Analyzing Microbial Communities and Co-Selecting for Resistance to Landfill Contaminants and Antibiotics in Soil Bacteria in Riverton Wyoming

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October 24th, 2019

Learning Actively Mentoring Program

University of Wyoming

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Project Summary

The Riverton Landfill has been identified as a high priority landfill in Wyoming, as levels of certain metals and organic compounds in its leachate have been found to be above Wyoming Department of Environmental Quality-Solid and Hazardous Waste Division (WDEQ-SHWD) groundwater protection standards (Inberg-Miller, 2018). This project was inspired by collaboration with the Riverton 7th grade teachers and students who have an interest on how this landfill is impacting their community. One aspect of the leachate that has not been tested at the Riverton site is its impacts on the soil's microbiology. Antibiotic resistance has become a difficult problem in human and animal disease treatment. While it is known that heavy metal resistance and antibiotic resistance are frequently co-selected in bacteria, co-selection for tolerance to common organic pollutants, such as polyvinyl chloride (PVC) and antibiotic resistance has not been assessed. This leads us to hypothesize that a shift in the soil microbial community will correlate to concentrations of organic and inorganic contaminants in the soil from the leachate.

The objective of our study is to identify the microbial communities within and at radial distances away from the landfill to determine if and how the microbial communities have changed. We will obtain samples from the landfill refuse area as well as three sites at distances radially away from the landfill refuse area. From each sample, we will extract the total microbial DNA. These DNA samples will be processed and sent to the Wyoming Department of Public Health Labs for sequencing. We aim to determine the microbial community within the soil and water using PCR and DNA sequencing, and metal and PVC concentration using atomic absorption spectra (AAS) and gas chromatography (GC) respectively. Finally, bacteria that are tolerant to iron, manganese, cadmium or PVC will be selected for using media containing varying concentrations of these components. Out of these selected microbes, antibiotic resistance will be determined using Kirby-Bauer assays. Further, we predict that there will be higher concentrations of iron, manganese, cadmium and PVC tolerant bacteria; and proportionally higher concentrations of antibiotic resistant bacteria closer to the refuse area.

Intellectual merit: This research will help fill gaps in knowledge about the effects of common organic contaminants on antibiotic resistance. Furthermore, this research could impact the Riverton community view on waste creation and removal methods, and how older landfills operating under these outdated EPA regulations can be a danger to the community and surrounding environment.

Broader impacts: By doing this research, we will better understand not only the chemical hazards of the landfill, but also the potential microbial dangers it poses. A change in soil or water communities can translate to human health in Riverton. Our research will inform Riverton community members, including the Riverton 7th grade teachers and students of the potential risks involved with the landfill and allow them to be active participants in its remediation. If we can isolate heavy metal and PVC degrading microorganisms from the soil and/or water, we can suggest bioremediation methods.

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Project Description

Statement of Problem Significance

Older, improperly lined landfills are sources of many toxins that can leach into the surrounding environment with consequences for both ecological and human health (Teta, 2017). According to a 2015 report done by the Wyoming Department of Environmental Quality-Solid and Hazardous Waste Division (WDEQ), it was determined that the Riverton Landfill was 10th in a list of the eleven highest priority landfills in Wyoming (Inberg-Miller, 2018). The main goal of our work is to determine the effects of the landfill leachate on iron, manganese, cadmium and PVC concentration and its correlation to microbial community composition in the soil and water surrounding the refuse area. If we can isolate heavy metal and PVC degrading microorganisms from the soil and/or water, we can suggest bioremediation methods. Further, we aim to determine whether iron, manganese, cadmium and PVC correlate with rates of antibiotic resistance in soil bacteria. This research has broad implications across disciplines of ecology, microbiology, medicine, and sociology.

The data from this research will support future studies of other landfills with similar problems. While it is known that many heavy metals co-select for antibiotic resistance in the environment (Nguyen et al, 2019) this has not been assessed with many organic contaminants, such as PVC, that are ubiquitous anthropogenic contaminants. This will fill in an important gap of knowledge about waste management and the effects common contaminants have on soil microbial communities. The research will be done on a landfill that has been noted as a concern by the Wyoming Department of Environmental Quality-Solid and Hazardous Waste Division (WDEQ). Knowing what microbes are present and how they are changed by the landfill will allow for better landfill engineering, management, and reclamation. The results will be communicated to members of the Riverton community, landfill managers, and 7th grade students at Riverton Elementary through a poster presentation session. This will not only aid in dissemination of our data but will also encourage civic engagement and pride.

Introduction

Relevant literature

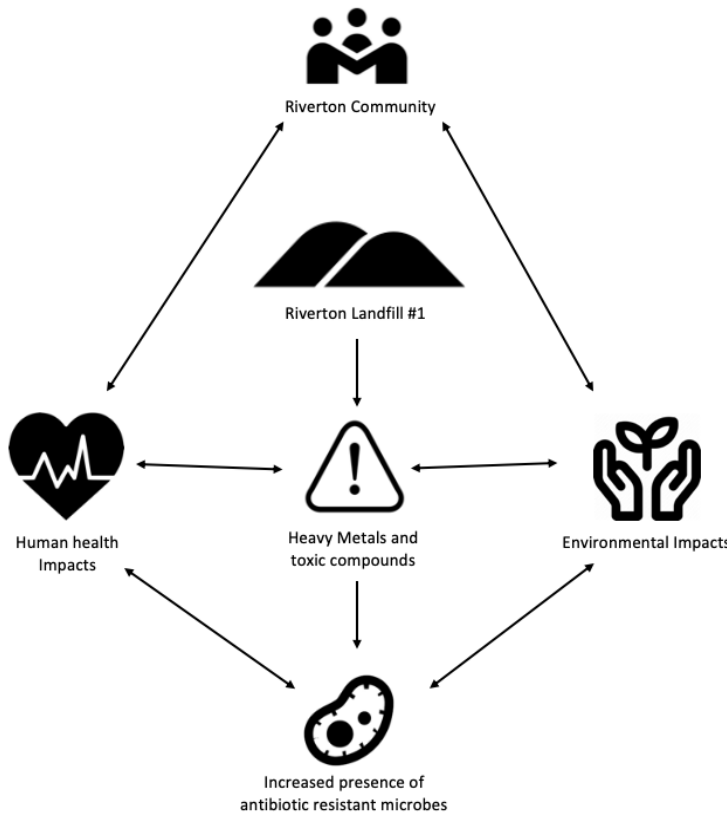
In 2018, groundwater monitoring wells were built to determine the quality of the ground water surrounding the Riverton landfill. It was found that there were a number of organic and inorganic parameters that are above Wyoming groundwater protection standards (Inberg-Miller, 2018). Among these contaminants were iron, manganese, cadmium, and PVC; all of which have important health implications. Iron and manganese are both essential for human physiology; however, at high concentrations they can cause serious health problems (O'Neil et al, 2015). Cadmium is inessential to human health and is toxic to humans and most microbes (Fazeli, 2010). A study done on the Jiaozho Bay in China showed that there was a strong correlation between heavy metal contamination and microbial communities when comparing contaminated and clean water samples (Yao et al, 2015). Other studies have shown that heavy metals co-select for antibiotic resistance in bacteria (Nguyen et al, 2019). PVC is a common soil contaminant of anthropogenic origin that is known to cause liver diseases and cancers (Thomas et al, 1975). A study found that when PVC is degraded in environments such as landfills or oceans, they can release dioxins (Garcia, 2016). Dioxins are persistent environmental pollutants which at levels higher than the background can cause reproductive complications, hormonal imbalances, and cancer (WHO, 2016). More recent studies have found that certain bacteria are capable of degrading PVC films (Giacomucci, 2019). When PVC has been degraded, vinyl chloride (VC) monomers are a significant pollutant that can cause conditions such as brain, liver, and lung cancer (NIH, 2018). Though the connection between heavy metal resistance and antibiotic resistance has been well studied, most literature focuses on metals such as zinc, copper, cadmium, and mercury. There has not been a thorough assessment of iron and manganese and no work has been done on PVC induced antibiotic resistance. We aim to elucidate these interactions if there are any.

Preliminary Data

The Inberg-Miller Engineers initial testing of the site indicates the presence of iron, manganese, vinyl chloride, and other organic salts at levels above Wyoming groundwater standards (Inberg-Miller, 2018). These samples were taken from a test site located immediately outside of the landfill refuse area. Little is known about the composition of the soil prior to the landfill leachate reaching the soil. Some of the most recent soil composition reports done by the department of agriculture predate the landfill's use (USDA, 1974). This informs our work in the following ways, it helps establish what the chemical composition of the soil before and after the landfill was used. This can help us establish if the leachate is increasing concentrations of heavy metals and vinyl chloride from PVC or if that was there prior to the landfill.

Parameter	Groundwater Protection Standard (mg/L)	Average (mg/L)	High (mg/L)	Low (mg/L)
Iron	0.3	15.7321	258	0.06
Manganese	0.05	2.7605	38.5	0.01
Vinyl chloride	2	2.6	20	0.1
Cadmium	0.005	0.0064	0.918	0.00007

Concept Map



Justification of Approach

Our approach uses chemistry, microbiology, and molecular biology standards for testing our hypotheses. Testing for heavy metals in environmental samples is commonly done using atomic absorption spectra (AAS) and we have a current published article that uses GFAAS for testing heavy metals in sediments (Yao et al, 2017). Gas chromatography is the method used to determine VC concentrations in many of the journals that are measuring VC in culture samples (Lintner, 2014). Polymerase chain reaction denaturing gradient gel electrophoresis (PCR-DGGE) for soil microbial community analysis is a field standard (Varma et al, 2007). For the Kirby-Bauer assays we will be using Muller-Hinton agar as it allows proper antibiotic diffusion, diverse bacterial growth, and is supported in most literature (Reller, 2009).

Research Plan

Objectives

- To determine if the Riverton Landfill #1 has an impact on the microbial diversity within the soil.
- To determine if soil and leachate with elevated heavy metals and organic polymers will contain a higher presence of heavy metal and organic polymer tolerant and antibiotic resistant bacteria.
- To inform citizens of the potential risks involved with the landfill and allow them to be active participants in remediation of their community.

- To understand the benefits of involving student learners and educators in researching and implementing solutions.
- To provide our stakeholders including city of Riverton, Inberg-Miller Engineering Firm and the Riverton Community with potential suggestions for remediation.
- To lead to future research in landfill reclamation and management.
- To evaluate the risks and benefits of different landfill reclamation options.

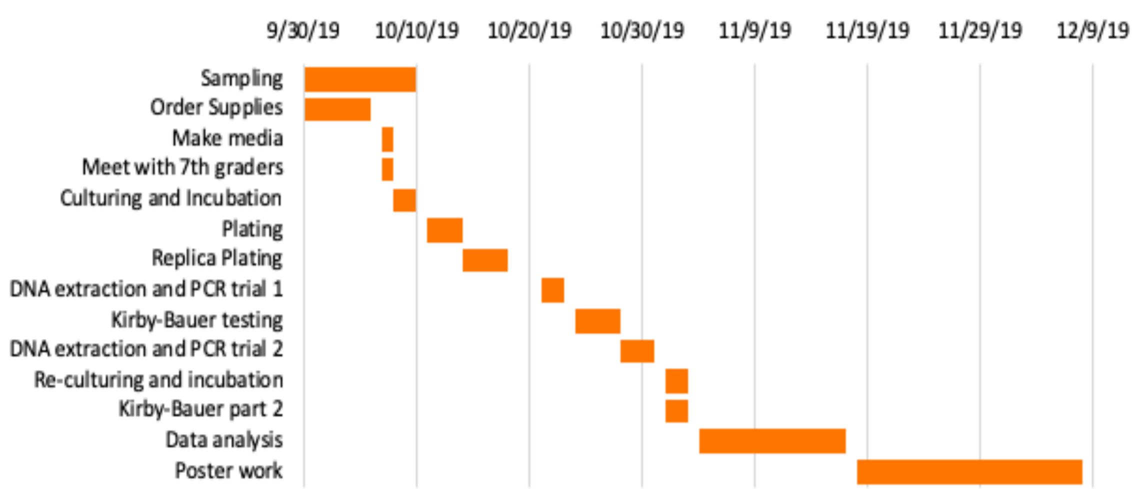
Hypotheses

H1: The concentration of manganese, iron, cadmium and vinyl chloride will be higher in soil closer to the refuse area and less prevalent with radial distance away from the refuse area.
H2: Leachate from the Riverton Landfill is shifting the microbial communities of the surrounding soil as compared to unaffected soil in the surrounding area.
H3: The prevalence of iron, manganese, cadmium and PVC tolerant microbes will be higher in soil closer to the refuse area and less prevalent with radial distance away from the refuse area.
H4: The prevalence of antibiotic resistant bacteria will be higher in soil closer to the refuse area and less prevalent with radial distance away from the refuse area.

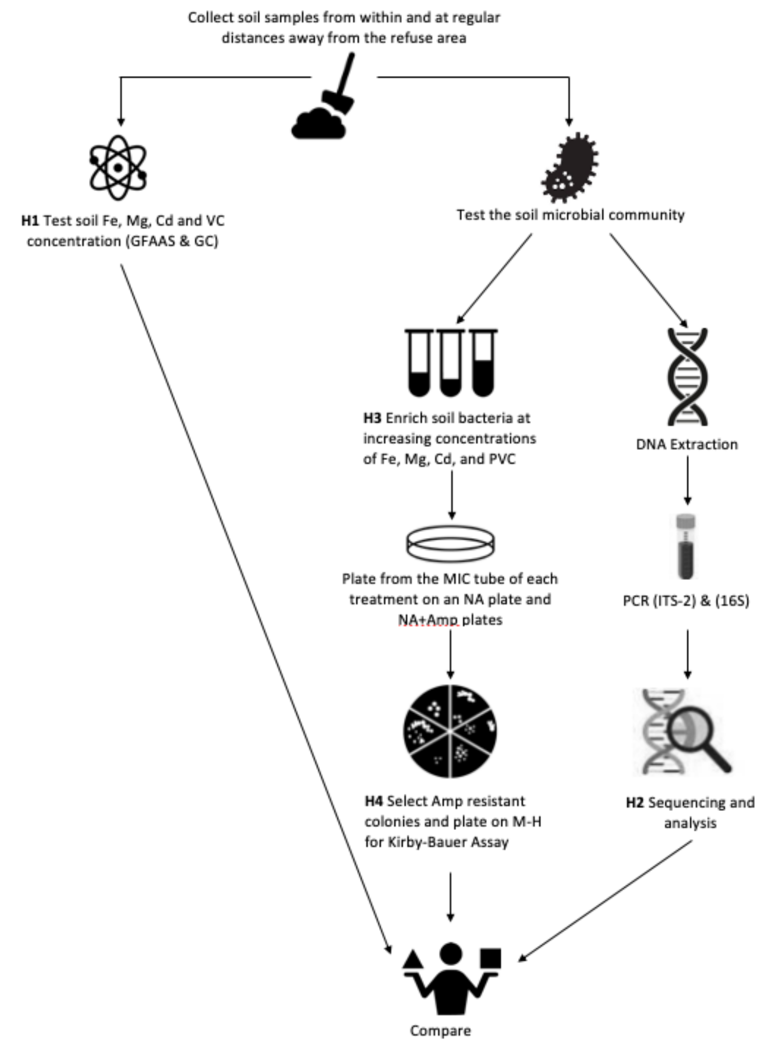
Specific Aims

- We will determine differences between microbial communities in soil samples within and at regular radial distances away from the landfill refuse area.
- We will determine the presence or absence of iron, manganese, cadmium and vinyl chloride tolerating microbes within and at regular distances away from the landfill refuse area.
- We will determine whether iron, manganese, cadmium, or PVC select for antibiotic resistance in soil bacteria.
- We will suggest methods to enrich iron, manganese, cadmium, and PVC degrading microbes for bioremediation of landfills.
- We will interface with community members including city officials, students and teachers, and the media to build a sense of civic engagement and pride.

Project Timeline



Design Schematic



Materials and Methods

H1

Soil samples will be collected alongside the Inberg-Miller Engineers from the Riverton #1 Landfill site. They will be collected from test pits P-49, P-19, and P-50 (Inberg-Miller, 2018). Three soil samples from each site will be taken from the top 5 cm of surface soil and placed into sterile whirl pack bags. Iron, manganese, and cadmium concentrations will be determined by atomic absorption spectrometry (AAS) as described by (Yao et al, 2017). PVC concentration will be determined using gas chromatography (GC) with flame ionization detection as described by (Lintner, 2014).

H2

Total soil DNA will be extracted using Omega Bio-Tek E.Z.N.A Soil DNA kits according to the manufacturer's protocol from 0.5g (wet weight) of soil sample. Yields of DNA will be determined using electrophoresis on a 0.8% agarose gel as described by (Yao et al., 2017). DNA samples will be sent to the Wyoming Department of Health Microbiology Lab for 16s and ITS-2

PCR to determine bacterial and fungal soil community composition. DGGE will be performed to separate the PCR products as described by (Yao et al, 2017) to get denaturing gradient gels. High-resolution scans will be taken of the denaturing gradient gels and analyzed using GELCompar II software to analyze the community fingerprints of each gel to determine the relative abundance of each phylotype as described by (Yao et al, 2017)

H3

1 g of soil from each sample will be put into 50 mL falcon tubes and filled to 10 mL with phosphate-buffered saline (PBS) and shaken for 30 seconds. 1 mL aliquots of the soil-PBS mixture from each sample will be transferred into twenty-five NB tubes each, with increasing amounts of iron ($FeSO_4$), manganese ($MgSO_4$), cadmium ($CdCl_2$) and PVC powder added while five tubes with no addition will be used as controls. Concentrations of each will be as follows:

	FeSO4 g/100mL	MgSO4 g/100mL	PVC g/100mL	CdCl2 g/L
1	0.013	0.013	0.100	0.009
2	0.025	0.025	0.200	0.018
3	0.050	0.050	0.300	0.092
4	0.100	0.100	0.400	0.147
5	0.113	0.113	0.500	0.183

These concentrations were determined by looking at previous findings at maximum and minimum concentrations of heavy metals (Alnaimat et al, 2017) (Patil, 2012).
NB tubes will be incubated until the tube with the highest concentration of contaminant that is able to show turbidity is determined. From these tubes, 0.1 mL will be taken and put into a tube of 0.99 mL PBS, mixed, and 0.1mL from that tube will be put into separate tube of 0.99 mL of PBS which will be repeated one more time to make a dilution scheme. 0.1 mL of inoculant from the final diluted inoculant-PBS tube will be spread on an NA plate as well as three NA+Ampicillin (Amp) plates and incubated for 48 hours at 31 °C.

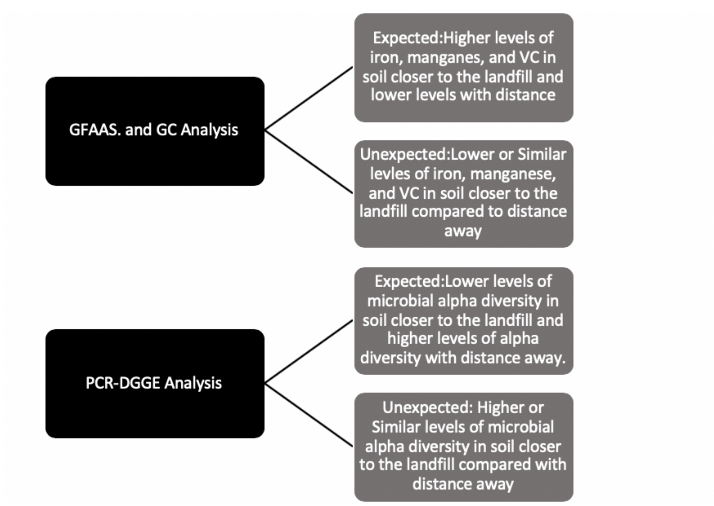
H4

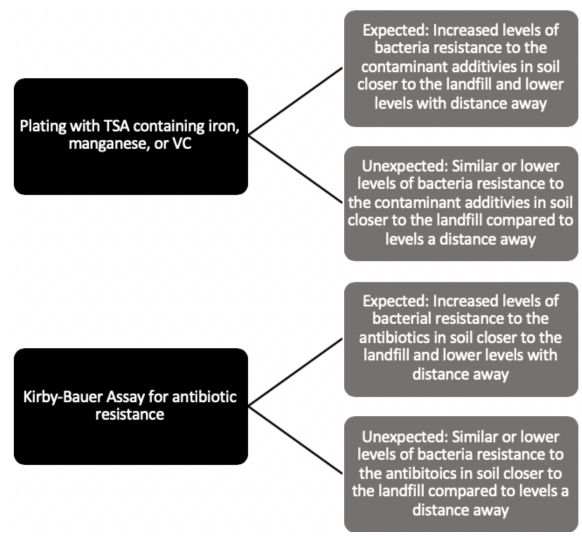
One to two of the most prevalent colony morphologies from the NA+Amp plate will be used to inoculate the entire surface of three Muller-Hinton (MH) plates each. The MH plates will then be divided into four quadrants, each with a different antibiotic diffusion disk containing each of the following: carbapenem, azithromycin, trimethoprim, and sulfa drugs. Plates will be incubated at 37 °C for about 48 hours. Using a table of standard zones of inhibition for each antibiotic, resistance and sensitivity will be determined for each isolate.

Analysis and Expected Results

Analysis on the AAS and GC results will be interpreted by or collaborators. We expect to find higher concentrations of iron, manganese, cadmium and PVC closer to the refuse area and lower levels with distance from the landfill. Iron, manganese, cadmium PVC, and antibiotic resistance will be compared by using colony counts and calculating iron, manganese, cadmium and PVC tolerant colonies over total CFUs on non-selective NA plates to determine the percent of bacteria with tolerance. These data will be used to determine whether higher concentrations of iron, manganese, and PVC correlates to higher levels of tolerance to the heavy metals and organic compound and if that increased rate of antibiotic resistance. We do not expect that higher levels of contaminants would decrease tolerance or decrease antibiotic resistance or to not affect these measures. We expect that higher levels of our leachate contaminants of interest will correlate to higher levels tolerance for them and increased levels of antibiotic resistance. Our PCR-DGGE analysis will be done with the help of collaborators such as Amy Rhoad (a PhD student in Microbiology), who is certified in instructing bioinformatics. We expect there to be shifts in the microbial community correlated to the higher levels of contaminants. We do not expect the microbial communities to stay the same or that alpha diversity will increase closer to the landfill. Shifts may be in alpha diversity with lower diversity closer to the landfill and higher diversity with distance from the landfill.

Cellular samples will be stored with glycerol cryoprotectant in 1 mL cryovials in a -80 ºC freezer. Data from chemical and genotypic analysis will be stored on Google Drive. Short-term culture storage will be refrigerated at 4 ºC.





List of References with Annotation

Alnaimat, Sulaiman, Saqr Abushattal, Osama Althunibat, Eid Alsbou, and Reda Amasha. "Iron (II) and Other Heavy-Metal Tolerance in Bacteria Isolated from Rock Varnish in the Arid Region of Al-Jafer Basin, Jordan." *Biodiversitas, Journal of Biological Diversity*18, no. 3 (January 2017). https://doi.org/10.13057/biodiv/d180350.

Accessed through Web of Science. This article determines the minimum and maximum quantities of iron sulfate and manganese sulfate that it takes to culture bacteria from samples. This allows us to grow the bacteria from our soil samples and add concentration to determine heavy metal resistance. Cited by 5.

Fazeli, M., P. Hassanzadeh, and S. Alaei. "Cadmium Chloride Exhibits a Profound Toxic Effect on Bacterial Microflora of the Mice Gastrointestinal Tract." *Human & Experimental Toxicology*30, no. 2 (2010): 152–59. https://doi.org/10.1177/0960327110369821.

Accessed through Web of Science. This article describes the health effects of cadmium on bacteria in our gut as well as for human health. This can help determine the effect of cadmium leachate getting into the environment. Cited by 41

García, Jeannette M. 2016. "Catalyst: Design Challenges for the Future of Plastics Recycling." *Chem*1, 6, 813–15. https://doi.org/10.1016/j.chempr.2016.11.003.

Accessed through Web of Science. This article discusses the degradation of PVC in the environment, the chemicals it makes, and by-products of the reaction. This shows us the process of PVC degradation that may be happening in the landfill. Cited by 18.

Giacomucci, Lucia, Noura Raddadi, Michelina Soccio, Nadia Lotti, and Fabio Fava. "Polyvinyl Chloride Biodegradation by Pseudomonas Citronellolis and Bacillus Flexus." 2019. *New Biotechnology,* 52, 35–41. https://doi.org/10.1016/j.nbt.2019.04.005.

Accessed through Web of Science. This article discusses the degradation of PVC film by different bacteria. If we can replicate the results with bacteria from the landfill refuse area. We can use this information to culture the bacteria that we might find in the landfill. Cited by 1.

Hassen, A., N. Saidi, M. Cherif, and A. Boudabous. "Resistance of Environmental Bacteria to Heavy Metals." *Bioresource Technology*64, no. 1 (1998): 7–15. https://doi.org/10.1016/s0960-8524(97)00161-2.

Accessed through Web of Science. This is a journal done on the effects of heavy metals such as cadmium had on the bacteria in the environment. We can use this article to determine the concentrations of cadmium in which the bacteria are resistant or sensitive. Cited by 317.

Inberg-Miller Engineers. 2018. "Supplemental Report of Corrective Actions." *Assessment of Corrective Actions Riverton #1 Landfill SHWD #10.215.*

Provided by the Inberg-Miller Engineers. This is a recent report done on our specific landfill that provides an assessment of what kinds of contaminants we can expect to find in the Riverton landfill. This will help guide us in what we expect to have an effect on microbial communities in the soil and water surrounding the landfill.

Lintner, Carly Faye. 2014. "Exploring the diversity of vinyl chloride assimilating bacteria in enriched groundwater cultures." *University of Iowa*, https://doi.org/10.17077/etd.wx2m4vxc

Accessed through Google Scholar. This article shows that there are environmental bacteria capable of assimilating vinyl chloride in groundwater cultures, and illustrates potential culturing methods as well as what bacteria we are most likely to find. This information will assist in guiding our research of the impact of environmental bacteria on polyvinyl chloride (PVC), and guide our methods as we prepare our samples.

Nguyen, Christine C., Cody N. Hugie, Molly L. Kile, Tala Navab-Daneshmand. 2019. "Association between heavy metals and antibiotic-resistant human pathogens in environmental reservoirs: A review." *Frontiers of Environmental Science & Engineering,* 13:46. https://doi.org/10.1007/s11783-019-1129-0

Accessed through Google Scholar. This article describes the association between heavy metals and antibiotic resistance in human pathogens in environmental reservoirs. We may want to look for whether the bacteria that are co-selected for heavy metal resistance and antibiotic resistance are pathogens, so this would be a useful article to refer back to. This article also focuses on heavy metals as co-selection agents that could promote antibiotic resistance, which is pertinent information that would be useful in our research.

NIH. 2018."Vinyl Chloride - Cancer-Causing Substances." *The National Cancer Institute,* https://www.cancer.gov/about-cancer/causes-prevention/risk/substances/vinyl-chloride.

Accessed through National Institute of Health archives. This articles shows the potential health effects that vinyl chloride has on humans. We can use this information in order to make sure that each sampler is safe from the effects of vinyl chloride that is in the landfill.

O'Neal, Stefanie L., and Wei Zheng. 2015. "Manganese Toxicity Upon Overexposure: a Decade in Review." *Current Environmental Health Reports*, 2:3, 315–328. doi:10.1007/s40572-015-0056-x.

Accessed through Google Scholar. This article describes the effects of manganese toxicity in humans and gives background information for the importance of studying this landfill. This information will be utilized in our research as we explore the potential health risks associated with the Riverton Landfill toxins. Having this knowledge of manganese toxicity on human health will give us a clearer understanding of the greater sociological impact of the landfill.

Patil, Rajashree. "Isolation of Polyvinyl Chloride Degrading Bacterial Strains from Environmental Samples Using Enrichment Culture Technique." *African Journal Of Biotechnology*11, no. 31 (2012). https://doi.org/10.5897/ajb11.3630.

Accessed through Google Scholar. This article tells us the maximum and minimum concentrations of PVC that is tolerated by bacteria. This gives us information on what concentration of PVC in our media to grow the vinyl-chloride resistant bacteria.

Reller, L. B., Melvin Weinstein, James H. Jorgensen, Mary Jane Ferraro. 2009. "Antimicrobial Susceptibility Testing: A Review of General Principles and Contemporary Practices." *Clinical Infectious Diseases*, 49:11, 1749–1755. https://doi.org/10.1086/647952

Accessed through Google Scholar. This article describes antibiotic susceptibility testing using Muller Hinton agar as a standard. This article will be utilized in our research as we are using Muller Hinton agar in our sampling. The information in this article will be useful when we

are testing our samples for antibiotic susceptibility on Muller Hinton agar and need to refer back to commonly used methods and outcomes. Cited 1022 times.

Stepanauskas, R. , Glenn, T. C., Jagoe, C. H., Tuckfield, R. C., Lindell, A. H., King, C. J. and McArthur, J. V. 2006. "Coselection for microbial resistance to metals and antibiotics in freshwater microcosms." *Environmental Microbiology,* 8: 1510-1514. doi:10.1111/j.1462-2920.2006.01091.x

Accessed through Google Scholar. This article shows that heavy metals select for antibiotic resistance. This is pertinent to our study of iron and manganese resistance and antibiotic resistance as we are funneling our research to focus on co-selection of antibiotic resistance and metal resistance. 136 citations.

Teta, Charles, and Tapiwa Hikwa. "Heavy Metal Contamination of Ground Water from an Unlined Landfill in Bulawayo, Zimbabwe." 2017. *Journal of Health and Pollution*, 15, 18–27. https://doi.org/10.5696/2156-9614-8.15.18.

Accessed through Web of Science. This article describes the leaching of landfill degradation in landfills without liners. This is information will utilize to understand what conditions are in the landfill prior to us sampling any soils from it. Knowledge is important to ensure safety in taking samples. Cited 8 times.

Thomas, Louis B., M.D., Hans Popper, M.D., Ph.D., Paul D. Berk, M.D., Irving Selikoff, M.D., and Henry Falk, M.D. 1975. "Vinyl-Chloride-Induced Liver Disease from Idiopathic Portal Hypertension (Banti's Syndrome) to Angiosarcomas." *New England Journal of Medicine*, 292:1, 17–22. doi:10.1056/nejm197501022920104.

Accessed through Google Scholar. This article describes liver diseases caused by vinyl chloride and gives background information for the importance of studying this landfill. This information will be utilized in our research as we further our knowledge of potential health risks associated with the Riverton Landfill. Knowledge of health risks associated with vinyl chloride are useful in our research as we explore the impact of polyvinyl chloride, a polymer of vinyl chloride.

USDA. 1974. "Soil Survey of Riverton Area, Wyoming." *United States Department of Agriculture*, 0–90.

Accessed through the United States Department of Agriculture Archives. This article talks about the chemical composition of the soil in Riverton, WY in 1974 which is closest chemical composition sample that we have to date.

Varma, Ajit and Oelmuller Ralf. 2007. "Advanced Techniques in Soil Microbiology." *Springer.*

Watson, Rachel M. 2017. "General and Medical Microbiology Laboratory Manual." *University of Wyoming. Creative Commons.*

WHO. 2016. “Dioxins and Their Effects on Human Health.” *The World Health Organization.* https://www.who.int/news-room/fact-sheets/detail/dioxins-and-their-effects-on-human-health.

Accessed through National Cancer Institute information database. This article talks about dioxins what they are and why they are a large health risk for those who are dealing with PVC pipe degradation. This information will inform us on pre-sampling condition of the landfill, it informs us of possible toxins that could hurt us in the landfill refuse area.

Yao, Xie-feng, Jiu-ming Zhang, Li Tian, Jian-hua Guo. 2017.” The effect of Heavy Metal Contamination on the Bacterial Community Structure at Jiaozhou Bay, China.” *Brazilian Journal of Microbiology,* 48. https://doi.org/10.1016/j.bjm.2016.09.007.

Accessed through Google Scholar. This study was done over a period of about a year showing the effects of heavy metals on the microbial communities of a Bay in china. This is exactly what we want to look at with our research of the Riverton Landfill. It has been cited about 12 times. This reference will guide us in potential sampling and analysis methods to determine the effect of heavy metals on the soil and water samples taken from the Riverton sites.