Returning Agency to Owens Dry Lakebed Through Exploration of the Microbial, Chemical, and Art-Making Capacity of a Desiccated Site

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Learning Actively Mentoring Program

Project Summary

-*Overview*-

Art has long been a tool to elicit emotion, spark discussion, and engage the viewer. However, recently art has been found to have another use – a tool in science (Braund et al. 2019). These two aspects of art are brought together by the work of Metabolic Studio, including one of their ongoing projects, the *Owens Dry Lakebed Developed Print* Collection. Owens Lake was once a large body of fresh water in California that dried shortly after the completion of the Los Angeles aqueduct. Due to this rapid desiccation, the lakebed now contains and emits toxins and is a patchwork of mitigation efforts, some of which include briny, alkaline pools wherein Metabolic Studio fixes their prints. This unique location not only serves as subject for the photographs produced by Metabolic Studio, but also contains compounds necessary to help in the removal of unexposed silver halide crystals from the film, known as the film fixation process (Duke et al. n.d.). Our research will address the question: to what extent does film development affect the microbial community in the soil and water, and to what extent does the microbial community and chemical conditions within Owens Dry Lakebed affect the variation of the prints. We hypothesize that the qualities of the photographs fixed on the lakebed can predict microbial populations and salt compositions. Additionally, we will explore the bioremediative capacity of microorganisms in Owens lakebed, explicitly those capable of converting methane and arsenic to less toxic forms. We will address these research questions by comparing the bacterial diversity of pre-film-fixation and post-film-fixation lakebed microbial communities. We will also determine how pH, soil texture, thiosulfate concentrations, salinity and salt composition may mediate film development and bacterial communities. Our approach will utilize growth cultures, PCR, and bioinformatics to elucidate microbial systems as well as a slew of chemical analyses such as specific conductivity and inductively coupled plasma – optical emission spectrometry (ICP-OES) to explicate how abiotic factors are connected to film development as well as microbial biodiversity of each sample. This study provides an avenue to advance the synergy of art and science.

Intellectual merit: This research promotes unprecedented transdisciplinary inquiry 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: Collaboration is a foundation of the proposed research – we will be partnering with Tristan Duke, Lauren Bon, and Rich Nielsen of Metabolic Studio, the Department of Geochemistry and the Department of Ecosystems Science and Management at the University of Wyoming, the Microbestiary art/science outreach project, and art students from Laramie High School. This research will also highlight the extent of ongoing environmental injustices while simultaneously seeking to give agency to Owens Dry Lakebed beyond its reputation as a wasteland. It will contribute to our understanding as a society of how humans can affect land, and how the land can in turn affect its own effects on us in the form of art and science.

Figure1: Photograph of Owens Dry Lakebed from Metabolic Studio. https:/www.metabolicstudio.org/optics-division

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Project Description

-Statement of Problem and Significance-

Owens Dry Lake has long been known as a toxic wasteland — a problem that needs to be solved. In 1913, Owens Lake was subjected to an accelerated drying process fueled by the rerouting of its water to the new Los Angeles Aqueduct. Due to the human driven desiccation, along with the high salinity of the area, toxic, and often carcinogenic, chemicals leach from the surface of the lakebed (Levy et al. 1999). The salinity and toxicity of the lake, however, provides an extraordinary environment that allows extremophilic microorganisms to thrive and land-based art to manifest the distinctive features of the dry lake. This environment—the salinity, basicity, toxicity, chemical makeup, and microbiome—can be mapped onto the land, mapped onto the body, and mapped onto art, giving a necessary reminder that the land is not simply a human health hazard, but a landscape capable of supporting diverse microbial life and creating art in a novel way.

The hazards of Owens Dry Lake can be visualized through the land. Salt crusts, which can be seen from 100km (about 62.14 mi) above the earth using Google Earth, are generated at the surface of the lakebed through the evaporation of up flowing groundwater (Levy et al. 1999). When high salinities are reached in the surface salts, ground waters indicate that toxins, such as arsenic and fluorine, are partitioned into these surface salts from the soil (Levy et al. 1999).

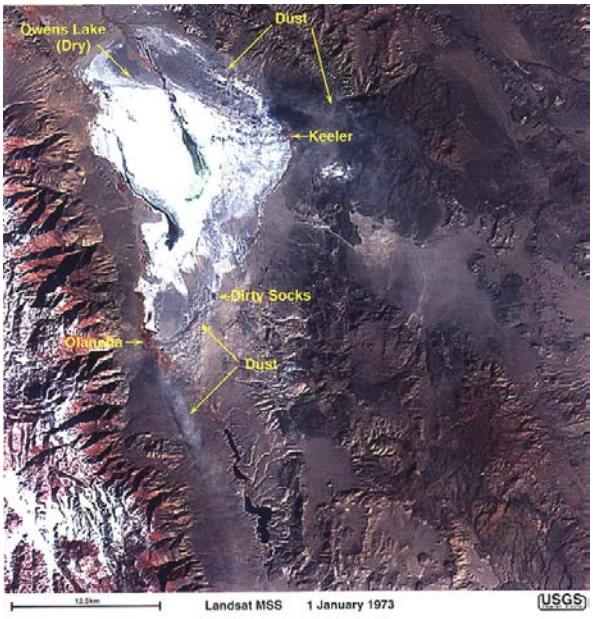


Figure 2: Aerial view of dust storm in 1973 from the United States Geological Survey.

https://geochange.er.usgs.gov/sw/impacts/geology/owens/

The harmful chemicals present in Owen's Lakebed can be mapped onto the body due to the health effects caused by the PM-10 pollution on the surrounding populations. By many estimates, Owens dry Lake is the largest sole source of PM-10 pollution in the United States. Dust storms containing PM-10 particles from the Owens Dry Lakebed can travel to neighboring regions (Figure 2). Exposure to PM-10 pollution is demonstrated to have both short- and long-term effects ranging from worsening of respiratory diseases, high blood pressure, heart attacks, and increased risk of cancer ("Inhalable Particulate." n.d.). Moreover, acute exposure to arsenic can alter cellular metabolism, causing problems for both energy production and biosynthesis ("Medical Management." 2014). These effects of PM-10 pollution and chemical exposures can be mapped onto the human-body, seen through the effects of the lakebed-produced dust storms on the surrounding population.

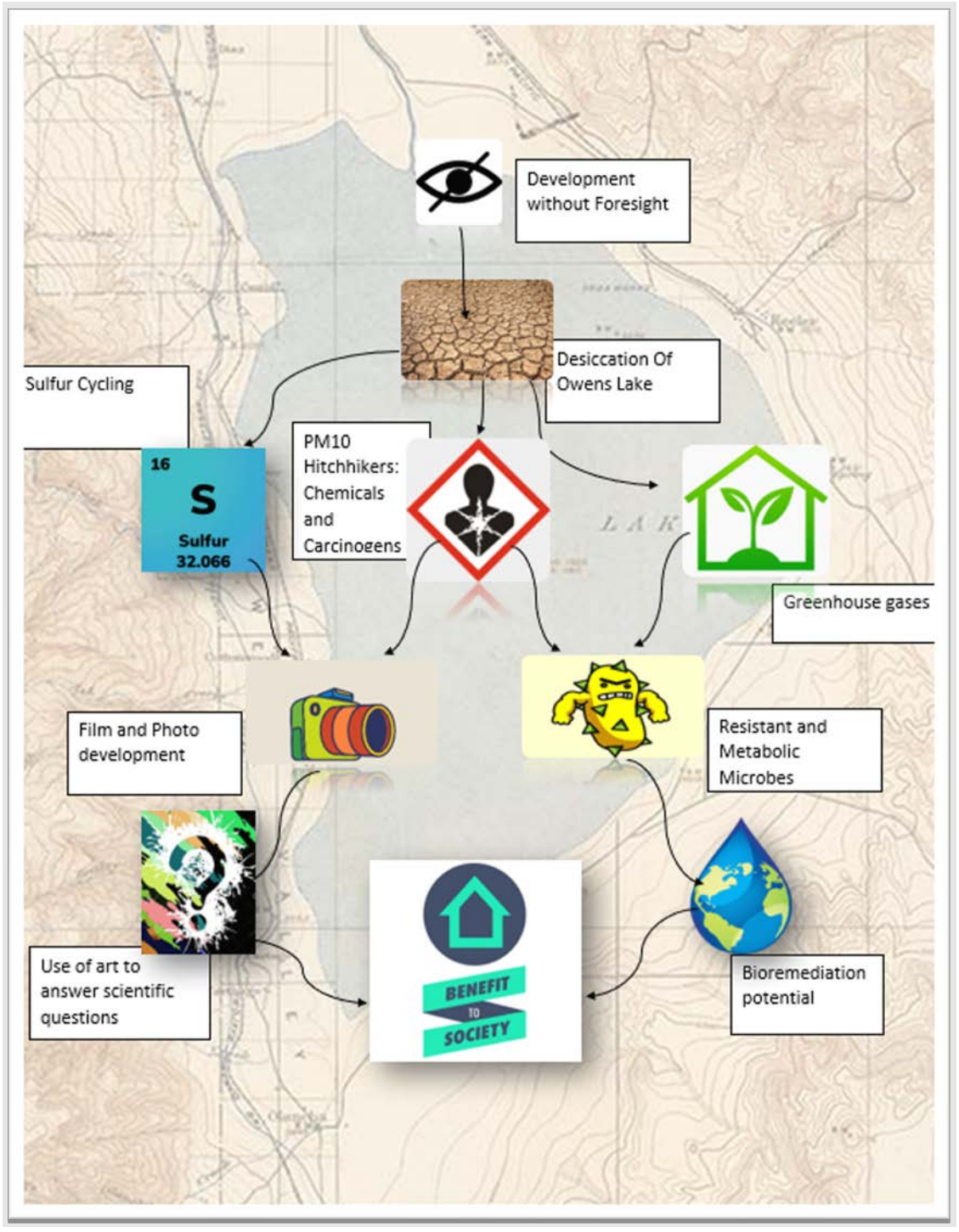
Not only are humans affected by the distinctive biome of Owens Dry Lake, but also microbial life. The toxic salt crusts of the dry lake offer an unusual microbial environment which allows halophilic, halotolerant, and alkaliphilic microorganisms to thrive as well as organisms that can utilize or resist the toxic chemicals present such as arsenic. Many of these organisms will metabolize arsenic into different, "less toxic" forms (Kabiraj et al. 2022).

The salts and chemicals of the lakebed not only create a diverse, eye-catching, toxic landscape, but also map onto land-based artwork created by Metabolic Studio. In one of their ongoing projects, *Owens Dry Lakebed Developed Print Collection*, Metabolic Studio creates land-based photo prints that are fixated in the surface waters on the Owens Lakebed (Duke et al. n.d.). These images show unique development patterns, lacking uniformity between the multiple images fixed. Different coloration patterns occur, with some veining and cloudiness. These images help to show differences in the development process, and potential roles of microbes and chemicals in this process.

Reflection on the ways the Owens Dry Lakebed maps on the land, on life, and on art, brings forth both a response to mitigating toxic output and the mechanism of art as a detection tool of specific microorganisms, salts, and consequently, portions of the lake with high toxins partitioned into surface salts. Knowing that the toxic environment of Owens Dry Lakebed causes detrimental human health hazards yet also allows microbial life to thrive, promotes the idea of understanding the capacity of, and potentially utilizing, microbes to clean up the toxicity of the

lakebed. Isolating the microbes capable of turning the carcinogenic elements present to less toxic forms, followed by quantification and analysis of the process, could lead to the microbes being used on a large scale to reduce the carcinogenic and toxic materials present in the inevitable dust storms caused by the lakebed. Utilizing photo development as a detection tool offers a new method to visualize and understand both the toxins of the lake and the microbes, creating a unique piece of art as a product. This method not only offers microbial and chemical information, but also helps to show that Owens Dry Lakebed is not simply a wasteland, but a unique environment capable of producing beautiful art. The processes of bioremediation via microorganisms and photo development as a detection tool could be used at other desiccated salt lakes to get multifaceted results and provide scientific evidence in a method that community members can appreciate and understand. The findings from this research could help to guide understanding of microbial populations, the capacity of microorganisms to bioremediate, and a new method to pinpoint toxic chemical partitioning of other desiccated salt lakes. This research will investigate the many ways that Owens Dry Lakebed continues to have agency, despite human interventions.

-Conceptual Model-



-Relevant Literature-

Owens Dry Lakebed, like other soda lakes, was formed by the continuous evaporation of flowing groundwater and the diversion to Los Angeles. Prior research has determined that salts of Owens Dry Lakebed are mostly trona, halite, and burkeite (Friedman et al. 1976); however, burkeite, mirabilite, and thenardite are other saline minerals that have been present in the salt flats ("Owens Lake, California"). Sodium carbonate specifically has been seen to be the dominant salt present in water samples (Herbst et al. 2014). This groundwater lacks magnesium and calcium, but is rich in carbon dioxide, resulting in the leaching of sodium from sodium-rich rocks (Sorokin et al. 2014). As a result, there is an abundance of sulfate-, carbonate- and chloride- salts which generate high basicity and salinity (Ryu et al. 2006). The groundwater also contains high concentrations of toxic trace elements, such as arsenic. Arsenic is only partitioned from the surface of the lakebed when high concentrations of salts are present, and it can then be "readily leached from lakebed salts when exposed to natural precipitation" (Levy et al. 1999). This results in higher concentrations of Arsenite (As (III)), the predominant form of arsenic found in shallow groundwater (up to 96 mg/L), which is less strongly absorbed, more soluble, and more toxic than Arsenate (As(V)) (Ryu et al. 2006). The Arsenate can be bound to miniscule soil particles. Thus, significant amounts of PM-10 molecules containing toxic arsenic, fluorine, as well as many salts, can be spread through several types of storms. PM-10 pollution refers to airborne particulate matter with a diameter of 10 micrometers or smaller — making it possible for inhalation and deep deposition of the matter. According to the Environmental Protection Agency, as of 2017, Owens Lake emits approximately 300,000 tons of PM-10 yearly with 0.01% (30 tons) of this emission being attributed to arsenic. Consequently, creating a health hazard for many animals, plants, and humans throughout Owens Valley, and potentially the world.

While arsenic is highly toxic to many forms of life, certain microorganisms have the capacity to tolerate high concentrations of the metalloid, while others can utilize it as a source of energy through redox transformations; 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 uptake arsenic to perform their metabolic processes because phosphate is

chemically similar 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 Dry Lake interact with these exclusive environmental conditions for their important metabolic and physiological processes. The major process regulating redox conditions for microorganisms in Owens Dry Lakebed is sulfate reduction (Ryu et al. 2006). The biological sulfur cycle is integral to microbial metabolism because it 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 Dry 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 (made 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 (Duke. n.d.; University of Houston n.d.). 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 brown (Pope. 1959).

-Preliminary Data-

In addition to sample collection, preliminary water chemistry data were collected July 27th, 2022, post photo fixation on the Owens Lakebed. A YSI meter was used to gather general water chemistry information at the photo fixation sites. Multiple readings were taken at each of the four lakebed sites. The measures of importance for this research are specific conductivity, pH, and temperature.

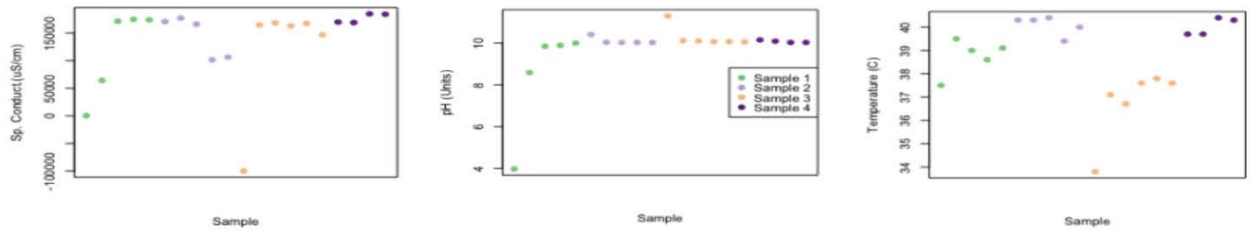


Figure 3(a) Specific conductivity of each sample from each treatment. 3(b) pH of each sample from each treatment. 3(c) Temperature of water of each sample from each treatment.

Preliminary data has shown a range in specific conductivity of the four sample sites (Fig. 3a). Sample sites 1 and 4 had similar conductivity, averaging 173,612.67 uS/cm and 177,096.5 uS/cm, respectively. Sample site 2 had the lowest conductivity averaging 144,446 uS/cm, while sample site 3's conductivity was in the middle of this range at 162,193.2 uS/cm. Variations in conductivity suggest differences in salt concentrations and the saline minerals present between sample sites. Figure 3b illustrates the pH average of 10 with an outlier in Sample 1. Similar temperatures for the four sample sites at around 40 °C (Fig. 3c.); however, sample site 3 is somewhat of an outlier at 37.36 °C. Differences in temperature of the sample sites could be reflected in the microbial species present. To determine the averages for conductivity and temperature for the sample sites, gross outliers were Q-tested out ("Q Test for Outliers." n.d.). Each site had three to five readings taken and averaged.

-Sample Collection-

Four treatments were designated (Figure 4). No-rinse indicates the film was removed from the stop-bath which contained 2% acetic acid and laid in the lakebed. 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. To prevent contamination of the samples were collected 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 shovels-full and was placed into large sterile Whirl-Paks. Three water samples in 50 mL falcon tubes were collected for each treatment. All four treatments of the 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 PST. 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.

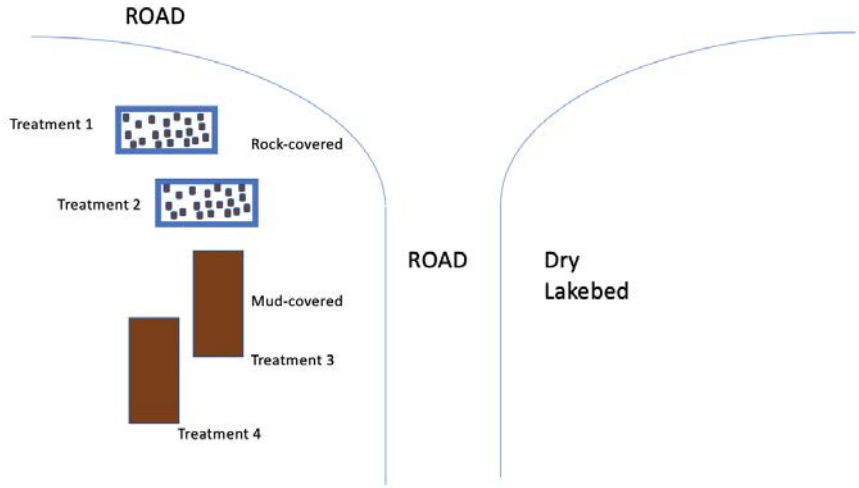


Figure 4 illustrates the site of sampling in Owens (dry) Lake, California. Treatment 1 was covered with rocks and not rinsed. Treatment 2 was covered with rocks and rinsed. Treatment 3 was covered with mud and not rinsed. Treatment 4 was covered with mud and rinsed.

-Future Methods and Materials-

To determine the microbial biodiversity pre-film-fixation and post-film-fixation, the samples used will be water pre-film fixation day, soil pre-film fixation day, water pre-film fixation night, soil pre-film fixation night, water post-film fixation, and soil post-film fixation. The soil samples have been stored in Whirl-Paks and kept at -80 °C. To characterize microbial populations of the soil samples, an Omega E.Z.N.A. Soil DNA Extraction kit will be used to isolate the DNA. DNA will be extracted from the water samples utilizing an Omega E.Z.N.A. Water DNA Extraction Kit. The extracted DNA will be cleaned and concentrated using a ZYMO kit. All extractions will be performed according to the manufacturer's instructions. At this point, the samples' DNA concentrations will be checked with a Nanodrop to confirm that DNA is present. The samples will then undergo PCR with primers flanking the V3/V4 region of the 16s rRNA gene. PCR will also include barcodes from a 16s Bar Coding Kit for MinION for differentiation of the products. The 16s amplicons will be pooled and differentiated by barcode. To verify the size and presence of the PCR product, amplicons will be run on an agarose gel with

a DNA ladder standard. These amplicons can then be sequenced using the MinION. Raw data will be acquired using MinKnow which is a proprietary software from Nanodrop. From there, GUPPY and Epi2Me will determine phylogeny of each bacterium and archaeon. A novel, computational software, known as *MelonnPan*, will then be used to predict the metabolic profiles of the microbes identified from the different samples. The predicted metabolic pathways of these microbes will be supported by plating the isolated microbes on soil extract agar. We will use this soil extract agar to mimic the environment in which these organisms are found, using soil directly from the lakebed. This will allow the microbes to grow in an ideal environment for determining their unique metabolic pathways. The measurement of the arsenic content found in the soil extract agar will be measured before and after streaking the microorganisms to measure the effect of the microorganisms on arsenic levels. From this, we can infer some of the arsenic metabolic abilities of the bacteria found in Owen's Lake. Additional analyses will also be used to establish patterns against soil abiotic factors such as arsenic.

We will retest the pH and specific conductivity (EC) to ensure accuracy using the Thermo Scientific Orion Star A meter in the Norton Soil Fertility Lab of the Ecosystems Science and Management department at the University of Wyoming. To determine the salt compositions pre-film-fixation and post-film-fixation, inductively coupled plasma – optical emission spectrometry (ICP-OES) will be used to identify the cations present in the salts. Ion chromatography (IC) will be used to identify the anions present in the salts. This will be done with the support and collaboration of Ellen Polites, a Ph.D. student in Geochemistry and the University of Wyoming Geochemistry Lab. To prepare samples for the testing, 1g of sample will be dissolved and diluted in buffer.

We will indirectly measure thiosulfate levels pre-film-fixation and post-film-fixation through cyanolysis of thiosulfate and subsequent colorimetric determination of the thiocyanate product with help from the University of Wyoming Geochemistry Lab. We will also partner with the University of Wyoming Geochemistry lab to determine arsenic concentrations of samples using ICP-MS.

Following determination of microbial biodiversity and chemical makeup, analysis of photos developed on the lakebed by Metabolic Studio will be completed. Photos will be analyzed utilizing a word bank describing the characteristics of the prints and made in collaboration with

Metabolic Studio and local art students, as well as quantification of differences between the photos. The quantification of the differences will be determined using the program ImageJ (National Institutes of Health. 2012). Finally, the specific salts and microbes discovered will be compared to the photo analyses.

-*Justification of Approach*-

Our first approach using the amplification process known as polymerase chain reaction (PCR) is used globally by microbiologists. 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 et al. 2007). According to the National Institute of Health, the function of this gene has not changed over time, and it will provide phylogenic information, perhaps to the species level. To allow our research group the opportunity to learn more about the amplification process, we will use the MinION and its associated pipeline to provide phylogeny. Additionally, we have designed our own primer to detect the methanogenesis of archaea since primer specifications must be precise. Moreover, to determine transcription levels of the methanogenesis target gene, we will utilize reserve transcription polymerase chain reaction (RT-PCR) (Mo et al. 2012). This method is widely used in biomedical and other research fields to provide semiquantitative information. We will also utilize primers created by a group of applied microbiologists at the Mie University in Japan as they have been successful at detecting the *ars* operon (Suzuki et al. 1998). With our genetic information we will employ several bioinformatic techniques.

Beta diversity will be used to determine how similar or how dissimilar the diversity of species in each sample and summarize these finding an NMDS graph (Syms. 2008). In an NMDS graph, the closer two samples are to each other, the more similar the microbial community is to each other. This information may elucidate nuances in the niches of each sample. This analysis is widely used in ecological studies. *Melonpann* is a computational package developed for R Studio used to predict which metabolites microorganisms can and will use. MelonnPan works by inferring the total number of metabolites. It does this by enabling data-driven identification of set of predictive microbial features, as well as quantification of the accuracy of metabolite predictions (Mallick. 2022). This novel approach provides our team a safe way to determine the metabolism of toxic compounds such arsenic. ImageJ is traditionally

used in the biomedical field and has been supported by the National Institute of Health for over 10 years (Ferreria. 2012). However, we believe we can use this computational method to identify miniscule differences between the developed film prints.

Figure 5 illustrates the soil ribbon test, which is an adopted, standardized approach that has been used for more than 40 years by the United States Department of Agriculture (USDA) to determine soil texture and is a free method (USDA. 2022). We will perform this test 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 incredibly useful in understanding the complete microcosm of each sample. To determine the composition of salts, IC will be used to determine the anions that comprise the salt. This method has high sensitivity and a wide operating range (Pohl. 2005) making it a useful approach. ICP-OES data will provide us an exhaustive list of cations present in our samples even at trace levels. This test has been adopted by chemists in multiple fields including geochemistry (Planeta. 2021). This is a novel approach to fully interpret the microcosms of our samples which influence both the microbial ecology and the film-fixation process. Moreover, this analysis provides our team with an opportunity to work alongside the Department of Geochemistry at the University of Wyoming.

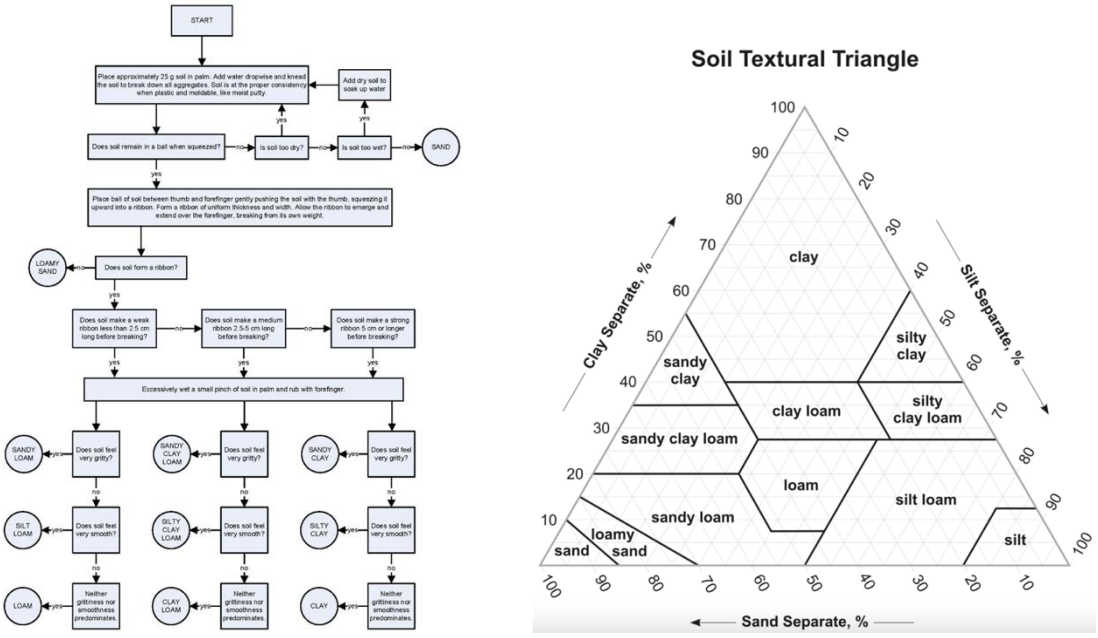


Figure 5 illustrates how to perform the soil ribbon test and how to determine the soil fraction percentages of the soil sample.
https://www.nrcs.usda.gov/wps/portal/nrcs/detail/soils/edu/?cid=nrcs142p2_054311

To measure arsenic concentrations, we will use ICP-MS. This is a particularly valuable tool used in modern physics and chemistry. ICP-MS stands for inductively Coupled Plasma Mass Spectrometry and the way it works is by pushing the sample, which is fed into the fog and is atomized in the ICP channel at 7000 K. These ions are then accelerated and focused into the mass spectrometer and is then analyzed with different mass to charge ratios, to analyze the different elements that will be found in the soil, including arsenic. This technique has better precision values when compared to other instruments such as the Atomic Absorption Spectroscopy instrument (Planeta. 2021).

Thiosulfate concentrations will be determined based on cyanolysis of thiosulfate and colorimetric determination of the thiocyanate product through a partnership with the University of Wyoming Geochemistry Lab. This technique has been used for decades and it supplements the addition of ferric ions to prevent the interference of other reducing compounds – an issue that exists in other methods (Sorbo. 1957).

Research Plan

-Objectives-

Our objectives are: to identify the bacterial and archaeal species of the Owens Dry Lakebed film photo fixation site (H1), to elucidate the extent to which these microbial communities are impacted by the salt concentrations and compositions, basicity, and soil texture (H2), to identify the chemicals present and how these factors influence the microbial communities and their metabolism (H3), to use computational methods to visualize microbial diversity and predict microbial metabolism (H4), to investigate the possibility of photo fixation as a detection tool, and product, of specific microbes and salts (H5), and to identify bacteria capable of bioremediation (H6).

-Hypotheses and Specific Aims-

H1: *Microbial biodiversity will differ pre-film-fixation and post-film-fixation.* We will compare microbial biodiversity before and after lakebed film-fixation using 16S rDNA sequencing. Moreover, the *pmoA* and the *ars operon* will help us determine metabolic capabilities of specific groups of microbes present in our soils to have a comprehensive knowledge of the community.

H2: *The film and microbial communities will differ with differing salt compositions and concentrations, thiosulfate levels, pH, and soil texture.* We will compare salt concentrations before and after lakebed photo fixation using a specific conductivity meter and compositions using ICP-OES and IC. We will indirectly compare thiosulfate levels before and after lakebed photo fixation using cyanolysis of thiosulfate and subsequent colorimetric detection of thiocyanate product. We will compare the communities of bacteria and archaea present in each sample along with the soil pH, texture, and concentrations of salts and thiosulfate to find patterns.

H3: *There will be an inverse relationship between the number of microorganisms present on the soil extract agar plates and the tryptic soy agar.* We will use both tryptic soy agar and soil extract agar plates and the DNA extract kit and the 16s rDNA extraction kit to identify the variety of microbes between the two plates.

H4: *The use of computational methods will allow us to predict if the microbes found within the lakebed samples are capable of arsenic metabolism leading to decreased overall arsenic concentrations.* We will use a novel computational method to predict metabolic profiles of microbes present in the lakebed samples. This method, known as MelonnPan, works by inferring the total number of metabolites.

H5: *The qualities of photos post-fixation can predict microbial population and salt compositions*. We will analyze photos fixed on the lakebed using a word bank made in collaboration with Metabolic Studio and local art students, as well as quantification of differences between the photos utilizing the program *ImageJ*. The photo analyses will then be compared with the chemical profiles and microbial populations to determine if there are significant correlations.

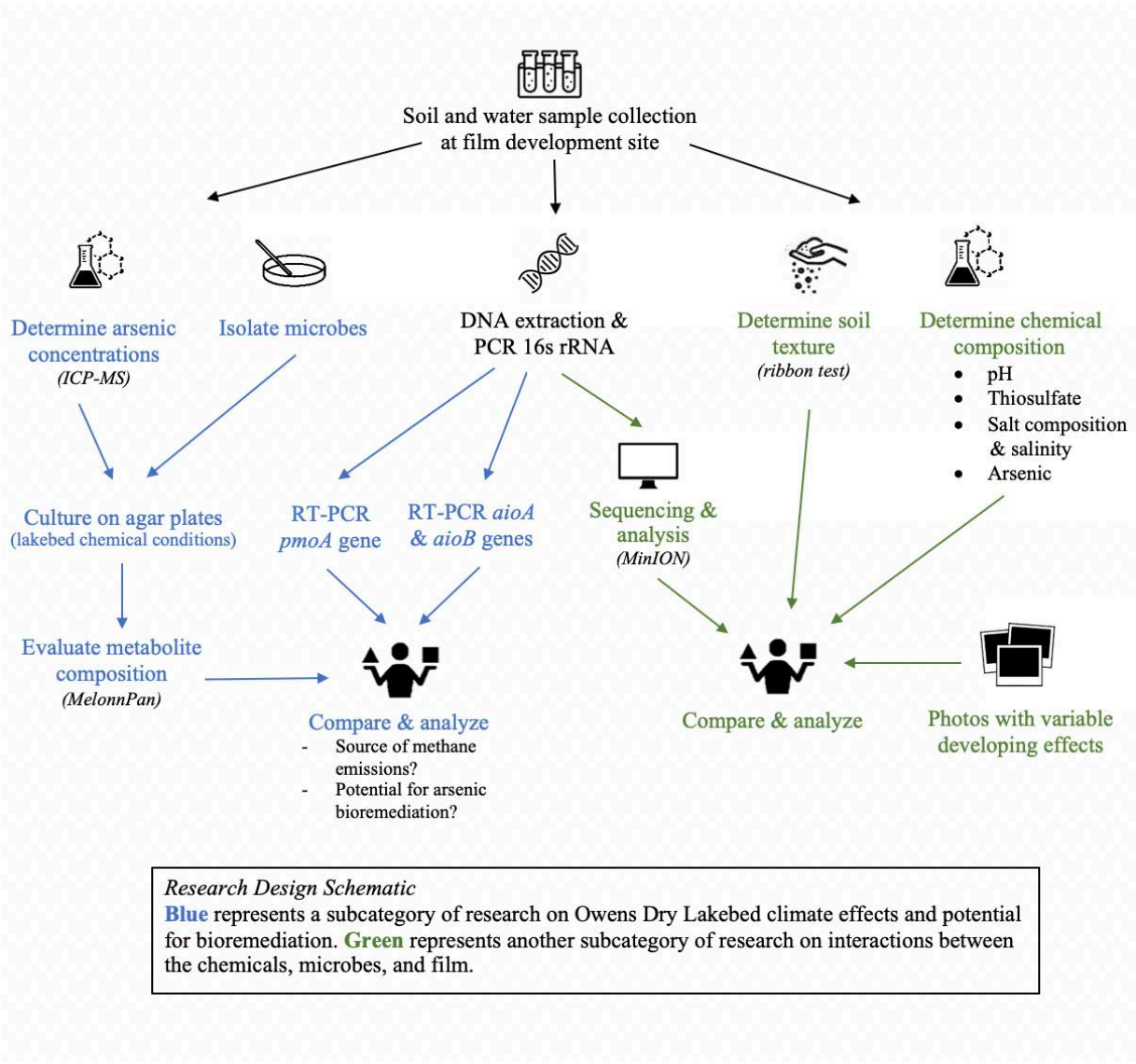
H6: *The microbes capable of metabolizing arsenic will prove to remediate and lower the concentration of arsenic on the soil extract agar plates.* We will take the plates and microbes found that grow on the soil-extract agar plates and will measure the amount of arsenic pre and post growth to find how much arsenic was metabolized over the course of growth.

H7a: *Archaea are present in the soils and at least one of the species present has the genes required for methane oxidation.* We will use PCR of 16s ribosomal DNA sequences specific to the domain to confirm the presence of Archaea species.

H7b: *Genes related to methane oxidation are being expressed in lakebed populations.* Combined with the results of the 16s ribosomal DNA PCR, RT-PCR will be then used to confirm the expression of the *pmoA* gene. *PmoA* is a subunit of the particulate methane monooxygenase enzyme necessary for methanotrophs to convert methane into methanol for metabolic purposes.

H7c: *If the genes for methane oxidation are present in the bacterial soil samples, then the lake is source of methane emissions.* By confirming the presence and expression of the *pmoA* gene, we can deduce that methane is being generated in the lakebed and that is being consumed by bacteria as an alternative source of energy.

-Research Design Schematic-



Research Design Schematic
Blue represents a subcategory of research on Owens Dry Lakebed climate effects and potential for bioremediation. **Green** represents another subcategory of research on interactions between the chemicals, microbes, and film.

-Analyses and Expected Results-

Data will be recorded in laboratory notebooks and stored in Google Sheets for ease of access among team members. It is expected that bacterial biodiversity will depend on arsenic, thiosulfate, and salt concentrations of the site/sample; and due to the microbes interacting with the chemicals in the film, microbial communities will differ pre-film-fixation and post-film-fixation. We expect to find archaea capable of methane oxidation, halophilic bacteria, alkaliphilic bacteria, and halotolerant bacteria. We also expect to find bacteria which metabolize arsenic and produce thiosulfate. It is also expected that salt concentrations and compositions will differ pre-film-fixation and post-film-fixation. We anticipate that the samples with increased salinity will also have increased thiosulfate levels. Photos from samples with low thiosulfate levels may contain black metallic spots or darkened areas due to the light-sensitive silver halide crystals not being completely dissolved in the fixation process (Pope. 1959). Whereas photos from samples with high thiosulfate levels are expected to have more brown toning due to excessive silver sulfide being formed from thiosulfate (Pope. 1959). Post-fixation, we expect to see an increase of chloride and a decrease of sulfate. There is also a link between what microbial species are present to the salt concentrations and the toxins partitioned into the salts. The photos developed on the lake are expected to act as a detection tool of what microbes and salts are present. We do not expect the chemical profile and microbial diversity of each treatment (1-4) to be the same and we also do not expect pre-film-fixation samples and post-film-fixation samples to be the same. Moreover, we do not expect to find halo-intolerant nor arsenic-intolerant bacteria to be present in our samples. 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 Dry Lakebed or that the methods described may need to be revised.

-Timeline-

Week	To Dos	Overarching Goals
Pre-0 (Sept. 26th-30th)	• Make media • Have finalized evolving draft complete • Order materials • Find recipe for SEA • Streak for initial growth of microbes	Ensure that everything needed to complete lab is ordered, planned, and is capable of being executed (excluding time)
0 (Oct. 3rd – 7th)	• Plan plating • Finishing Rough Draft of Proposal	Getting started: plan drying, plating and DNA work
1 (Oct. 10th – 14th)	• Dry Soil • DNA Extraction • Plate agar • Streak Microbes	Prepping to start the media development and prep for the extraction of DNA from soil and water samples.
2 (Oct. 17th – 21st)	• Begin data analysis • Streak microbes on TSA/ SEA • Measure pH, EC, and texture	Begin streaking and continue with data analysis via DNA extract.
3 (Oct. 24th - 28th)	• Replate for isolation and DNA extraction • Continue data analysis and DNA analysis • Prepare samples for Geochemistry analysis	Analyze microbial growth on plates and measure and view the genetic information for diversity and specific genes and primers.
4 (Oct. 31st – Nov. 4th)	• Data analysis for geochemistry of soil and water samples • Computational analysis of metabolic pathways • Salt makeup and development analysis • Begin poster and presentation to stakeholders	Run and prepare samples for geochemistry readings and analysis. Prepare our presentation for stakeholders.
5-9 (Nov. 7th – Dec. 10th)	• Finalize report, poster, speech, etc.	Finalize our presentation for stakeholders and prepare for a public audience.
10 (Dec. 15th)	• Communicate our findings and explain our poster	Communicate our findings with the public at the art exhibit.

References and Annotations

Abbaspour, Madjid, Amir Hossein Javid, S. A Mirbagheri, Farid Ahmadi-Givi, and Parvin Moghimi. 2012. “Investigation of lake drying attributed to Climate Change.” I*nternational Journal of Environmental Science and Technology.* SpringerLink. Retrieved September 24, 2022.

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 conditions are considered extreme conditions.

Braund, Martin and Michael J. Reiss. 2019. “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 how that allows us to use art to improve science. It was accessed through Springer Link.

“Climate change evidence: How do we know?” *NASA*. 2022. https://climate.nasa.gov/evidence/

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

Creason, Glen. 2016. CityDig: “Here's what Owens Lake looked like before Los Angeles drank it dry.” *Los Angeles Magazine*. Retrieved September 24, 2022, 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. “Effort to limit dust pollution in Owens Valley is advancing, but still room to improve.” *UCLA Newsroom*. https://www.ioes.ucla.edu/article/effort-to-limit-dust-pollution-in-owens-valley-is-advancing-but-still-room-to-improve/.

This online article provides a history of the issue as well as discussing current efforts for improvement at Owens Dry Lakebed. Although it is not a peer-reviewed article, it comes from a reliable source: The Institute of the Environment and Sustainability

de la Guardia, Miguel, and Sergio Armenta. “Multianalyte Determination versus One-at-a-Time Methodologies.” *Green Analytical Chemistry* 57 (2011): 121–56. https://doi.org/10.1016/b978-0-444-53709-6.00006-9.

This article was viewed on Science Direct. It offers information on the mechanisms of ICP-OES and the advantages of running ICP-OES on a sample. The article comes from a peer-reviewed, open-access journal.

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%20=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.

Duke, T. “EPFC Chemistry of Film Edit.” Vimeo Video, 18:57, n.d., https://vimeo.com/745260327/0c78aa8c55?embedded=false&source=video_title&owner=40859862.

Provided by learning coach Rachel. This lecture video by Tristan Duke, an artist of Metabolic Studio, discusses the chemistry of film fixation and the role of different chemicals in the process.

EPA. “Air Actions, California.” Environmental Protection Agency. Accessed September 4, 2022. https://19january2017snapshot.epa.gov/www3/region9/air/owens/qa.html.

This webpage was published by the Environmental Protection Agency. It was accessed through the EPA's previous website. This source has been used for information of PM-10 emissions from Owens Dry Lake. There is no publication date on the webpage; however, it was most recently updated on February 14, 2017.

Ferreira, Tiago, and Wayne Rasband. 2012. "ImageJ User Guide." *National Institute of Health.* https://imagej.nih.gov/ij/docs/guide/user-guide.pdf.

This is a user guide created by the National Institute of Health which provides thorough information on the computational program. It will be utilized in our attempt to appropriate this program for non-medical use.

Friedman, Irving, George I. Smith, and Kenneth G. Hardcastle. "Studies of Quaternary Saline Lakes—II. Isotopic and Compositional Changes during Desiccation of the Brines in Owens Lake, California, 1969–1971." *Geochimica et Cosmochimica Acta* 40, no. 5 (1976): 501–11. https://doi.org/10.1016/0016-7037(76)90218-0.

This source was accessed through the University of Wyoming Libraries website and viewed on Science Direct. The source lacks recency but offers valuable information on the salt contents of Owens Lake. This journal article was peer reviewed and includes ample citations for its work.

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

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

Herbst, David B, and Michael Prather. "Owens Lake – From Dustbowl to Mosaic of Saltwater Habitats." *Lakeline Magazine* 34, no. 3, 2014.

This magazine article was accessed as a PDF online. The article is lacking in that it is not peer reviewed; however, the authors are extremely qualified to provide information of interest. The article is about the habitat of Owens Lake but includes useful information on the salt contents of Owens Lake.

"Inhalable Particulate Matter and Health (PM2.5 and PM10) | California Air Resources Board." California Government. California Air Resources Board. Accessed September 4, 2022. https://ww2.arb.ca.gov/resources/inhalable-particulate-matter-and-health.

This webpage was accessed through the state of California's government website. The webpage does not offer a publication date. The webpage includes information on PM-10 pollution, exposure health risks, and national standards. It is beneficial for this paper due to its thorough assessment and explanation of PM-10 pollution.

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.

Levy, D.B., J.A. Schramke, K.J. Esposito, T.A. Erickson, and J.C. Moore. "The Shallow Ground Water Chemistry of Arsenic, Fluorine, and Major Elements: Eastern Owens Lake,

California." *Applied Geochemistry* 14, no. 1 (January 1999): 53–65. https://doi.org/10.1016/s0883-2927(98)00038-9.

This is a peer-reviewed article. It was accessed through the University of Wyoming Libraries website and viewed on Science Direct. It is relevant to discussions about what chemicals are present in the groundwater at Owens Lake (eastern). Additionally, this article offers information on the dust storm contents. This article is not recent, published in 1999, yet information was accurate for its time. There have been changes in the past 20 years.

Lloyd, Jonathan R., and Ronald S. Oremland. 2006. "Microbial Transformations of Arsenic in the Environment: From Soda Lakes to Aquifers." 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.

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.

"Medical Management Guidelines for Arsenic (As) and Inorganic Arsenic Compounds." Centers for Disease Control and Prevention. Centers for Disease Control and Prevention, October 21, 2014. https://wwwn.cdc.gov/TSP/MMG/MMGDetails.aspx?mmgid=1424&toxid=3.

This article was published on the Centers for Disease Control and Prevention website on the Agency for Toxic Substances and Disease Registry. It offers information on different routes of consumption of arsenic and the possible long and short-term effects caused by

ingesting arsenic. It is important for this paper to understand the effects arsenic can cause on Lake Owens surrounding populations.

"National current conditions." *Drought.gov*. 2022. 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*.

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.

"Optics division." *Metabolic Studio.* https:// www.metabolicstudio.org/optics-division.

This website is the central location of information for our stakeholders. It is one of the most germane websites to this study. This site is authentic and provides an abundance of knowledge necessary to complete this proposal and ultimately this research. Here you will find their ongoing projects of Metabolic Studio. It is where I found the panorama photo of Owens (Dry) Lake. Here you can contact the creators, and dive deep into how as well as why their art is created.

"Owens Lake, California." Owens lake. Accessed September 2022. https://saltworkconsultants.com/owens-lake-/.

This source gave information on the salt compositions of Owens Lake. While it is not a peer-reviewed journal article, the source is expert consultants in saline geosystems. They provide professional expertise in saline geosystems and carbonate reservoirs. This source was useful for information on the salt composition of Owens Lake.

Planeta, Karolina, Aldona Kubala-Kukus, Agnieszka Drozdz, Katarzyna Matusiak, Zuzanna Setkowicz, and Joanna Chweiej. The assessment of the usability of selected instrumental techniques for the elemental analysis of biomedical samples. *Sci Rep* **11**, 3704 (2021). https://doi.org/10.1038/s41598-021-82179-3.

This article is from Nature.com. At first glance, Nature.com looks like an ordinary website. However, this is a peer-reviewed open-access article. It is a review of ICP-MS, ICP-OES, and AAS and describes both the pros and cons of each instrument.

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.

“Q Test for Outliers.” Chemistry and Biochemistry Florida State University. Florida State University. Accessed September 2022. https://www.chem.fsu.edu/chemlab/Mastering/Q.html.

This webpage is published on the official Florida State University website as part of the Chemistry and Biochemistry resources. The webpage gives information on how to conduct a statistical Q test for outliers, as well as a critical value table to determine what values can be rejected with what confidence level.

Reheis, Marith, C. 2016. “A human-induced dust problem.” Owens (Dry) Lake, California. https://geochange.er.usgs.gov/sw/impacts/geology/owens/#:~:text=Water%20was%20first%20diverted%20from,side%20of%20the%20la ke%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 the events, photos of the region, and graphs pertaining to the abiotic factors. It was accessed through the USGS (United States Geological Survey).

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 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

Shrivastava, Pooja, and Rajesh Kumar. 2015. "Soil Salinity: A serious environmental issue and plant growth promoting bacteria as one of the tools for its alleviation." *Saudi journal of biological sciences.* 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.

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 affects 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.

Sorokin, Dimitry Y., Tom Berben, Emily D. Melton, Lex Overmars, Charlotte 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.

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 educational 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.