Melding Science and Art: Examining the Impact of Microbial Metabolism on Land-Based Art in Owens Lakebed, California

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Project Summary

As a result of accelerated desiccation, Owens Lakebed in California contains a multitude of salts, toxic elements, and unique extremophile bacteria (Maxmen 2018). While it is frequently seen as a toxic wasteland, Owens Lakebed plays a major role in the land-based art created by Metabolic Studio due to interactions between its unique chemical composition and microbial diversity (Duke, Bon, and Nielsen n.d.). We aim to determine the effect that microorganisms have on film fixation and how their metabolic processes and products are being influenced by Owens Lakebed. Artists at Metabolic Studio observe variable fixation effects on film developed overnight at the Owens Lakebed. However, the complex interactions taking place between the microbes, chemicals, and film are unknown. We will characterize the bacterial biodiversity and chemical profile in the Owens Lakebed film development site, evaluate the interactions/relationships between the chemistry, microbes, and film, and determine synergistic effects on photo development.

Bacterial diversity will be characterized using 16s rDNA gene sequencing with samples gathered at pre-, during, and post-film development. Through chemical testing, thiosulfate levels, arsenic concentration, and salinity will be measured from pre-, during, and post-film development samples. This data will be compared and analyzed to determine the relationship between the chemical profile of Owens Lakebed and the microbial diversity on film fixation and the overarching film development process.

Intellectual Merit:

This research promotes transdisciplinary learning by utilizing art to ask and answer scientific questions. The implications of our work will challenge conventions and address matters that go beyond each discipline alone.

Broader Impacts:

This research will highlight the extent of environmental injustices while simultaneously seeking to give agency to Owen's Lakebed beyond its destructive past. It will contribute to our understanding as a society of how humans can affect land, and how the land can reciprocate its own effects on us in the form of art and science.

Table of Contents

Project Description

Statement of Problem Significance

Due to the Los Angeles aqueduct in 1913, Owens Lake in California did not go through the natural development of forming a playa, and instead, it was subject to an accelerated desiccation process (Maxmen 2018). As a result, Owens Valley became what is often considered a wasteland containing toxic particulate matter with metals such as arsenic and cadmium (Maxmen 2018). Owens lakebed became the source of extensive dust storms as a consequence of changed ground chemistry, leading to a human-created health disaster where carcinogenic dust was frequently spread. For countless people in the surrounding areas, dust accumulated in the tissues and caused many health complications (Maxmen 2018). At the same time, this new extremely alkaline and saline environment created a place for unique microbial species and extremophile bacteria to colonize and thrive (Ryu et al. 2006). Since there are toxic metals in this environment, there exists a complex metabolic shift in these microorganisms where they have evolved mechanisms to detoxify, and in some cases utilize the pollutants, to enable their own survival (Kabiraj et al. 2022).

While Owens Lake is frequently seen as a toxic wasteland, artists of Metabolic Studio have developed land-based art approaches to create an opportunity to reshape the perception of the environment while also bringing attention to the extent of its destruction. Metabolic Studio has suggested that the combination of the salts, metals, and extremophile bacteria within the lakebed forms a photochemical environment where the photo development process can take place (Duke, Bon, and Nielsen n.d.). After acting as a dark room, the lakebed leaves its unique print on the developed image with a variable degree of metallic deposits and coloration (Duke, Bon, and Nielsen n.d.). Owens lakebed is able to make a lasting impression on both art and science. Determining the role of its unique environmental conditions in microbial physiology and metabolism may lead to artistic innovation while simultaneously offering an opportunity to give agency to the land that is a source of environmental injustice. We aim to characterize the bacterial biodiversity before, during, and after the lakebed film development process while also defining the lakebed's chemical profile during these periods. Analyzing the relationship between the lakebed's chemical profile and the microorganisms that inhabit it will allow us to determine how these interactions influence the film development process.

Introduction and Background

Relevant Literature

Owens Lake, like other soda lakes, was formed due to the continuous evaporation of up flowing groundwater. This ground water that is lacking magnesium and calcium, but rich in carbon dioxide, leads to the leaching of sodium from sodium-rich rocks (Sorokin et al. 2014). As a result, Owens lake accumulated abundant deposits of sulfate- and carbonate-rich salts (Ryu et al. 2006). The brine pool is dominated by sodium salts such as carbonate, sulfate, and chloride (Ryu et al. 2006). The high concentration of sodium carbonate, in combination with increased evaporation rates, creates a sodium carbonate/bicarbonate-buffered system with extremely high pH and salinity (Sorokin et al. 2014). The groundwater also contains high concentrations of toxic trace elements such as arsenic. Due to its strong reducing conditions, As (III) is the most predominant form of arsenic in shallow groundwater (up to 96 mg/L), and it creates many human health concerns in Owens Valley as it is less strongly absorbed, more soluble, and more toxic than As (V) (Ryu et al. 2006).

While arsenic is highly toxic to many forms of life, certain microorganisms have evolved to be able to tolerate high concentrations of the metalloid, and others can utilize it as a source of energy through redox transformations (Lloyd et al. 2006). In anaerobic conditions, specialist bacteria can use arsenate as an electron acceptor in place of oxygen (Lloyd et al. 2006). Bacterial arsenic tolerance is possible through the *ars* operon, which uses reduction to convert arsenate to arsenite (Kabiraj et al. 2022). Bacteria can also oxidize arsenite to arsenate through a key enzyme called arsenite oxidase, which is encoded by *aioA* and *aioB* genes (Kabiraj et al. 2022). The microorganisms are able to uptake arsenic to perform these processes because phosphate is analogous to arsenate (Kabiraj et al. 2022). As a result, two pentavalent phosphate transporters can be utilized for arsenate uptake (Kabiraj et al. 2022).

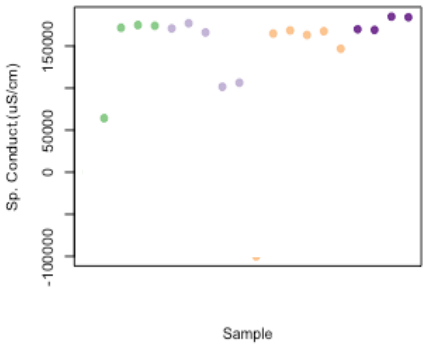
The microorganisms inhabiting Owens Lakebed interact with these unique environmental conditions in order to perform important metabolic and physiological processes. The major process regulating redox conditions for microorganisms in Owens Lake is sulfate reduction (Ryu et al. 2006). The biological sulfur cycle is integral to microbial metabolism because is connected to many other main element cycles such as nitrogen, carbon, and metal cycles (Sorokin et al. 2011). Sulfate reduction is also extremely sensitive to the presence of abundant minerals and salt saturation, both of which are characteristic of Owens Lakebed (Kulp et al. 2007). This imposes

several chemical shifts in the cycle. Consequently, sulfide and insoluble sulfur can interact, forming soluble polysulfides that are stable in extremely alkaline conditions (Sorokin et al. 2011). These polysulfides can then be rapidly oxidized to thiosulfate, which acts as a favorable electron donor (Sorokin et al. 2011). Obligatory anaerobic and obligatory haloalkaliphilic bacteria perform these reactions, and they can also obtain energy by thiosulfate or sulfite disproportionation (Sorokin et al. 2014).

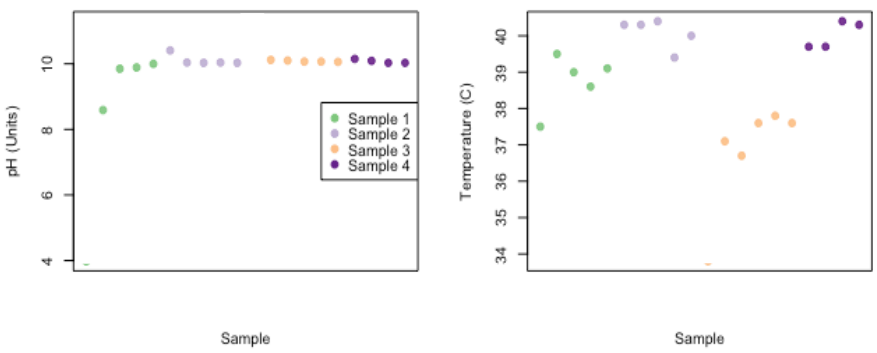
In photographic processing, an image is fixed (permanent) after soaking in a solution containing thiosulfate (Pope 1959. Sodium thiosulfate, a salt that is the most important ingredient in fixation, inactivates light-sensitive silver halide crystals that were unexposed during the prior developing step (University of Houston). During fixation, a silver thiosulfate complex is formed which eventually decomposes to produce silver sulfide, and the rate of this reaction depends on the composition of the fixing bath, temperature, and acidity (Pope 1959). The degree of this reaction during fixation effects the permanence of the image and excessive reactions and formation of silver sulfide can cause the image tone to become brownish (Pope 1959).

Preliminary Data

Key
Sample 1: Rocks, No Rinse
Sample 2: Rocks, Rinsed
Sample 3: Buried, No Rinse
Sample 4: Buried, Rinsed

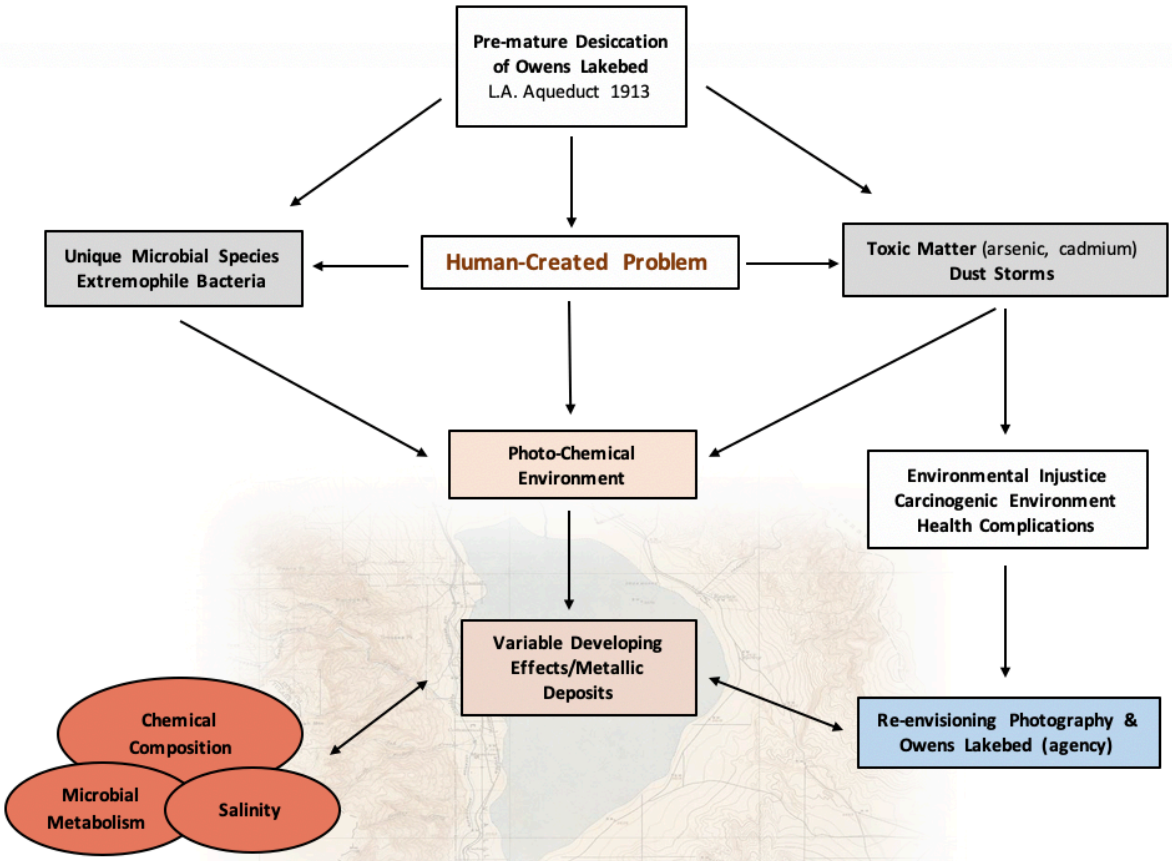


At the site of sample collection, a YSI meter was used and measured several parameters such as specific conductance, pH, and temperature. Four to five measurements were taken



per sample (1-4). pH was generally similar for all samples whereas temperature and specific conductance varied slightly. Salinity measured by specific conductance, pH, and temperature are all factors that affect both microbial metabolism and the film development process. By comparing these environmental conditions with chemical profile and microbial diversity data that is collected during our research, we may gain an understanding of the resulting variable effects on the processed photos and any correlations that may exist.

Conceptual Model



Justification of Approach

Our research utilizes techniques in molecular biology and chemistry that are commonly used and supported by literature. 16s rDNA gene sequencing is the gold standard for the identification of microorganisms within a sample as extensive gene sequence databases exist for analysis (Church et al. 2020). In terms of chemical analysis, our collaboration with the

University of Wyoming EcoBGC lab to determine thiosulfate concentrations is supported as they possess specialized equipment and are experts in their field. Regarding salinity measurements, the most accurate way to measure salinity is through conductivity as it is provides a more accurate measurement than a refractometer and other devices. An arsenic detection kit will be used to measure arsenic concentrations in samples and it is highly sensitive with an ability to measure arsenic in various ppb concentrations.

Research Plan

Objectives

- To characterize and determine the effect of bacterial biodiversity in Owens Lakebed on photo development.
- To characterize and determine the effect of the chemical profile in Owens Lakebed on photo development (thiosulfate, salinity, arsenic).
- To determine relationships between the chemical profile of Owens Lakebed and the diversity of the microorganisms that inhabit it, and how these relationships influence photo development.

Hypotheses

- H1: Bacterial biodiversity will differ between pre-, during, and post-photo fixation samples.
- H2: The chemical profile in Owens Lakebed will differ between pre-, during, and post-photo fixation samples.
- H3: The chemical profile concentrations and degree of bacterial biodiversity of Owens Lakebed are correlated and can be linked to photo development effects.

Specific Aims

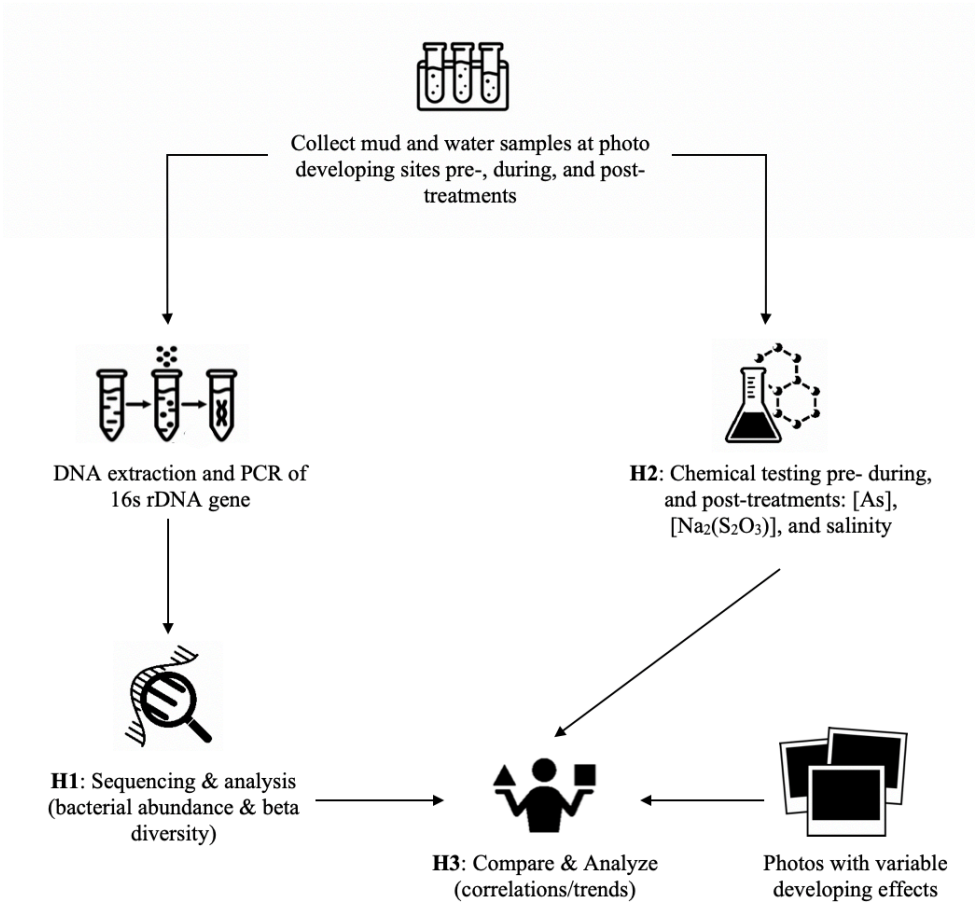
- Aim 1: We will characterize the bacterial biodiversity using 16S rDNA gene sequencing with samples gathered at pre-, during, and post-photo fixation.
- Aim 2: We will characterize the chemical profile in Owens Lakebed using:
    I. Electrical conductivity meter – to measure salinity

II. University of Wyoming EcoBGC Laboratory collaboration – to measure sodium thiosulfate concentrations

III. Arsenic detection kit – to measure arsenic concentrations

- Aim 3: We will compare the data from Specific Aims 1 & 2 through graphical and statistical analysis to determine the relationship between the chemical profile of Owens Lakebed and the microbial diversity pre- and post-photo treatment.

Research Design Schematic



Methods and Materials

Sampling Collection – Owens Lakebed, California:

Pre-treatment samples for both water and soil were collected on July 27th, 2022 at 5:30 p.m. Three water samples in 50 mL falcon tubes and three water samples in small glass amber bottles were collected at random, variable sites that were in the general vicinity of the future

print burial area. While wearing nitrile gloves, falcon tubes and glass amber bottles were dipped into the water until full. For soil collection, the trowels were rinsed with vinegar to remove chemical residue followed by sterilization through an ETOH rinse and natural evaporation. 4-5 shovels full of mud were collected and shoveled into sterile whirl packs at random, variable sites that were in the general vicinity of the future burial area.

Night samples for both water and soil were collected on July 27th, 2022 at 9:45 p.m. right before photo-treatment. Three water samples in 50 mL falcon tubes and three samples in whirl packs were collected at the sites that the prints would be buried using the same techniques as pre-treatment sample collection.

The prints were then treated overnight: treatments 1 and 2 were rock covered face down in Owens Lakebed, and treatments 3 and 4 were mud covered face down in Owens Lakebed. Prior to laying the prints out on Owens Lakebed, treatments 2 and 4 were rinsed in water after the stop bath to remove residual acetic acid before being placed on the lakebed for fixation, and treatments 1 and 3 were taken directly out of the stop bath (without being rinsed) and laid in the lakebed.

Post-treatment samples for both water and soil were collected on July 28th at 10:30 a.m. Three water samples in 50 mL falcon tubes were collected for each treatment (1-4) immediately after the prints were pulled from the sites using the same techniques as pre-treatment and night sample collection. Mud samples were collected for treatments 3 and 4 on top of the prints and under the prints with the same techniques for mud sampling used in pre-treatment and night sample collection.

Bacterial Biodiversity

To determine the bacterial biodiversity of mud samples, we will first collect the samples in the -80 freezer and thaw them. We will then use sterile technique to measure the correct amount of mud needed for the OMEGA E.N.Z.A Soil DNA kit and extract the DNA, per manufacturer's protocols. Following this, the DNA will be cleaned and concentrated with ZYNO kit. The Nanodrop will be used to determine if DNA is present and at what concentration. PCR will be performed with primers to the V3-V4 region of the 16s rRNA gene from 16s Barcoded Kit for MinION. The 16s DNA amplicon pool samples will be differentiated by bar code from MinION kit. We will then sequence using MinION with minKnow software. To analyze this data we will use RStudio to determine bacterial/archaeal abundance, and Guppy to determine beta

diversity for each site. To determine the bacterial biodiversity of water samples, we will use the same techniques as described for the mud samples, but the OMEGA E.N.Z.A Water DNA kit will be used to extract the DNA, per manufacturers protocols.

Chemical Profile

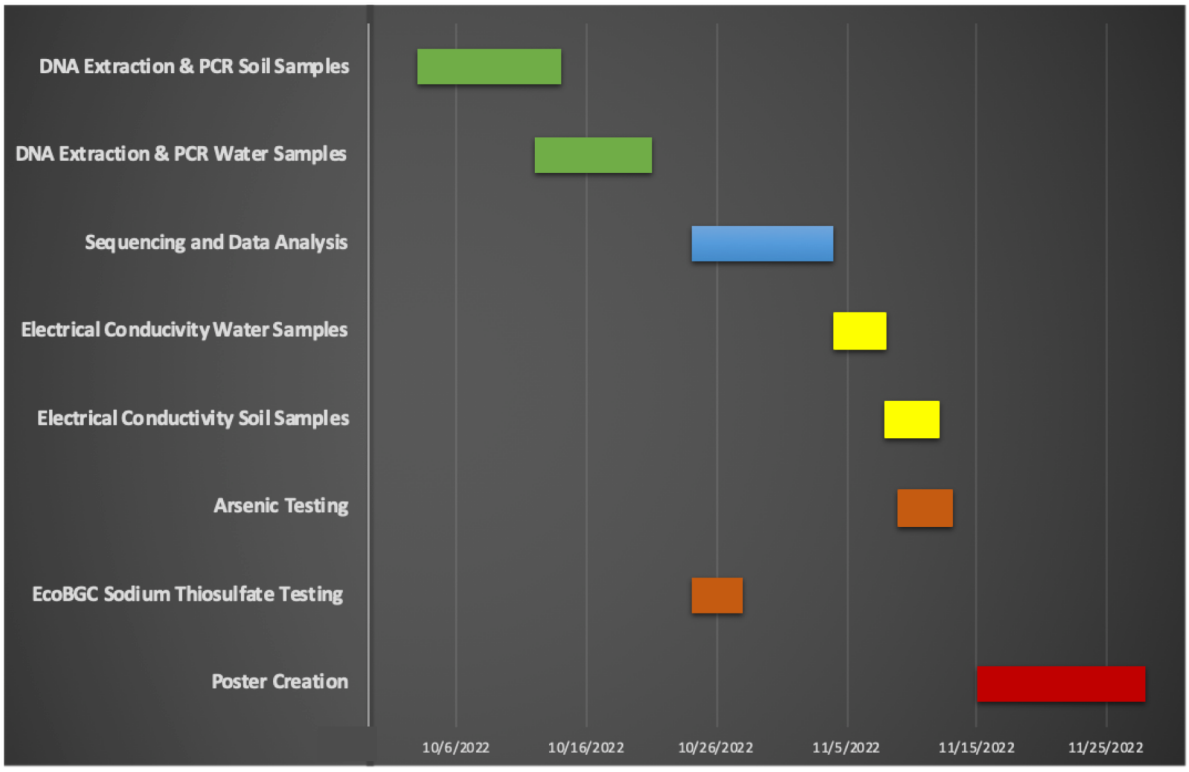
We will partner with University of Wyoming EcoBGC lab to determine sodium thiosulfate concentration of pre-treatment, night, and post-treatment samples for both water and soil. We will use an electrical conductivity meter to measure the salinity of water samples and the 1:5 weight-to-volume ($EC_{1.5}$w/v) method as described by the Department of Primary Industries and Regional Development to measure the salinity of soil samples. $EC_{1.5}$ will then be converted to EC_e as described by the Department of Primary Industries and Regional Development for better comparison with water samples. Arsenic concentrations will be measured using an arsenic detection kit.

Analysis and Expected Results

Data will be recorded in laboratory notebooks and stored in google sheets for ease of access among team members. Bar graphs will be created for chemical profile data and to compare the samples to one another. Chemical profile data for each sample will be graphed in order to determine any correlations/relationships. We will analyze and sequence bacterial biodiversity data using R package Guppy to compare alpha and beta diversity between each sample. It is expected that bacterial biodiversity will be similar between treatments 1& 2 and treatments 3 & 4 due to the differences in conditions for microbial metabolism (buried in mud or covered in rocks with space between). It is also expected that bacterial biodiversity will depend on arsenic concentrations and salinity of the site/sample, and due to the circadian rhythm of the microbes and their interactions with the chemicals in the film this diversity will differ in pre-, during, and post-photo fixation samples. We anticipate that the samples with increased salinity will also have increased sodium thiosulfate levels. Photos from samples with low sodium thiosulfate levels may contain black metallic spots or darkened areas due to light-sensitive silver halide crystals not being completely dissolved in the fixation process, whereas photos from samples with relatively high sodium thiosulfate levels are expected to have more brown toning due to excessive silver sulfide being formed from sodium thiosulfate (Pope 1959). We do not expect the chemical profile and microbial diversity of each treatment to be the same, and we also

do not expect salinity and arsenic concentrations to be noticeably low. Unexpected results or results not described may indicate that there are other factors contributing to the fixation process occurring in Owens Lakebed or that the methods described may need to be revised.

Timeline



References

Sorokin, Dimitry Y., J. Gijs Kuenen, and Gerard Muyzer. 2011. "The Microbial Sulfur Cycle at Extremely Haloalkaline Conditions of Soda Lakes." *Frontiers in Microbiology*, 2(44): 1-16. https://doi.org/10.3389/fmicb.2011.00044

Article provided by learning coach Rachel. This article draws connections between salinity, high pH, haloalkaliphilic microorganisms, and the sulfur cycle. It discusses the populations of microbes found in these environments and how they grow by thiosulfate disproportionation in salt-saturating conditions without an external electron donor. Varying salinity in soda lakes effects the sulfur cycle and the "key" microbes involved. This article will help to determine why thiosulfate levels were so high in an environment that they were not expected to be present in at all and may lead to findings about the role of thiosulfates/sulfur cycle in photo fixation.

Ryu, Ji-hun, Robert A. Zierenberg, Randy A. Dahlgren, and Suduan Gao. 2006. "Sulfur Biogeochemistry and Isotopic Fractionation in Shallow Groundwater and Sediments of Owens Dry Lake, California." *Chemical Geology*, 229(4): 257-272. https://doi.org/10.1016/j.chemgeo.2005.11.001

Article provided by learning coach Rachel. This article analyzes groundwater and sediment samples from Owens Dry Lake, California. It discusses the concentrations of metals found and the interaction it has with the microorganisms and human health. It also discusses the effect of high salinity on sulfate reduction and the importance of sulfur biogeochemistry. This article will help to determine the composition of Owens Lake.

Sorokin, Dimitry Y., Tom Berben, Emily D. Melton, Lex Overmars, Charlottte D. Vavourakis, and Gerard Muyzer. 2014. "Microbial Diversity and Biogeochemical Cycling in Soda Lakes." *Extremophiles*, 18: 791-809. https://doi.org/10.1007/s00792-014-0670-9

Accessed through Google Scholar. This article discusses the effect of high concentrations of sodium carbonates and stable elevated pH on microbial metabolism in soda lakes. Many microorganisms have adapted to allow them to perform enzymatic reactions under these conditions. This article will contribute background knowledge about cellular adaptations to soda lake environments.

Lloyd, Jonathan R., and Ronald S. Oremland. 2006. "Microbial Transformations of Arsenic in the Environment: From Soda Lakes to Aquifiers." *Elements*, 2(2): 85-90. https://doi.org/10.2113/gselements.2.2.85

Accessed through Google Scholar. This article discusses microbial interactions with arsenic and strategies evolved to protect them from arsenic. Several species of arsenic-metabolizing bacteria in two soda lakes were isolated. It talks about the possibility of microbes playing a role in mobilizing sediment-bound arsenic into water that is abstracted for drinking and irrigation. This article will help to determine the relationship between microbes and arsenic in the environment and how they can be used.

Kabiraj, Ashutosh, Raju Biswas, Urmi Halder, and Rajib Bandopadhyay. 2022. "Bacterial Arsenic Metabolism and Its Role in Arsenic Bioremediation." *Current Microbiology*, 79(131): 1-15. https://doi.org/10.1007/s00284-022-02810-y

Accessed through University of Wyoming Web of Science Database. This article discusses bioremediation of arsenic species using arsenic loving bacteria. It talks about the ways in which these bacteria are able to uptake arsenic, detoxify themselves from it, and utilize it. This article will help to determine how microorganisms in Owens Lakebed could be used for bioremediation of arsenic.

Kulp, T. R., S. Han, C. W. Saltikov, B. D. Lanoil, K. Zargar, and R.S. Omerland. 2007. "Effects of Imposed Salinity Gradients on Dissimilatory Arsenate Reduction, Sulfate Reduction, and Other Microbial Processes in Sediments from Two California Soda Lakes." *Applied and Environmental Microbiology*, 73(16): 5130-5137. https://doi.org/10.1128/AEM.00771-07

Accessed through University of Wyoming JSTOR Database. This article discusses the effect of salinity on arsenate reduction, sulfate reduction, and other microbial processes in soda lakes that are similar to Owens Lakebed. It indicates that there may be shifts in microbial diversity depending on the relative concentration of salts. This article will contribute to background knowledge on how the microbes are influenced by the environmental conditions in Owens lakebed, and the effect this environment could have on arsenic bioremediation.

Duke, T., L. Bon, and R. Nielsen. "Lakebed Developing Process (2013-to Present)." *Vimeo Video*, 7:55, n.d., https://vimeo.com/532105773/08a1c2bc02?embedded=false&source=video_title&owner=40859862

Provided by learning coach Rachel. This lecture video by Metabolic Studio discusses their land-based art project at Owens Lakebed, California and the significance behind it. This video contributes to our understanding of the stakeholder's objective and how it fits into a bigger picture with environmental injustice.

Pope, C. I. 1959. "Formation of Silver Sulfide in the Photographic Image During Fixation." *Journal of Research of the National Bureau of Standards*, 64(1): 65-73. https://nvlpubs.nist.gov/nistpubs/jres/64C/jresv64Cn1p65_A1b.pdf

Provided by learning coach Rachel. Although this article is fairly dated, it is very pertinent to our research because it discusses the fundamentals of film development and describes the role of thiosulfate in film fixation and how it can lead to different effects. This article will allow us to gain an understanding of the interactions that occur when a photo is placed in a fixation bath and how that relates to the conditions of Owens Lakebed in land-based art.

University of Houston College of Technology. N.d. “Chemistry of Photographic Processing.” *Digital Media Materials Handout*, 1-11. https://web.tech.uh.edu/digitalmedia/materials/3351/PHOTCHEM.pdf

Accessed through Google. Although it is not a review article, it is education material provided by the University of Houston College of Technology that describes the steps and chemistry involved in photographic processing. This article will increase our understanding of the elements involved in the steps of photographic processing (development, stop bath, fixation, etc.) and the purpose behind them.

Department of Industries and Regional Development. 2022. “Measuring Soil Salinity.” *Agriculture and Food*. https://www.agric.wa.gov.au/soil-salinity/measuring-soil-salinity

Accessed through Google. This article discusses techniques behind measuring water and soil salinity. This article is pertinent to our research because it allows us to measure soil salinity in a way that can be converted to units involved in water salinity for proper comparison between samples.

Church D. L., L. Cerutti, A. Gurtler, T. Griener, A. Zelazny, and S. Emler. “Performance and Application of 16S rRNA Gene Cycle Sequencing for Routine Identification of Bacteria in the Clinical Microbiology Laboratory.” American Society for Microbiology Journal, 33(4). https://doi.org/10.1128/CMR.00053-19.

Accessed through Google Scholar. This article discusses the accuracy of 16s gene sequencing for the identification of microorganisms. This article supports our justification of approach for identifying bacterial diversity in samples.