Transdisciplinary Beauty: From Photo Development to Bioremediation Through Metabolic Studios

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Microbial Capstone in Collaboration
with Metabolic Studios

Project Summary:

Overview: Arsenic poisoning has been a large factor in our world for thousands of years, and it continues to wreak havoc in our world today. Most research done today is on developing ways to treat the problem once it arises, while minimal research has been done on developing a preventative measure beyond wearing proper personal protective equipment (PPE) when you know you are around it, but what happens if you are unaware of the presence? In LA, California, they are sitting in this exact seat from the aerosolized arsenic content found in the ground and air from Owens Dry Lakebed, they are unaware of the toxic, carcinogenic arsenic they ingest on a consistent basis; this needs to be addressed and the problem needs to be fixed, not ignored. This leads us to our study from Metabolic Studios, and their photo development site at Owens Dry Lakebed. Our objective is to test the hypothesis that microbes found associated with the Owens Lakebed photo development process can be grown and used to decrease the amount of highly toxic, inorganic arsenic and convert that to less toxic, organic arsenic. If this could be used to decrease the amount of arsenic overall, our world would become a less toxic place for life to continue to thrive without hindrance. Some prior research has proven and showed that we are aware of microorganisms that are capable and could metabolize arsenic in their everyday lives, and that produce an organic form of it. The organic form is less toxic to life that has yet to adapt to these environments. The focus of this study is purely testing to see if in Owens Lakebed, photo development site, there are microorganisms that live in this area that perform this act and if it could be used as a form of bioremediation for Owens Lakebed, as well as other hyper-salinized areas tainted by toxic, inorganic, arsenic. Our approach will test the questions: 1) in areas of high arsenic concentrations, the higher presence of microorganisms that process and metabolize arsenic will have a greater biodiversity, opposed to areas without those microbes? And 2) can we suggest bioremediation as a plausible alternative to what we are using and doing today?

Merit: This study will be valuable to everyone, but it will also be extremely valuable to microbiologists, environmentalists, and many other persons involved in land and water management systems. It is unique for multiple reasons, one reason is that we are melding and playing in the world of transdisciplinary work, solving problems while creating and developing art. A second reason our work is unique is because we may be able to solve problems in toxic areas, using and promoting life in those areas to clean up the mess, like how certain microorganisms can clean up oil disasters. Such an approach sets the stage to a whole new world of potential for cleaning up our polluted world. And this may just be the key to opening that gate, to both worlds of transdisciplinary work and bioremediation.

Broader impact: By doing this research, we will better understand the chemical impact of this area on life and microorganisms living in this world of injustice. This will also show and support the movement that we may be able to lower the numbers to be less risky and overall increase the health of those living in these areas. Our research will act as an informant to many people living in the Inyo County of California, as well as those involved in the clean-up of this area. We will also be able to bring to light the importance of transdisciplinary work, through showing the use of working with experts, undergraduates, and high school students to combine efforts to find solutions to problems within our world.

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Project Description

Statement of Problem Significance

Older, improperly reclaimed land and improper actions taken while mining have led to toxic elements surfacing on our planet and leading to life being affected in many unexpected ways, examples of this include plants being unable to grow, animals being unable to live, and organisms being unable to thrive in these areas. Owens Lake, California was once a beautiful blue lake, fed by natural sources, till early 20th century when the LA aqueduct was built and began to redirect the water that fed the lake, to the aqueduct (Reheis, 2016). As a result of this, Owens Lake ended up drying up and is now a large source of aerosolized carcinogens, and of those carcinogens, arsenic is of highest concern. This is due to many factors, one of those factors is that out of 300,000 tons of PM-10, 30 tons of this particulate matter is aerosolized arsenic (Biland, 2017). The concern with Arsenic is it is a natural metalloid chemical that can "occur in all rock, air, water, and soil." Unfortunately, for most plants, animals, and other forms of life it causes certain elements to become displaced within the cells of that lifeform, causing changes to the cells structure, such as conformations with the surface proteins, causing a change in the cells functions and ability and ultimately damaging the host (Paddock, 2018). This has aroused the concern for the population in Los Angeles, CA, as well as other cities and populations facing similar aerosolized toxins coming from toxic elements of the Earth. Although these can be considered toxic to most life forms, it isn't entirely true for many bacteria and other microorganisms as they are capable of metabolizing and using arsenic in their habitats and environments to survive. Most of these organisms will metabolize arsenic into different "less toxic" forms (Kabiraj et al., 2022). This is being studied and researched from multiple platforms and approaches, one side coming from Metabolic Studios who is looking into the utilization of these microbes and elements in the area and applying them to the development and treatments of photography, and another approach coming from our research. Ultimately, allowing us to investigate cleaning up toxic areas and developing photos using life and chemicals found in this new world. This research and these studies on these microorganisms have several broad and useful implications across the world in all fields, this includes studying how microbes can be used in the development of beautiful pieces of art going all the way to using microbes to clean up toxic lands like Rocky Flats and Owens Dry Lakebed. This may be the door to a whole new world of bioremediation.

The goal for this research is to potentially solve a problem that the globe is facing in places where mankind has left a footprint that has grown to affect the life around those areas. These locations to name a miniscule few include Owens lakebed, the Great Salt Lake, the Dead Sea, and many others. The research will be done using Owens lakebed to find microorganisms living in the water and soil and see how they are either capable of metabolizing the arsenic, or how they are able to withstand its toxic effects (Kruger et al., 2013). And then it will be taking measures to either improve the quantity of microorganisms in those areas to clean up the toxins, or to incorporate the study into some other form to allow for the clean-up of these locations without taxing the Earth any more than we already have.

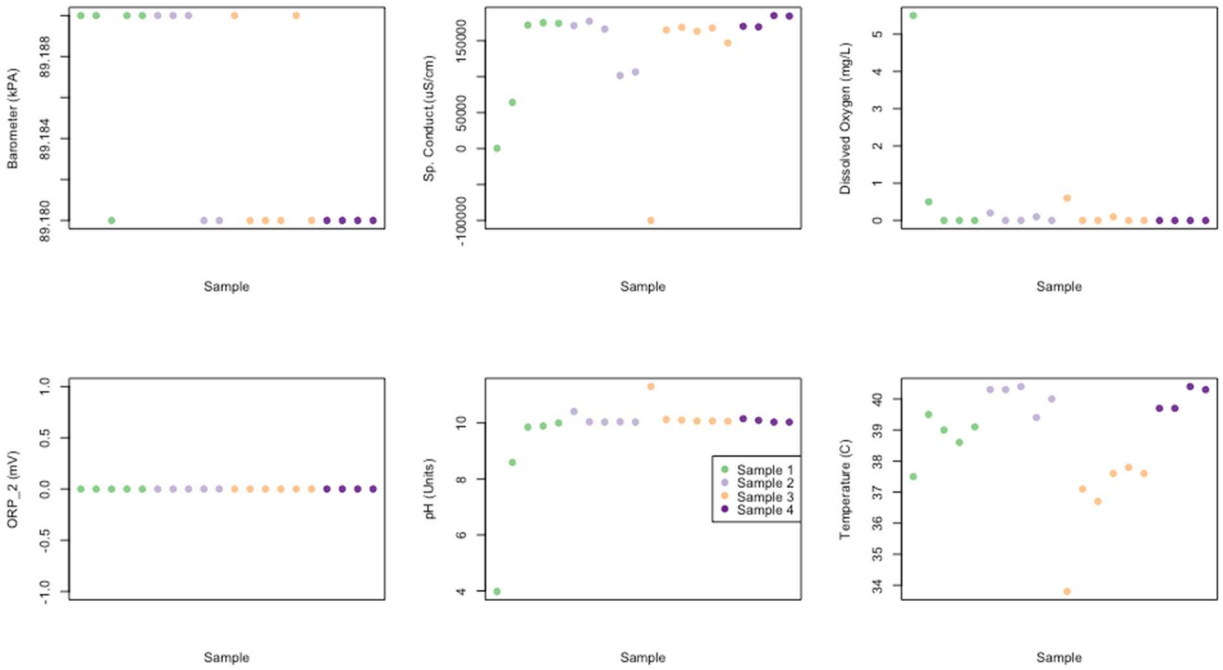
Introduction

Relevant Literature

Since before we knew what it was, arsenic has been a large concern for many reasons. These reasons include its ability to be used as a poison and its ability to be found in just about everything we live off on a day-to-day basis. But this concern is well earned, as arsenic is found in our waters, in the air, the ground, in soil contamination from mining and fracking as well as our lumber that is treated in arsenic coats (NIEHS, 2022). This has brought about the concern in how to remove and limit the exposure of mankind to arsenic as well as other toxic chemicals that is found naturally in our Earth's crust (WHO, 2018) . In 1994, studies were conducted to show the measurements of arsenic in the ground and soil and water there at Owens Lake. It was found that the mean arsenic concentration was 50 ppm and in the north and east of the lake it was as high as 150 ppm (Reheis, 2016). The concern with this was the particulate matter of the air and it read that the largest single source of PM-10 pollutant in the U.S. with a large portion containing aerosolized arsenic (Biland, 2017). In 2018 and to this day, the concern with arsenic is that short term exposure will cause minor health issues, long term exposure can lead to complications such as: cancer, liver disease, diabetes, and many other concerning medical conditions, because of this the desire to limit and reduce the amount of arsenic exposure to oneself has increased, especially in places such as Owens Lake (Paddock, 2018). Other places where arsenic poisoning and arsenic in the water sources has caused detrimental damage to would be the lakes and water around the Yellowknife area in Canada. This has caused fishing, harvesting, swimming, and consuming water in these areas to become unsafe as it clearly could pose to become a health hazard (Anonymous #1, 2019). This however has lead to a vast number of studies done from 1996 to 2007, where it has been found that "not only most living organisms have developed arsenic resistance mechanisms but some of them are able to utilize" arsenic in their respiration, redox reactions, and other physiological necessities (Paez-Espino et al., 2009). Now that there has been a connection between the arsenic content of Owens Lakebed, as well as it can be assumed that there are arsenic resistant microorganisms living in the area that potentially use this arsenic in their metabolism, that we may be able to find this organism and aim to find a bioremediation for this environmental disaster.

Preliminary Data:

The experts at Metabolic Studios, in partnership with the University of Wyoming Microbiology Program, initial testing of the site at Owens Dry Lakebed indicates the possible presence of halophiles and other extremophiles given the data below. These samples were taken from Owens Dry Lakebed, where the photographs being developed by Metabolic Studios had laid their photos. Little is known of the soil composition regarding the materials found in the soil such as metals, microbes, and other substances. It is known that the soil does contain several harmful components, but the amounts are unknown. This data helps to establish a rough picture of what may be found in the soil during the study and analysis of the soil and the microorganisms that may be found throughout the process.


Barometer (kPA)
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1
2
3
4
5
Sample
ORP_2 (mV)
-1.0
-0.5
0.0
0.5
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Sample
pH (Units)
4
6
8
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Sample 1
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Temperature (C)
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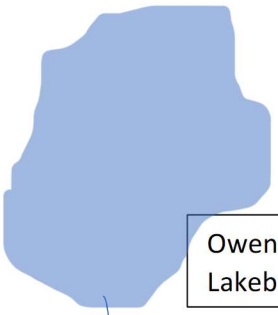
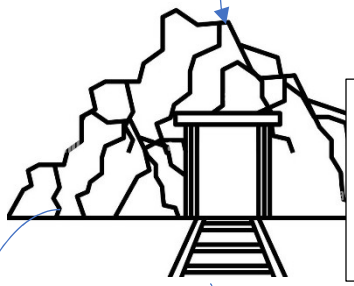
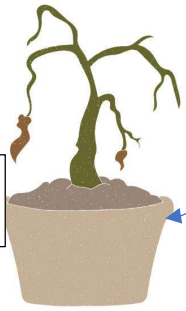
Concept Map:



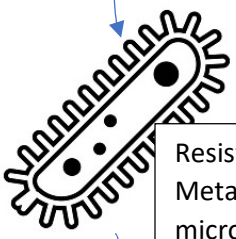
Owens Dry Lakebed



Mining and other related activities releasing arsenic and other chemicals



Toxic to most plants



Resistant/ Metabolic microbes

Toxic to humans and other animals

Bioremediation of these lands

Benefits for society

Justification of Approach

Our approach utilizes chemistry, microbiology, geology, and molecular biology standards for testing our hypotheses. By testing for the toxic metals (arsenic) in the soil and water samples is commonly done using atomic absorption spectra, and we have a lab who is willing to potentially run our soil and water samples to get this amount (Hill and Fisher, 2017). Polymerase chain reaction, or PCR will be used for the soil microbes and the microbial community as a field standard for reading and synthesizing DNA polymerase and this will be done according to methods by the National Center for Biotech Information (NCBI, 2017). We will also be using an E.Z.N.A DNA soil kit and water kit to be able to grab our DNA from our microorganisms so that we may use this in PCR to identify the microbes. This will be done according to the E.Z.N.A. Kit instructions.

Research Plan

Objectives:

- To determine if Owens Dry Lakebed has an impact on the microbial diversity within the soil and water.
- To determine if the soil and water contain a toxic amount of arsenic and other carcinogens and if that plays a role in the microbes capable of growing there.
- To inform the public about the risks of carcinogens involved in Owens Dry Lakebed, and other similar lakes, and allow them to take part in the remediation of the land that was impacted by a serious injustice.
- To evaluate the risks and benefits of bioremediation, and to find if there is a future in this form of remediation.
- To pave the way for giving justice back to the land, in the best and safest way possible.
- Highlight the vast benefits of transdisciplinary work, using it, to solve real world problems with people of many different backgrounds and expertise.

Hypotheses:

H1: The concentration of microorganisms will increase when pulled from arsenic contaminated soil and water samples.

H2: Microorganisms from Owens Dry Lakebed will have a natural resistance to arsenic and other toxic elements in the soil and water and have developed a way metabolize these elements and compounds.

H3: The concentration of arsenic in the soil will decrease as the number of microorganisms growing and living in the soil increase, due to their ability to potentially metabolize it.

Specific Aims:

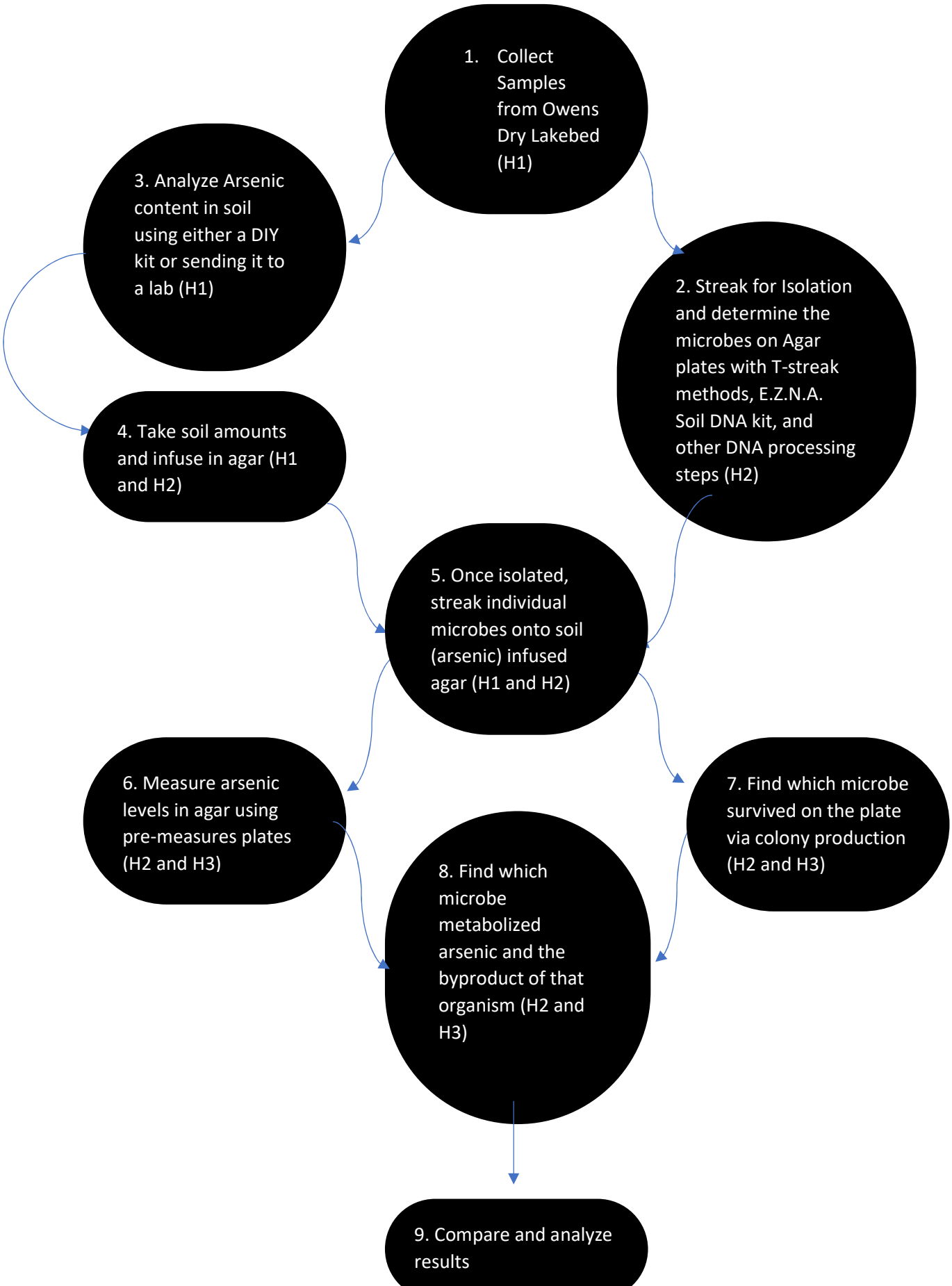
- We will determine difference between microorganisms and microbial communities from the soil samples and water samples taken from the photograph development sites by Metabolic Studios.
- We will determine the presence or absence of arsenic in both the soil and water samples and the amounts found in both.
- We will test the microbe's ability to survive on arsenic agar plates and find if the soil microbes or water microbes grow better with differing amounts of arsenic.
- We will suggest methods, and hopefully test methods to find if microbes capable of living where arsenic is found, are also capable of metabolizing this arsenic to a more stable, less toxic form.
- We will interface and collaborate with community members, community experts, students, and teachers to build and focus on civic engagement, and the importance of transdisciplinary work, and it impact on society.

Project Timeline:

Week	To Dos	Overarching Goals
0 (Sept. 26th-30th)	• Make media • Have finalized evolving draft complete • Order materials • Find recipe if not capable of ordering arsenic media • Streak for initial growth of microbes	Ensure that everything needed to complete lab is ordered, planned, and is capable of being executed (excluding time)
1 (Oct. 3rd – 7th)	• Streak for isolation • initial measurements of samples (pH, metal content, etc.) • Analyze soil microbes with E.Z.N.A Soil DNA kit • Clean, extract, PCR, and DNA sequencing	Getting started, we will need to streak for isolation and getting a
2 (Oct. 10th – 14th)	• Repeat/ complete week 1 tasks if needed • Find a way to create and plate the arsenic agar • Plate it, and prep it to be streaked on in the up coming weeks.	This will give us our baseline to work with and test our hypotheses.

3 (Oct. 17th – 21st)	• Once isolated, plate and streak microbes onto arsenic agar. • Let microbes grow and develop over the week to see if content changes.	We are looking to see if the microbes will grow and colonize or not. But if the do, if the arsenic levels will decrease over the span of time.
4 (Oct. 24th - 28th)	• Read the results of the agar content, via either a lab or self-test. • Re-peat week 3 to gather multiple forms of data • Find microbe capable of arsenic metabolism.	Get the agar plates read somehow and re-run to better support data and hypothesis.
5 (Oct. 31st – Nov. 4th)	• Begin poster and presentation to stakeholders	Prepare our presentation for stakeholders.
6 (Nov. 7th – 11th)	• Finalize report, poster, speech, etc.	Finalize our presentation for public audience.
7 (Nov. 14th – 18th)	• Communicate our findings and explain our poster.	Communicate our findings with the public.

Design Schematic:



Materials and Methods:

H1:

Soil and water samples will be collected from the -80ºC freezer, as the samples were collected in collaboration with University of Wyoming and Metabolic Studios, in LA, California. Soil and water samples from each photograph that was developed will be taken and sent to one of the community labs for atomic absorption spectrometry (AAS) as described by (Hill and Fischer, 2017). Soil and water samples will also be taken and will be ran through the E.Z.N.A. soil and water test kits as explained by omega biotech. These samples will then be cleaned and concentrated according to our ZYMO PCR Purification Kit. Once that has been completed we will be able to move to using the Nanodrop technique per their instructions, to determine the presence of DNA. Once shown proof of DNA presence, PCR will be done giving us our primers and sequences and this will be accomplished according to (NCBI, 2017). We will then read the sequences through the 16S barcode identifiers from Nanopore, per their instructions, and through the MinIon program we will be able to sequence the DNA, and tell what kind of microorganisms we have found in the samples pulled.

H2:

Once the microorganisms have been identified, research through the Web of Science will be used to find information on each microorganism found through the DNA extraction process. The organisms from the isolation of the T-streak method will be spread onto the agar containing a certain amount of soil correlating with the amount of arsenic that will be found and done according to H1. The agar plates will then be stored in the 37ºC container, as that seems to be a good average temperature for Owens Dry Lakebed over day and night. After 24 to 48 hours, they will be pulled out and documented which microbes were capable of growth on the sample infused plates.

H3:

After the time spent were the colonies have grown, the agar will then be autoclaved according to (*Autoclaving Procedures).* Once autoclaved, the agar will be taken and will be ran through our AJ-Low Arsenic Detectors by methods of (Chemsee) or through the community lab from initial arsenic reading. Taking initial data of soil samples and comparing those samples to post lab will be crucial in figuring out if these microorganisms are capable of arsenic metabolism. If this deems possible, further studies will be conducted to determine to what scale this would work for.

Analysis and Expected Results:

Analysis on the AAS results will be interpreted by our collaborators. We expect to find high amounts of inorganic arsenic in both the water and soil samples. We expect the samples to be relatively similar across the board from one photograph to another. Once this data is determined we believe that we will be able to isolate a fair number of microbes, which we will

place onto the agar arsenic or soil plates. It is expected that growth will occur across the board and that from the DNA analysis we will be able to know which bacteria, archaea, etc. is capable of this metabolism. Once this is determined we will follow through with the growth of the microbes on the agar plates, then those agar plates will be sent back to the lab through another level of AAS to read the arsenic content before and after microorganism growth and exposure. We expect to find that agar that had growth, will also have less arsenic content than what was initially placed on them through calculations of soil content and water content.

References with Annotations:

#1, A. (2017, August 11). *Polymerase chain reaction (PCR)*. National Center for Biotechnology Information. Retrieved September 24, 2022, from https://www.ncbi.nlm.nih.gov/probe/docs/techpcr/

Accessed through the Web of Science. This article discusses the process and role of PCR. This allows us to understand the role that it has on our tests and our results.

#2, A. (n.d.). *Autoclaving procedures*. Environmental Safety, Sustainability and Risk. Retrieved September 25, 2022.

Accessed through Web of Science. This article discusses the methods and procedures on how to autoclave.

Biland, L. (2017, February 14). *Air Actions, California*. EPA. Retrieved September 2, 2022, from https://19january2017snapshot.epa.gov/www3/region9/air/owens/qa.html

Accessed through the Environmental Protection Agency. This article discusses the air quality and air readings of Owens Lake. This article gives us and allows us to understand the PM-10 quality and the arsenic content in the air.

Hill, S., & Fisher, A. (2017). *Atomic Absorption, Methods and Instrumentation*. Elsevier Enhanced Reader. Retrieved September 24, 2022.

Accessed through the Web of Science. This article discusses the procedure to run Atomic Absorption. This allows us to understand that there are ways to read and measure the amount of metals in the soil and water samples.

Kabiraj, A., Biswas, R., Halder, U., & Bandopadhyay, R. (2022, March 15). *Bacterial arsenic metabolism and its role in arsenic bioremediation*. Current microbiology. Retrieved September 2, 2022, from https://pubmed.ncbi.nlm.nih.gov/35290506/#:~:text=Arsenate%20and%20arsenite%20are

%20mostly%20uptaken%20by%20bacteria,%28e.g.%2C%20reduction%2C%20oxidation%2C%20methylation%2C%20etc.%29%20into%20different%20forms.

Accessed through the Web of Science. This article discusses the ability of bacteria to metabolize arsenic and how it can potentially be used as a bioremediation for high arsenic contaminated sites. It allows us to understand that there are others looking similar research and that microorganisms can be used as a bioremediation technique. Cited by 1.

Kruger, M., Bertin, P., Heipieper, H., & Arsene-Ploetze, F. (2013, April 2). *Bacterial metabolism of environmental arsenic--mechanisms and biotechnological applications*. Applied microbiology and biotechnology. Retrieved September 2, 2022, from https://pubmed.ncbi.nlm.nih.gov/23546422/

Accessed through the Web of Science. This article discusses the metabolism of bacteria and their abilities to metabolize Arsenic through their own mechanisms. This article allows us to understand that there are microorganisms capable of metabolizing arsenic on their own and that it can be converted into something less toxic. Cited by 115.

Paddock, M. (2018, January 4). *Arsenic poisoning: Causes, symptoms, and treatment*. Medical News Today. Retrieved September 2, 2022, from https://www.medicalnewstoday.com/articles/241860

Accessed through Medical News Today. This article discusses the affects of arsenic on the human body and arsenic poisoning. This article allows us to understand just how many lives are potentially at risk in this world due to the arsenic content in their area and why some form of remediation is needed.

Paez-Espino, D., Tamames, J., de Lorenzo, V., & Canovas, D. (2009, January 7). *Microbial responses to environmental arsenic - biometals*. SpringerLink. Retrieved September 2, 2022, from https://link.springer.com/article/10.1007/s10534-008-9195-y#ref-CR45

Accessed through Web of Science. This article discusses how microbes have built a natural response to arsenic, whether that response is resistance or the ability to metabolize it they have that ability. This allows us to prove and show that in a world full of toxins bacteria are capable of living and surviving in these areas and can be used to potentially clean them up. Cited by 246.

Reheis, M. (2016). *A human-induced dust problem*. Owens (Dry) Lake, California. Retrieved September 2, 2022, from https://geochange.er.usgs.gov/sw/impacts/geology/owens/

Accessed through U.S. Geological Survey. This article discusses the problem developed at Owens Lake and how this has problem has grown and developed as humans have grown in the area. This article allows us to trace the history of Owens Lake to understand what occurred to benefit or cause the problem with carcinogens in the air and ground.

Services, H. and S. (2019, July 5). *Arsenic in Lake water around Yellowknife*. Arsenic in Lake Water Around Yellowknife. Retrieved September 2, 2022, from https://www.hss.gov.nt.ca/en/newsroom/arsenic-lake-water-around-yellowknife

Accessed through Government of Northwest Territories. This article discusses the problem of arsenic in many of the water sources and grounds near Yellowknife, Canada. This allows us to understand that the problem Owens Lake isn't the only place this problem has developed and thus providing more reason for this research to advance.