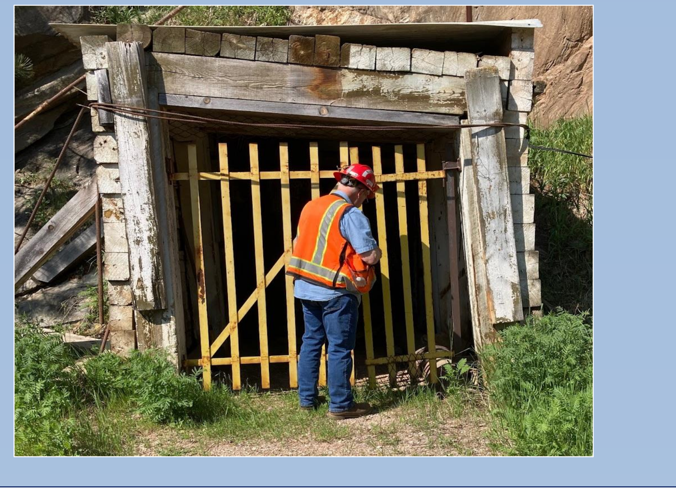


Figure 12: Davis Campus clean room entry

Figure 13, 14, & 15: (Top, Middle, bottom) 800 level entry, 4850 entry, & 300 level entry

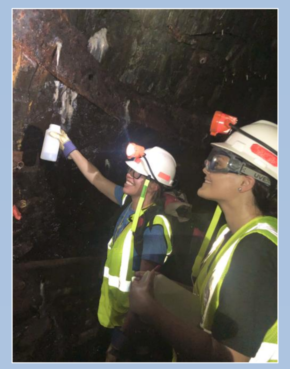
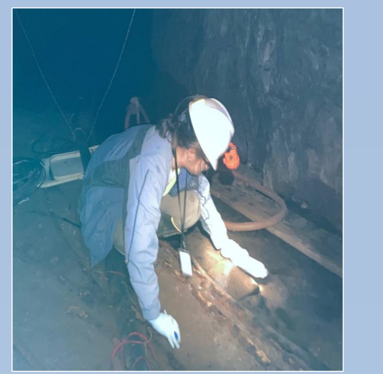


Figure 16 & 17: (Top to bottom) 300 level sampling



CONCLUSION

In previous experiments from past REU students and their mentor, a trend that is seen is emphasis on water sampling via Inductively coupled plasma mass spectrometry (ICP-MS), titrations, and Flame Atomic Absorption (AA). This year, the REU chemistry and biology groups decided to investigate more solid samples which had biofilm or microbiological traces living in the surfaces of many rock samples. Using acid digestion, the rock samples allowed for an approachable method of understanding the chemical characteristics in which the bacteria live in underground. These prepped samples allowed for ICP-MS analysis to be observed. With the help of ICP-MS, trace elements in the rock samples can be further analyzed while assisting further research in the biological world. Overall, a lot of the work done this summer was insightful in practicing analytical chemistry. The work done this summer was a continuation, however, a lot of the data storage from previous years lacked in sufficient information which lagged the overall research timeline this summer. The experience going down at SURF was helpful in understanding what kind of environment houses the microbial life underground.

REFERENCES

Baird, C.; Cann, M. The Chemistry of Natural Waters. In Environmental chemistry; Freeman: New York, NY, 2012.

Kegley, S. E.; Andrews, J. The Chemistry of Water; University Science Books: Sausalito, CA, 1997.

Science | Sanford Lab Homestake Visitor Center, Lead SD http://sanfordlabhomestake.com/science (accessed Jul 21, 2021).

Smith. L, et. al. Laboratory Notebooks and Poster Presentations from past REU students. (Augustana University, South Dakota University, etc.) Summer 2017-Summer 2019

Harris. D.C. Quantitative Chemical Analysis. (8th edition) W.H. Freeman & Co. New York , NY. 2010..

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