Comparing the Diversity of Bacterial Communities Before and After Film Fixation in Owens (Dry) Lake, CA

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Project Summary

-Overview-

Art has long been a tool to elicit emotion and can be used as a tool in science. These two aspects of art are brought together by Metabolic Studio's projects including one of its ongoing projects *Owens Dry Lakebed Developed Print* Collection. Owens Lake was once a large body of fresh water in California that dried as a result of the creation and completion of the Los Angeles aqueduct. This unique location not only serves as the subject of their art but also contains compounds necessary to help in the film fixation - the removal of unexposed silver halide crystals from the film - process. Our research will address the question of to what extent this film development process affects the microbial community in the soils. We will address this research question by comparing the bacterial diversity of pre-film-fixation, and post-film-fixation communities. Our approach will utilize the 16s rRNA gene information to compare pre-film-fixation and post-film-fixation bacterial communities (H1) and will diversity between the treatments of how each photo was fixated (H2).

This study provides an avenue to explore the synergy of art and science which is currently lacking. *Intellectual merit:* No past study has focused on not only the influence the landscape has on art but also on how art influences the landscape and its ecology. *Broader merit:* One significant impact of this study is the collaboration with undergraduate researchers, expert stakeholders, and community partners in both Wyoming and California. Furthermore, this study provides an opportunity to refocus our attention back on Inyo county and the marginalized global communities affected by human-created environmental injustices and desertification brought on by climate change.

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Owens Lake Panorama 1. Silver gelatin processed in Owens Dry Lakebed. [2016.4273]. *https:/www.metabolicstudio.org/optics-division*

Project Description

-Statement of Significance-

Art is multifaceted. The artist is commissioned by their desire to bring joy, bring disdain, or bring focus to an aspect of culture and life. Once it leaves the creator's hands, it takes on a unique meaning for each viewer. This allows works of art to become more than just pieces to admire, this also allows works of art to become tools of change. Moreover, the ever-increasing media an artist can use to create these pieces provides viewers not only with new ideas of what art can be but also allows viewers to look into how and why the art is made. Metabolic Studio exemplifies this statement with its novel concepts and obligation to facilitate equity of environmental injustices that are the subject of their art. Tristan Duke, Laura Bon, and Rich of Nielsen of Metabolic Studio aim to shed light on environmental injustices by infusing ongoing issues into their art. Although they have many present projects, one project of which has taken their photography to a valley east of the Sierra Nevadas. Owens (Dry) Lake was once the largest lake in California (Creason, 2016). The water of Owens Lake and Owens River has been diverted more than 200 miles to Los Angeles since its completion in 1913. This previously thriving lake is now the nation's largest contributor to dangerous dust storms in the United States since the dust bowl era of the 1930s (Colgan, 2020). Now, minimal water occupies the lake bed, and the accumulation of minerals and harmful toxins increases annually from the leaching of soils of the Sierra Nevada range. This grim setting has recently become the site of awe-inspiring prints from Metabolic Studio to draw our attention to this injustice felt by Inyo County residents and the country as a whole.

This study site is a hub of transdisciplinary fields. Although the human-created desiccation has harmed the land and the people of the land, it has also established its own significance through art. The rare chemical compounds found in Owens lakebed are critical for fixing - a thiosulfate salt solution, commonly sodium thiosulfate, used as a treatment to prevent the film from darkening or fogging - film and provide a rare opportunity for us to study the microbial ecology of this process which is unique to this land. The microbial community data can serve as a model for future desiccation of the land regardless if it is from the extraction of fresh waters or global climate change by demonstrating how this microcosm functions. Additionally, the basicity, temperature, and salinity of this site are of eco-economic importance. The dust storms of Owens (Dry) Lake are a health hazard to the residents near Owens Valley which may increase medical costs for those residents, and the dust storms also deposit salt throughout California which has a greater than 10 billion dollar export of agricultural goods each year (USTR, 2022).

-Conceptual Model-

Environmental Injustice: harmed land and people

Metabolic Studio not only uses Owens (Dry) Lake as the focus of the art, but uses the land to make the art.

How does the microbial community impact the art, and how does the art impact the microbial community?

Synergy of art, science, and other disciplines to appreciate the research

Model microbial communities of extreme environments from global change or water diversion.

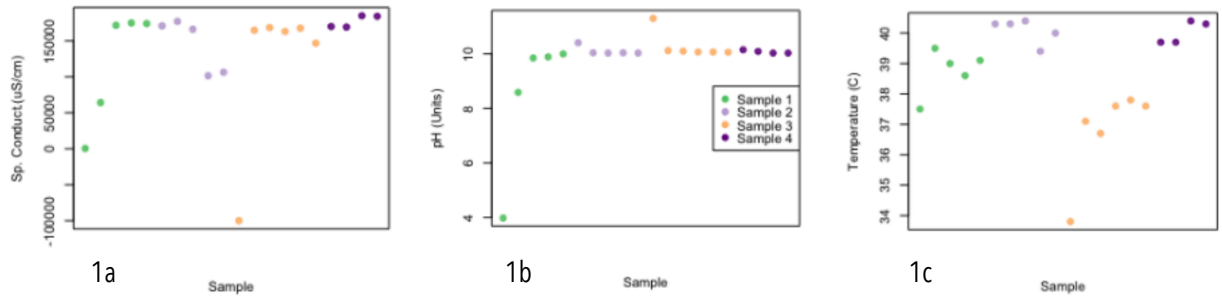
-Relevant Literature-

In recent decades, the great divide between arts and sciences has been closing rapidly because of our current understanding that the synergy they create is authentic and relevant to education that is needed for the advancement of research (Braund & Reiss, 2019). With this new awareness, transdisciplinary studies should be at the forefront of research such as this study. The study of the microbial communities of Owens (Dry) Lake congregates knowledge from microbiologists, geologists, artists, chemists, economists, ecologists, and beyond. Currently, no literature has addressed how microorganisms can contribute to the development of photographs, nor does it address how the fixation of film impacts the microbial community. Furthermore, climate change has been a global issue for several decades and one of the main impacts of climate change is endangered water sources (NASA, 2022). Water evaporation is one of our most critical issues (Hassani, et al. 2021). Currently, more than 45% of the lower states here in the US are experiencing drought (NOAA, 2022), and freshwater sources are drying around the globe in countries such as Iran (Abbaspour et. al, 2012). Desiccation has led to the accumulation of chemical compounds and the salinization of these soils. Henceforth, the dust that is deposited in nearby areas of California has the potential to affect vegetation (USGS, 2016), and this new salinity is detrimental to crop productivity decreasing yields by 20-50% (Sheivastava and Kumar, 2015). Although Owens Lake is a human-created dry lakebed in that same capacity, it can be a great opportunity to study extremophiles and how they assist in the development of the film, the opportunity to inform us of the functionality of this extreme environment, and provide the opportunity to renew our knowledge of how desertification strains agriculture.

-Preliminary Data-

I have gathered preliminary data on the water abiotic factors from Owens (Dry) Lake using a YSI meter. Figure 1a demonstrates the extreme salinity of the soil of 15 dS/m; the USDA considers soils with a reading of 4 dS/m to be saline. Figure 1b demonstrates the basicity of the soil with an

Figure 1a. Specific conductivity of each sample from each treatment measuring salinity. 1b. pH of each sample from each treatment measuring basicity. 1c. Temperature of water of each sample from each treatment.



1a 1b 1c

average pH of 10. Figure 1c demonstrates the water temperature with noticeable differences between each sample. These abiotic factors combined create an environment beyond the optimal range of water and soils. Note there are sample readings in these figures that will be retested since there were accidental readings and not significant outliers.

-Methods and Materials-

Sample collection:

Four treatments were designated (Figure 2). No-rinse indicates the film was removed from the stop-bath which contained 2% acetic acid and laid in the lake-bed. Rinse indicates the film was rinsed in water after the stop-bath and before being laid in the lakebed. Treatment 4 had no pre-fixation processing. All four treatment photos can be viewed in supplemental Pre-film-fixation soil samples were collected randomly at each of the four treatments for the future burial site of the photographs on July 27th, 2022 at 17:30. Post-film-fixation soil samples were taken from Owens (Dry) Lake on July 27th, 2022 at 21:45 PST in the same manner as above. To prevent contamination of the samples were as follows: the shovel was first cleaned with vinegar to remove chemical residues followed by a cleaning with 95% ethanol and air-dried for sterilization. The wind at the site prevented the use of flame sterilization of the shovel. Each sample is between 4-5 shovel-fulls and was placed into large sterile whirlpacks.

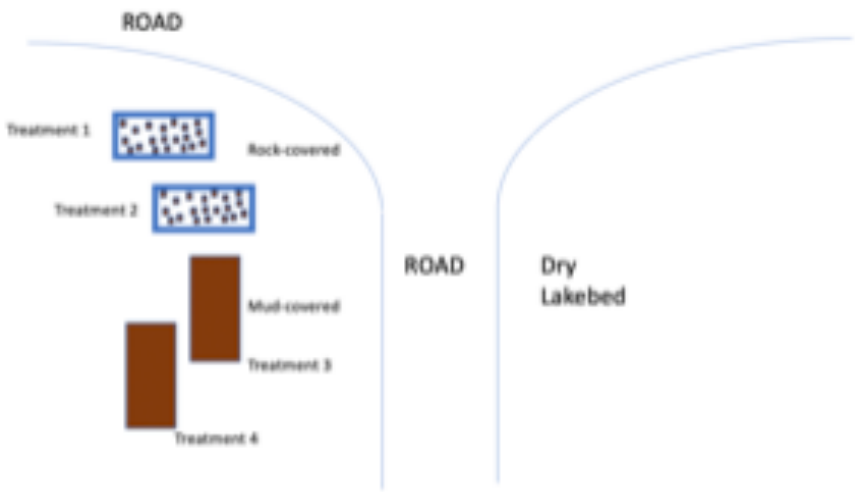


Figure 2 illustrates the site of sampling in Owens (Dry) Lake, California. Treatment 1 was not rinsed and covered with rocks. Treatment 2 was rinsed and covered with rocks. Treatment 3 was not rinsed and covered completely with mud. Treatment 4 was rinsed and covered completely with mud (printout).

-Future Methods and Materials-

Sub-samples will be obtained from the original whirlpacks after the soil has been homogenized. I will determine texture using the USDA's standardized ribbon method

protocol. I will also re-test the pH and specific conductivity (EC) to ensure accuracy using the *Thermo Scientific Orion Star A* meter in the Norton Soil Fertility Lab at the University of Wyoming. I will use the OMEGA Bio-Tea E.Z.N.A SOil DNA Kit per the manufacturer's protocol to extract the bacterial DNA. I will use the ZYMO Research kit to clean and concentrate the DNA extractions. I will use primers of the 16s rRNA gene to amplify during PCR. I will sequence the amplicon-prepped DNA using the *16s Barcoding Kit 1-24* of the MinION machine according to the manufacturer's protocol. Raw data will be acquired using the MinKnow software compatible with the MinION. The data will be stored on my personal device as well as shared with my team. To analyze the data, I will read the files into R. Packages such as *GUPPY* and *EPI2ME* will determine the phylogeny of each bacterium or archaeon. To compare the beta diversity, I will use non-metric multidimensional analyses (NMDS). I will also analyze the relative abundance of each species in each sample, and test these data against soil abiotic factors to identify patterns.

-Justification of Approach-

Figure 3 illustrates the soil ribbon test which is an adopted, standardized approach that has been used for more than 40 years by USDA to determine soil texture and is a free method (USDA, 2022). I will be able to determine the percent of clay, silt, and sand for each sample. Measuring the salinity and pH of each sample can illustrate the heterogeneity of each sample. These data are used in determining soil fertility, and this information can be highly useful in understanding the complete microcosm of each sample. Next, the 16s rRNA gene is a highly conserved region of both bacteria and archaea. The changes in this gene are usually random and provide information about evolution (Janda and Abbot, 2007). According to the National Institute of Health, the

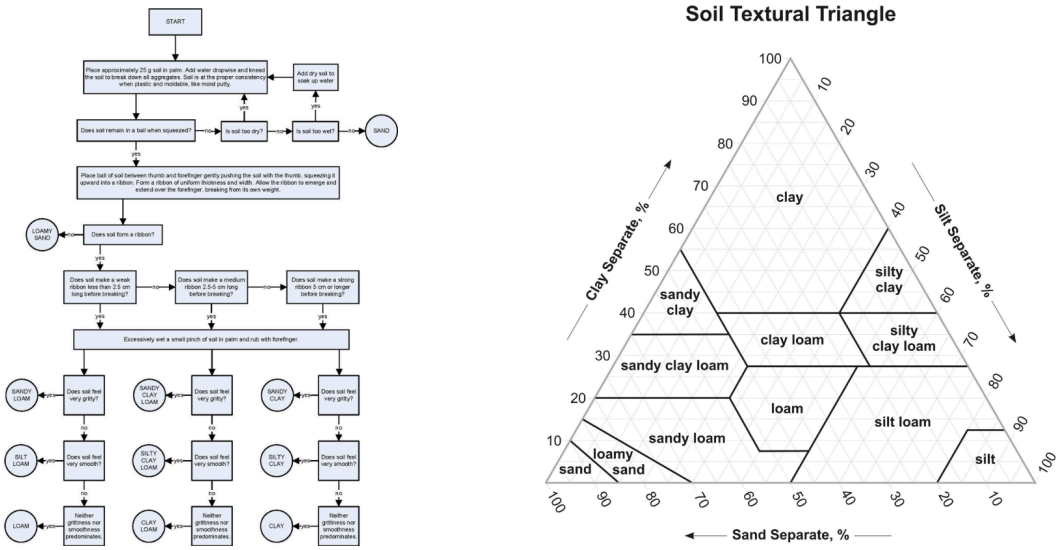


Figure 3 illustrates how to perform the soil ribbon test and how to determine the texture by identifying the sand, silt, and clay percentages of the soil sample.

function of this gene has not changed over time, it will provide phylogenic information, perhaps to the species level. This approach is used globally by microbiologists. Beta-diversity is used to determine how similar or how dissimilar the diversity of species is in each sample and summarize it into an NMDS graph (Syms, 2008). In an NMDS graph, the closer two samples are to each other the more similar they are in regard to the species. This information may elucidate nuances in the niches of each sample. This analysis is widely used in ecological studies.

Research Plan

-Objectives, Specific Aims, and Hypotheses-

To elucidate the unique microbial communities of Owens (Dry) Lake are both impacted by the film fixation and impact the film fixation. I will use genetic identification and analyses to compare these communities. Additionally, I will use standard soil abiotic factors such as texture, pH, and salinity to identify differences in each sample. Data from each sample will be analyzed and compared. Therefore, it is expected that the microbial relative abundance will differ in pre-film-fixation and post-film-fixation (H1), and the microbial relative abundance will differ between treatments (H2). It is expected to find species that may be present in each sample.

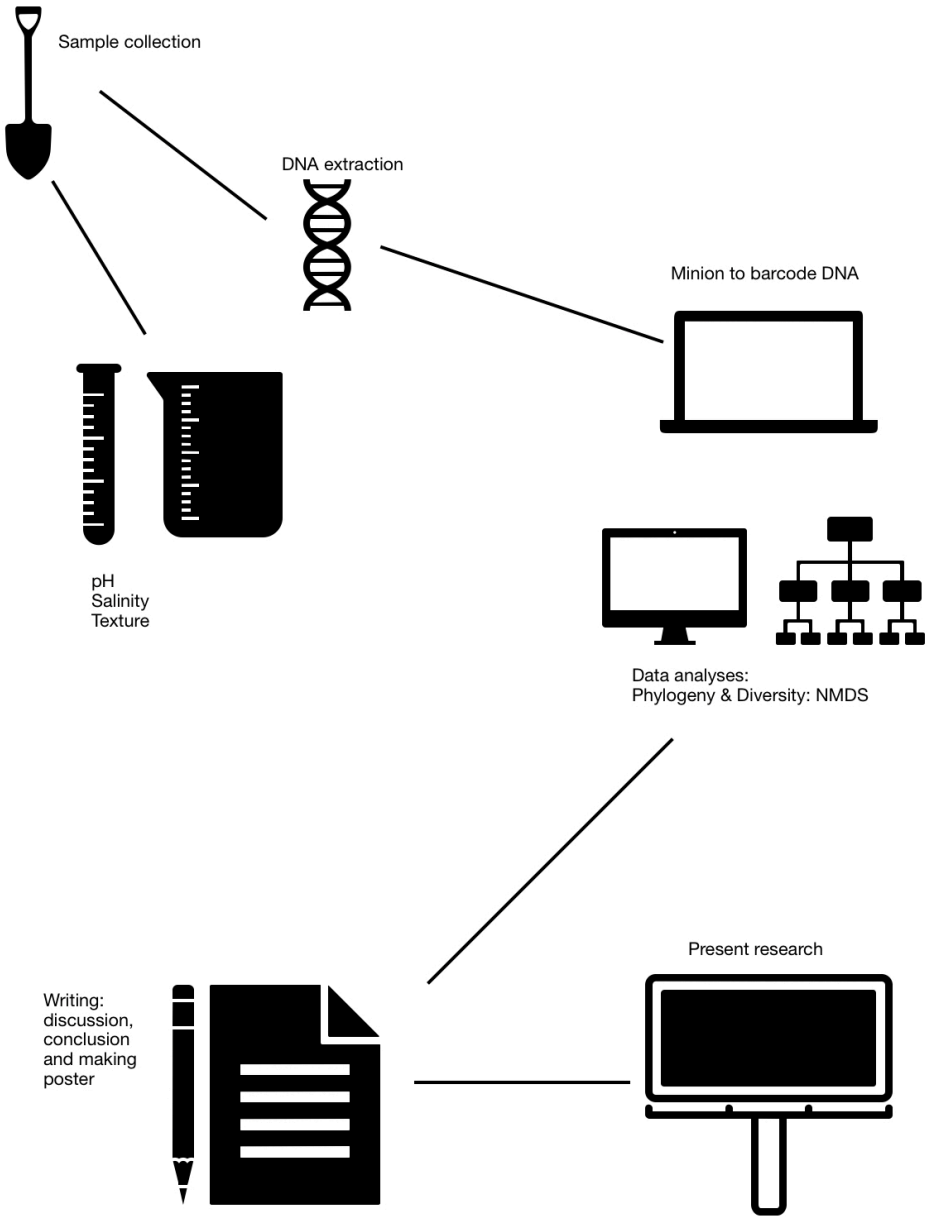
-Analysis and Expected Results-

This unique environment will have unique bacteria Table 1 illustrates the microbes we expect to find in the samples because of the basicity and salinity. It is not expected to find microbes that cannot survive the temperature that is typical for Owens Valley. All of my data will be stored in two locations. First, I will have a copy of the data on the cloud which is connected to both of my laptops, iPhone, and iPad. The redundancy is necessary to ensure data is not lost. Additionally, I will upload the data to the University drive, which is another cloud service. A physical notebook will also have data regarding experiments, results, and conclusions. The physical notebook will be kept on campus, in a locked room, and in my personal drawer. I will also take daily pictures of the physical notebook to have a "back-up" copy.

Microbes we expect to find	Organisms we do not expect to find
Halophilic microbes	Cyanobacteria
Halotolerant microbes	Temperature sensitive microbes
Alkaliphilic microbes	Acidophilic microbes

Table 1 illustrates the microorganism I expect to find during my analyses as well as microorganisms I do not expect to find because of their specific non-optimal growth conditions.

-Design Schematic-



-Timeline-

Month	Date(s)	Task
September	27	Application phase begins
	29	Perform soil ribbon test with visiting class
October	3-6	DNA extractions and clean up
	10-13	Sequence samples
	13	Midterm: eval and digital media
	17	Determine pH and salinity
	20- 31	Phylogeny, diversity, and NMDS analyses
November	1-15	Continued analyses
	16-22	Results and conclusions
	23-28	Poster work
	29	Poster due
December	1-5	Group poster work
	6	Group poster due
	15	Present in exhibit hosted by Art Museum at UWyo 4-7

References

- Abbaspour, M., Javid, A. H., Mirbagheri, S. A., Ahmadi Givi, F., & Moghimi, P. (2012, March 13). *Investigation of lake drying attributed to Climate Change - International Journal of Environmental Science and Technology*. SpringerLink. Retrieved September 24, 2022, from *https://link.springer.com/article/10.1007/s13762-012-0031-0*

- Abbaspour et al. was accessed Springer Link which requires signing into the University of Wyoming to get behind the paywall. Only the first paragraph in the text was used to identify what is considered extreme conditions.

This article is also behind a pay-wall, but I was able to access it by signing into the University of Wyoming. This article reviews how the desertification of lakebeds has occurred through global climate change. I like that it illustrates this problem is larger than in the US.

- Braund, M., & Reiss, M. J. (2019, June 20). *The 'great divide': How the arts contribute to science and Science Education - Canadian Journal of Science, Mathematics and Technology Education*. SpringerLink. Retrieved September 24, 2022, from https://link.springer.com/article/10.1007/s42330-019-00057-7

This article is concerned with evaluating how art and science education is the only way to have a complete education. It aims to shine a light on how art is more than what we usually think of and how that allows us to use art to improve science. It was accessed through Springer Link.

- *California*. United States Trade Representative. (n.d.). Retrieved September 24, 2022, from https://ustr.gov/map/state-benefits/ca

Although the information on this page is provided by the US government, it is easy to read and understand. It provides information of exports of California both agricultural and non-agricultural.

- Creason, G., & -. (2016, April 6). *CityDig: Here's what Owens Lake looked like before Los Angeles drank it dry Los Angeles Magazine*. Los Angeles Magazine. Retrieved September 24, 2022, from https://www.lamag.com/citythinkblog/citydig-heres-what-owens-lake-looked-like-before-los-angeles-drank-it-dry/

Although this is not a scientific article nor is it peer-reviewed, this article does provide the reader with a lay-person overview of the Owens (Dry) Lake environmental injustice.

This article was found when trying to find pictures of Owens Lake pre-Los Angeles aqueduct.

- Colgan, David. (2020, March 3). Effort to limit dust pollution in Owens Valley is advancing, but still room to improve. *UCLA Newsroom*. Retrieved from https://newsroom.ucla.edu/releases/owens-valley-dust-mitigation.

- Garcia, R. (2014). *Irrigation Guide - USDA*. Retrieved September 24, 2022, from https://www.nrcs.usda.gov/Internet/FSE_DOCUMENTS/nrcs144p2_033068.pdf

Thisis a 800 page guide to how irrigation and improve or cause salinity in soils. It provides management practices for many scenarios. I was able to download it free through the USDA.

- Gómez, F. (2011). Extreme Environment. In: *et al.* Encyclopedia of Astrobiology. Springer, Berlin, Heidelberg. https://doi.org/10.1007/978-3-642-11274-4_566

This chapter was accessed through the University of Wyoming. It provides information on extreme environments. Although I was not able to finish the entire chapter, I believe it contains fascinating information.

- Hassani, A., Azapagic, A., & Shokri, N. (2021, November 18). *Global predictions of primary soil salinization under changing climate in the 21st Century*. Nature News. Retrieved September 24, 2022, from https://www.nature.com/articles/s41467-021-26907-3

This page was accessed through Nature Communications which is a free journal. The information in this article provides information about how salinity may increase or decrease depending on the region by using data-driven models.

- *Optics division*. About. (n.d.). Retrieved September 24, 2022, from https://www.metabolicstudio.org/optics-division

This page is where most information can be found regarding the ongoing projects of Metabolic Studio. It is where I found the panorama photo of Owens (Dry) Lake. Here you can contact the creators, and deep dive into how as well as why their art is created.

- NASA. (2022, September 20). *Climate change evidence: How do we know?* NASA. Retrieved September 24, 2022, from https://climate.nasa.gov/evidence/

This website is a great place to find any and all information that is evidence-based about global climate change. It provides a history of events and has great optics.

- *National current conditions*. Drought.gov. (2022, September 20). Retrieved September 24, 2022, from https://www.drought.gov/current-conditions

This website gives up-to-date accurate conditions nationwide. It provides both state and crop drought data. There are also interactive maps on this website.

- *Natural Resources Conservation Service: Soils*. Guide to Texture by Feel | NRCS Soils. (n.d.). Retrieved September 24, 2022, from https://www.nrcs.usda.gov/wps/portal/nrcs/detail/soils/edu/?cid=nrcs142p2_054311

This online guide provides a complete guide to perform the ribbon test with soil, the triangle to calculate percentages of clay, silt, and sand, and provide access to soil education sponsored by the NRCS and USDA.

- Reheis, M. *A human-induced dust problem*. Owens (Dry) Lake, California. (2016, December 16). Retrieved September 24, 2022, from https://geochange.er.usgs.gov/sw/impacts/geology/owens/#:~:text=Water%20was%20first%20diverted%20from,side%20of%20the%20lake%2C%20figs.

This online article has been the most beneficial to understanding the land, the problems the land is facing, and the history of Owens (Dry) lake. It provides timelines of events, photos of the region, and graphs pertaining to abiotic factors. It was accessed through the USGS.

- Shrivastava, P., & Kumar, R. (2015, March). *Soil Salinity: A serious environmental issue and plant growth promoting bacteria as one of the tools for its alleviation*. Saudi journal of biological sciences. Retrieved September 24, 2022, from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4336437/

This article was accessed through the National Institute of Health and was published in the Saudi Journal of Biological Sciences. This article discusses how we may be able to utilize halophilic bacteria to help grow plants in saline conditions.