

Isolating Arsenic Metabolizing bacteria associated with Land-based art in Owens Lakebed in Order to Determine Bioremediation Potential

Project Summary:

This research addresses the question, if there are microorganisms present in Owens lake bed that have the capacity to metabolize toxic chemicals, and if there are, can their metabolic processes can be utilized for bioremediation. This will be addressed by studying the different microorganisms that are isolated from the soil and water present at the site. Our objective is to determine the capability of these microbes to withstand toxic levels of arsenic as well as their ability to transform arsenic from a highly toxic in-organic to a less toxic organic form.

This model will be used to shine a light on a relatively unexplored area of research. The effects of artificial desiccation on microbial communities, as well as the effects of arsenic metabolizing microbes on the toxicity of locations such as Owens Lake bed, have not been explored to the fullest extent. This research strives to provide new insights into this area of study.

Several social benefits will be realized as a result of better understanding how microbial life has the ability to provide potential bioremediation of the land as well as influence local land-based art. This experiment will be carried out by undergraduate students working alongside stakeholders and community partners. As a result, this study will integrate several groups across disciplines while working towards a greater overall understanding of how specific environmental processes occur and how they impact both the land and the people that live on the land.

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Statement of Problem Significance:

As a result of leaching from surrounding silver mines as well as rapid artificial desiccation, Owens lakebed has become a landscape reminiscent of a wasteland. The artificial desiccation has created high levels of toxicity in the soil and water that comprise the lakebed (Reheis 1997). Despite the inhospitable environment that was created, extremophilic bacteria seem to thrive in the pools and salt deposits that make up the lakebed. In order for these microbes to survive, they must metabolize the toxic chemicals and heavy metals that surround them. This ability to metabolize such harsh substances points to bioremediation as a potential solution. Remediation of toxic components such as: radioactive waste, toxic heavy metals, and other harmful substances can be quite difficult under extreme conditions (Jeong, Choi 2020). However, a closer look at the metabolic cycles of halophilic microorganisms that are already present in the environment could yield treatment methods for the reduction and potential removal of toxic pollutants in extreme environments, such as Owens lakebed.

Relevant Literature:

By the mid-1920's, Owen's Lake was completely dried due to artificial desiccation. As a result of this desiccation, the lake bed has become the single largest source of PM-10 dust in the United States (Reheis 1997). These dust storms have become a major health hazard to the people that live in and around the Owens Valley. Owens Lake is an extreme example of the potentially destabilizing effect on land surfaces and vegetation by the extraction of surface and ground water in desert regions (Reheis 1997). The dust storms in the area are not the only human health hazard that has resulted from the artificial desiccation. Significant quantities of toxic chemicals and heavy metals such as arsenic and antimony have been found (Shi et al. 2013).

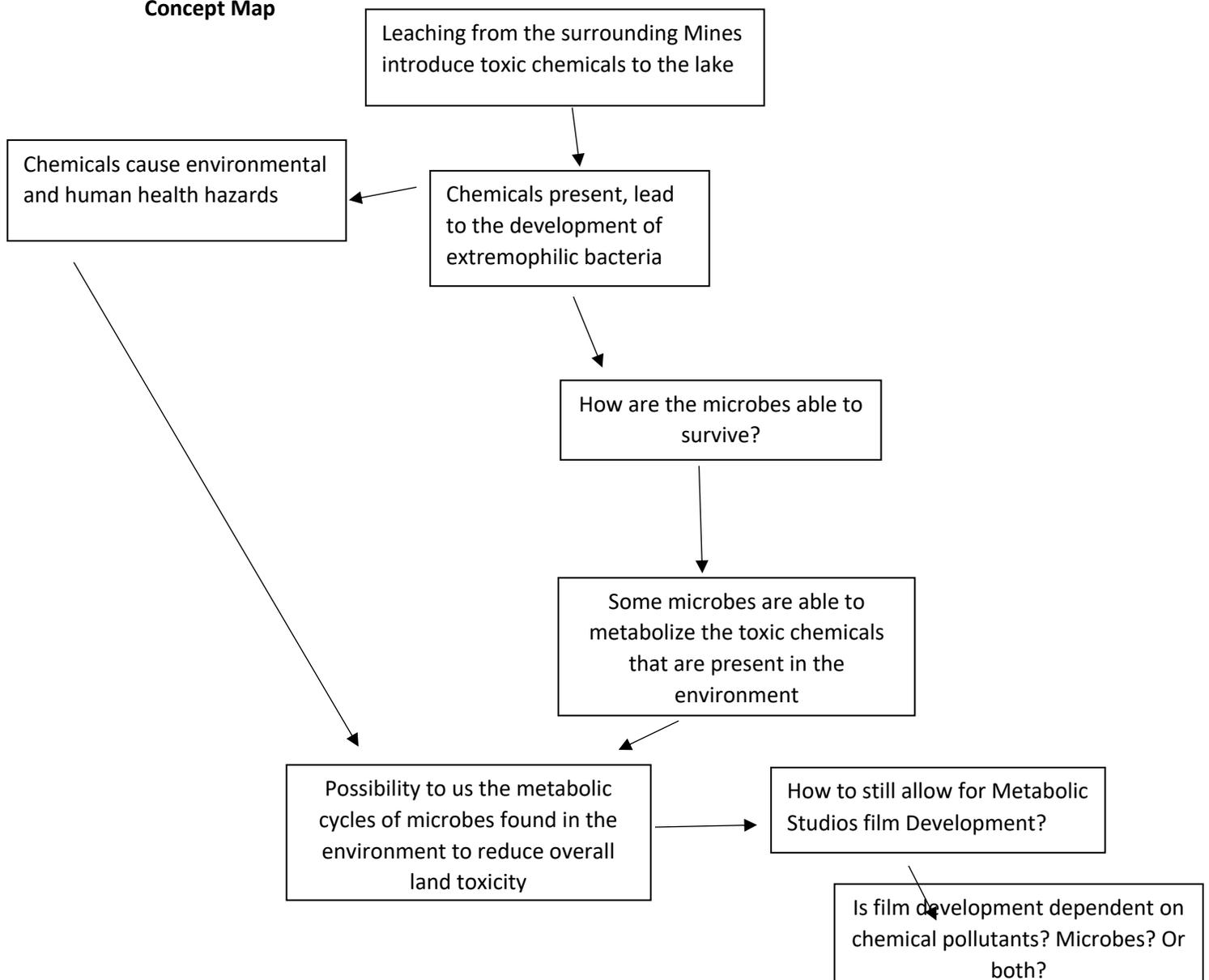
Arsenic contamination is a significant problem, which has impacts that can be seen in environments around the world. Despite the existence of such toxic substances, several different kinds of microorganisms have been found in the same areas (De Francisco et al. 2021). Analysis of different microbial communities in the presence of arsenic revealed that eukaryotic microorganisms may present many different arsenic tolerance or resistance mechanisms (De Francisco et al. 2021). Some of these mechanisms could prove to be pertinent for bioremediation.

Certain extremophilic microorganisms have proven to be effective bioagents with in their environments. These microbes are being explored for use in the clean-up of toxic pollutants that are contaminating the environment (Jeong, Choi 2020). Extremophilic microbes are a viable candidate for this form of bioremediation due to their unique characteristics such as toughness, adaptability, and strong resistance to extreme conditions (Jeong, Choi 2020). While more research is still needed on this topic, combining extremophilic microorganisms with biotechnology and nanotechnology is a promising endeavor and has the potential to open new avenues toward developing highly efficient and eco-friendly approaches for the treatment of toxic pollutants in harsh environments (Jeong, Choi 2020).

Preliminary Data

Preliminary data samples were collected from the Owens lakebed, at sites where photos were actively being developed by Metabolic Studios. Each Sample refers to a specific location where a specific photograph was developed. In sample 1, there was an average pH of 9.91, an average temperature of 38.9 C, and an average conductivity of 173612.667 uS/cm. In sample 2, there was an average pH of 10.11, an average temperature of 40.08 C, and an average conductivity of 144446.00 uS/cm. Sample 3 there was an average pH of 10.084, an average temperature of 37.36 C, and an average conductivity of 162193.20 uS/cm. Finally, sample 4 had an average pH of 10.075, an average temperature of 40.025 C and an average conductivity of 177096.50 uS/cm. While all of these photos were developed in the same general area of the lakebed, they were far enough apart to allow for noticeably different readings.

Concept Map



Justification of Methods:

In an effort to prove that arsenic metabolizing extremophilic bacteria are present in Owens Lake bed and have the potential to provide bioremediation, a variety of methods will be used. An E.Z.N.A. DNA kit will be used to extract any microbial DNA from the soil. This kit will be used because it has been proven as an effective way of extracting different DNA from soil samples at the same time (Garibyan, Avashia 2013). Polymerase Chain Reactions, or PCR, will also be used. This will be used to amplify the 16s rRNA gene. PCR was chosen because it is an efficient way to isolate and replicate different segments of DNA. Following this, DNA sequencing of the isolated segments will be done using the minION and minKNOW software. This software will be used because it is a well-known tool that has proven to be highly effective for DNA sequencing (Pruel et al 2018). Soil Extract Agar will also be utilized in this project. For this, some of the soil samples would be turned into a form of agar that contains soil and all the nutrients found in it. This method was determined to be the best because it would allow for the microbes grown from the samples taken to grow in an environment almost identical to the landscape they were taken from. Finally, MelonnPan will be used to predict the metabolic profiles of the different microbes isolated from the sample. MelonnPan is a novel, computational method for predictive metabolic profiling (Mallick et al 2019). This method is highly cost effective compared to traditional metabolic profiling, as all that it requires is R studio and the provided user manual. While, this is only predictive, not definitive metabolic profiling, simple predicting metabolic cycles is adequate for the purposes of this research project, since the results will be confirmed using plating methods.

Objective

By isolating the microorganisms that are native to the landscape and predicting their metabolic profiles using MelonnPan, we will be able to determine what microbes have the highest potential for bioremediation. This can then be confirmed by varying the arsenic levels of the growth medium in order to determine through physical means which microbe has the highest ability to metabolize arsenic.

Aims

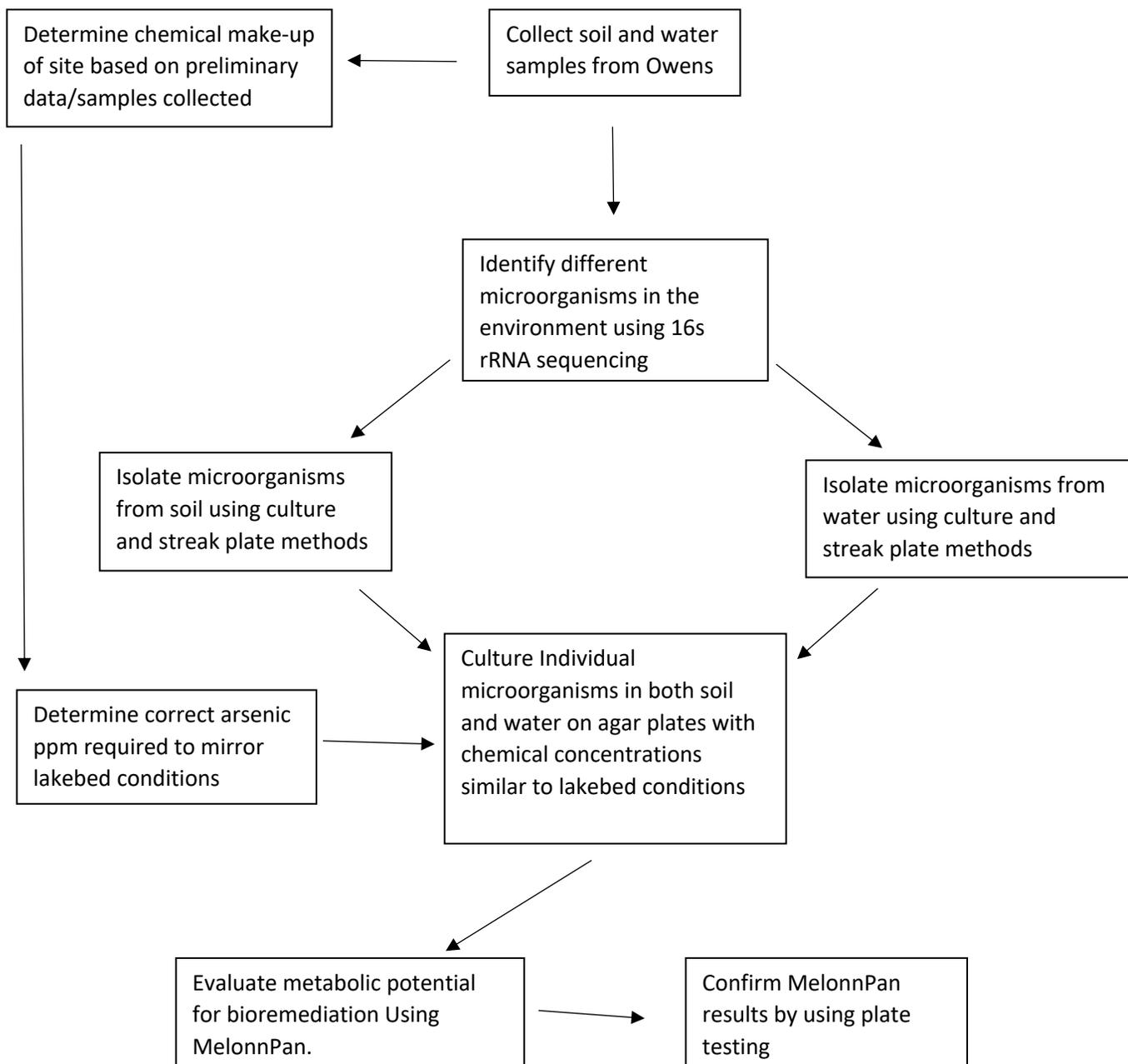
- 1) We will identify and isolate the different microorganisms present in the environment through PCR 16s rRNA sequencing
- 2) Prepare Soil Extract Agar (SEA) so that the growth medium contains nutrients in similar concentrations to Owens lakebed.
- 3) Once different species are isolated and plates are prepared, inoculate plates with individual species and incubate at 35 to 45 degrees C (temperatures mimicking Owens Lake).
- 4) Utilize MelonnPan to predict the metabolic profiles of the bacteria present in the samples.

Hypothesis:

H1: If we use computational software to predict the metabolic profiles of the different isolated microorganisms then we will be able to determine which of the microbes present in the environment have the highest potential for arsenic bioremediation.

H2: If we raise the arsenic levels in the growth medium then the microorganisms that have the capacity to metabolize arsenic into a less toxic form will show more growth.

Schematic



Methods

1. Collect soil and water samples from Owens lake bed.
2. Create Soil Extract Agar (SEA) following the recipe and instructions provided by the University of Wyoming Soils Lab.
3. Extract microbial DNA from the soil using the Omega E.Z.N.A. DNA extraction kit according to manufactures instructions.
4. Clean and concentrate DNA, confirm DNA presence using nanodrop according manufactures instructions.
5. PCR with primes to the V3/V4 region of the 16s rRNA gene to obtain 16s DNA amplicons.
6. Pool according to barcode and sequence using minION an minKNOW software.
7. Utilize a computational method for predicting metabolite composition from the 16S sequencing data (MelonnPan) according to the user manual provided
8. Confirm predicted results from MelonnPan by plating individual bacteria on agar with chemical concentrations mirroring Owens Lakebed.
9. Compare MelonnPan results with the concentrations of Toxic chemicals before and after the plates were cultured in order to determine which bacteria is most effective in transforming toxic chemicals into less toxic forms.
10. Use this determination to identify which bacteria that occurs naturally in the landscape would be the most efficient for bioremediation of the land.

Data Collection

This Data will be collected using various scientific methods. These methods include, 16S rRNA sequencing to determine bacterial genome sequences, as well as soil DNA kit to extract DNA from the soil. MelonnPan, a computational method for predicting metabolite composition, will be used to predict the metabolism of the bacteria isolated from the samples.

Analysis and Expected Results

From this experiment I expect to be able to isolate and identify a variety of microorganisms from the samples taken from Owens Lakebed. After the plates with chemical concentrations similar to the area have been cultured and analyzed, I expect to be able to determine which naturally occurring bacteria is most efficient at transforming toxic chemicals, such as arsenic, into less toxic forms.

From this experiment I expect to be able to isolate and identify a variety of microorganisms from the samples taken from Owens Lakebed After identifying the microorganisms that are present in the lakebed, I expect to be able to determine their metabolic profiles using a novel computational software.

From these results I expect to determine which naturally occurring bacteria is most efficient at transforming toxic chemicals, such as arsenic, into less toxic forms.

Timeline:

<https://padlet.com/rmimwatson/3uy2kcev6dzemqag>

*The specific dates in this timeline are by no means fixed dates. I am not 100% sure how long some of these steps will actually take. Right now, it shows how I am thinking things will progress rather than when things will truly happen.

Annotated Bibliography

Banciu HL, Muntyan MS. Adaptive strategies in the double-extremophilic prokaryotes inhabiting soda lakes. *Curr Opin Microbiol*. 2015 Jun;25:73-9. doi: 10.1016/j.mib.2015.05.003. Epub 2015 May 27. PMID: 26025020.

Haloalkaliphiles are microorganisms common to soda lakes because they are able to survive in both alkaline and high salinity conditions. Through extensive research, it has become apparent that several adaptations have arisen that allow them to survive such an environment. While these adaptations are not well known, researchers have proposed a multi-level adaptive strategy as a potential answer for how these microbes are able to survive. This source was found through the NCBI database.

De Francisco P, Martín-González A, Rodríguez-Martín D, Díaz S. Interactions with Arsenic: Mechanisms of Toxicity and Cellular Resistance in Eukaryotic Microorganisms. *Int J Environ Res Public Health*. 2021 Nov 21;18(22):12226. doi: 10.3390/ijerph182212226. PMID: 34831982; PMCID: PMC8618186.

This article describes how selective pressure induced the development of metabolic systems allowing microorganisms to use Arsenic an energy source. While the true effects of these biochemical processes are not well known, effort is made to explore both positive and negative impacts on microbial life. The authors also call attention to the potential for bioremediation that an understanding of these metabolic processes may offer. This source was found via the NCBI database.

Garibyan, L.; Avashia, N. Polymerase Chain Reaction. *Journal of Investigative Dermatology* **2013**, *133* (3), 1–4.

This article details the purpose behind using PCR for scientific research purposes. It explains how it has been found to be an effective way of amplifying selected DNA sequencing.

Jeong SW, Choi YJ. Extremophilic Microorganisms for the Treatment of Toxic Pollutants in the Environment. *Molecules*. 2020 Oct 23;25(21):4916. doi: 10.3390/molecules25214916. PMID: 33114255; PMCID: PMC7660605.

This article explores the use of extremophilic microbes for the remediation of toxic components such as radioactive waste, toxic heavy metals, and other harmful substances. This point of research is important because it explains in detail how extremophilic microorganisms could be the key to bioremediation in

areas where the environmental conditions prevent normal microbial growth. This source was found via the NCBI database.

Mallick, H. The huttenhower lab. <https://huttenhower.sph.harvard.edu/melonnpan/> (accessed Sep 24, 2022).

This article details MelonnPan, a computational program for predicting the metabolic profile of microorganisms. This program will be utilized as a method in this project. This source was found through the Huttenhower Lab at Harvard.

Preul, M. C.; Patel, A.; Belykh, E.; Miller, E. J.; George, L. L.; Martirosyan, N. L.; Byvaltsev, V. A. Minion Rapid Sequencing: Review of Potential Applications in Neurosurgery. *Surgical Neurology International* **2018**, *9* (1), 157.

This source details the benefits of using the minION software. While this particular article explains its applications in neurosurgery, the emphasis on the effectiveness of rapid sequencing through minION can still be seen.

Reheis MC. A human-induced dust problem.

<https://geochange.er.usgs.gov/sw/impacts/geology/owens/> (accessed Sep 1, 2022).

Owens Lake is a prime example of what artificial desiccation can do to an environment. It has created the largest source of PM-10 dust in the United States. The resulting dust storms from the lake bed pose a substantial health risk to people that reside in the Owens Valley and nearby areas. They also greatly impact air quality in a larger region surrounding the lake. Owens Lake has proved to be an extreme example of the potentially destabilizing effect that artificial desiccation can have on the environment. This source was found through the U.S. Geological Survey.

Shi Z, Cao Z, Qin D, Zhu W, Wang Q, Li M, Wang G. Correlation models between environmental factors and bacterial resistance to antimony and copper. *PLoS One*. 2013 Oct 29;8(10):e78533. doi: 10.1371/journal.pone.0078533. PMID: 24205252; PMCID: PMC3812145.

This article discusses certain types of bacteria that have highly specified metabolic pathways. These metabolic pathways allow these microbes to convert toxic Sb(III) to the less toxic form, Sb(V). Microbes that are capable of could prove to be useful for environmental Sb bioremediation. This source was found via the NCBI database.

Vavourakis CD, Andrei AS, Mehrshad M, Ghai R, Sorokin DY, Muyzer G. A metagenomics roadmap to the uncultured genome diversity in hypersaline soda lake sediments. *Microbiome*. 2018 Sep 19;6(1):168. doi: 10.1186/s40168-018-0548-7. PMID: 30231921; PMCID: PMC6146748.

This article explores the highly diverse microbial life that can be found in soda lakes. Metagenomic sequencing was performed on the sediments of soda lakes in order to provide insight into the lineages of such highly specified microorganisms. This process led to the isolation of dominant lineages with functional groups involved in carbon, sulfur, and nitrogen cycling. This source was found via the NCBI database.

