MICROBIOLOGY OF A RECLAIMED URANIUM MINE, LAGUNA PUEBLO, NM

By

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ABSTRACT

The Jackpile Mine near Laguna Pueblo is located in the New Mexico uranium belt and was once the world's largest open pit uranium mine. After the mine closed, it was reclaimed by the addition of soil on top of the mine tailings and leftover ore. This study aimed to characterize the microbial communities in the soil in relation to uranium concentrations and the effects of mining. Soil was sampled aseptically, DNA was extracted, and high-throughput metagenomic sequencing was performed at the National Center for Genome Resources. The results showed a wide range of typical arid soil microbes and a strong representation from metal reducers, e.g., *Geobacter* spp. There was also evidence that the relative abundances of some genera, including *Rhodopseudomonas, Bradyrhizobium,* and *Rubrobacter*, are affected by concentrations of nickel, selenium, and zinc. Uranium does not appear to be driving functional gene or phylogenetic differences among the six soils sampled.

Keywords: metagenome, uranium, reclamation, soil, bacteria

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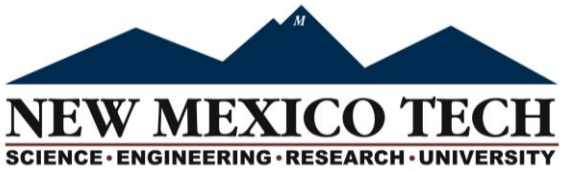
LIST OF ABBREVIATIONS AND SYMBOLS

µ	Micro
As	Arsenic
Ba	Barium
Bp	Base pair
Cd	Cadmium
cm	Centimeter
Cr	Chromium
Cu	Copper
DNA	Deoxyribonucleic acid
Fe	Iron
g	Gram (weight)
ICP-MS	Inductively coupled plasma mass spectrometry
JMP	"John's Macintosh Program" statistic analyzer and figure generator
km	Kilometer
MG-RAST	Metagenomic rapid annotations using subsystem technology
mL	Milliliter
Mn	Manganese
Mo	Molybdenum
Ni	Nickel

NMT	New Mexico Tech
OTU	Operational Taxonomic Unit
Pb	Lead
PCoA	Principle Component Analysis
Se	Selenium
SEED Subsystem	The database and infrastructure for comparative genomics
U	Uranium
U(IV)	Uranium, oxidation state four
U(VI)	Uranium, oxidation state six
US BIA	United States Bureau of Indian Affairs
US BLM	United States Bureau of Land Management
US EPA	United States Environmental Protection Agency
V	Vanadium
Zn	Zinc



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Olivia Chavez August 2016

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CHAPTER 1 : INTRODUCTION

It is evident that the human species is subject to an overwhelming bacterial abundance on Earth. Bacterial diversity greatly outnumbers eukaryotic diversity, and microbes are able to adapt to almost any environment. Microbes can thrive in conditions where eukaryotes cannot exist, such as hydrothermal vents (Muyzer et al., 1995), and can also withstand extreme radioactive conditions (Mattimore and Battista, 1996). As humans progress, we use and change our environments while exploiting resources in such extremes that it affects humans and many other organisms negatively (Hoekstra and Weidmann, 2014). One of these cases is mining, which is essential for the lifestyle of many humans, especially in developed countries. Mining methods and the hazards associated with them are fairly well understood, but occasionally accidents, such as spills, can occur, and can cause extreme shifts in ecosystem chemistries. Mining misfortunes can be in the form of heavy metal or acid spills, structural collapse or reclamation failure (Solà et al., 2004; Emad et al., 2014; Artiola et al., 2016). Shifts in ecosystem chemistries can occur not only with mining misfortunes, but also from the process of reclaiming a mine.

In this study, the microbiology of surface soils at a reclaimed open pit uranium mine at Laguna Pueblo was investigated. Previous research regarding heavy metal contamination within soils has shown changes in the bacterial communities (Chodak et al., 2013) and overall soil chemistries (Romero-Freire et al., 2016). What has not been thoroughly investigated is the aftermath of heavy metal mining and reclamation on microbial soil communities. To our knowledge, there are no previous soil microbial studies conducted on reclaimed uranium mines. This study aimed to characterize the microbial communities in a reclaimed uranium mine at Laguna Pueblo, the Jackpile Mine.

Heavy metals are naturally present in many soils. Occasionally, with mining of these metals, the concentrations can increase in the surrounding environments when mining operation procedures are not in place or adhered to or if reclamation of the mine is not completed properly. Due to the nature of the element, the uranium mining industry faces several problems when it comes to environmental safety and reclamation. Uranium has three major isotopes: uranium-234, uranium-235, and uranium-238, with the bulk of naturally occurring uranium being uranium^{238}. This metal decays slowly, emitting alpha particles, and small amounts of gamma particles. In addition, the isotopes have a half-life range of about 0.7-4.5 billion years, depending on the isotope. Uranium is found primarily in two oxidation states: uranium (IV) and uranium (VI). In addition to its radioactive characteristic, uranium (VI) occurs mostly in a soluble state and can travel easily in water. The other form, uranium (IV) is generally in solid, mineral form, and thus immobile, but

can be transported by dust (Feng et al., 2015; Brown, 2015). Often, uranium is naturally found bonded with oxygen as uranium dioxide, and uranium trioxide. The most common form is triuranium octoxide.

Uranium contamination from mines and ore-processing facilities, including inadequately reclaimed former mines, is not unusual; this is evidenced by sites such as the Rifle Integrated Field Research Challenge site in western Colorado (Hug et al., 2015) and a large portion of the Navajo Nation (Arnold, 2014). When the environment is contaminated with heavy metal, plants and animals are affected negatively. The extent of the negative effects depends on dosage and chemical species of the metal. Heavy metal exposure, even in small amounts, may lead to a variety of health issues in humans, including disease and failure of kidneys and lungs (Rage et al., 2015). Further, the presence of metals at non-natural concentrations provides selection pressure on living organisms within that environment, ranging from macroorganisms to microorganisms (Hemme et al., 2010).

Although heavy metal contamination is often detrimental, many organisms are able to adapt to the new challenge. Some plants take up heavy metals, and there are a few species of bacteria that are able to metabolize and change the redox states of metals. In some cases, this natural metabolic process is useful in that it prevents the contamination from spreading. In the case of uranium, bacterial metabolic processes reduce the metal from its mobile to immobile state. Harsh environments select for microbes that are able to adapt and proliferate in extreme conditions, and eliminate those that cannot survive. Through metagenomic sequencing, one can determine the microbial species and the genes that promote adaption to a heavy metal environment. Mouser et al. (2009), who sequenced a specific bacterium that selectively reduces uranium, *Geobacter uraniireducens*, found that the genes superoxide dismutase (sodA) and cytochrome D ubiquinol oxidase subunit I (cydA) were upregulated in the presence of uranium contamination. Studies have found the genus *Geobacter* has been represented at several sites of uranium contamination (Tapia-Rodriguez et al., 2014), and that the phylum *Proteobacteria* dominates microbial communities in other heavy metal-containing or bearing soils (Chodak et al., 2013). Among others, *Proteobacteria,* are known to reduce uranium (VI) to uranium (IV), which is generally immobile. Importantly, the bacteria are able to reduce uranium, either directly or indirectly via Fe-reduction (Sani et al., 2005). If there are other, more energetically favorable electron acceptors present, they will most likely be reduced by bacterial metabolism. This, of course, depends on the phase the metal is in and the accessibility of metallic ions.

The site of this study is the Jackpile Mine at Laguna Pueblo, New Mexico. At its height, the Jackpile Mine was the world's largest open pit uranium mine (Ulmer-Scholle, 2015). Twenty five million tons of uranium ore were mined and shipped out of the Jackpile Mine (US EPA, 2015). The Jackpile Mine is in a semiarid region, where grassland and pinyon-juniper woodland are the original vegetation. It has recently been classified as an EPA superfund site because of a failed reclamation assessment (US EPA, 2015). In hopes of better reclaiming the site in the future, a New Mexico Tech research team is characterizing various aspects of the site, including its microbial communities. There are a few other U-rich sites whose microbial activity has been characterized with the aim to understand microbially-mediated uranium transformations. A mining site in Rifle, CO, where vanadium and uranium were processed, has also been reclaimed; ongoing research

is examining the effects of remediating contamination by using microbes (Campbell et al., 2011). This Colorado site, located in a mountainous region, has been found to be mostly oxidized (Campbell et al., 2011). The Jackpile Mine differs from this site in that it is in a semiarid desert rather than in a fluvial sediment and its uranium is a mixture of uranium (IV) and uranium (VI) minerals (Blake et al., 2016) with the former being soluble and easily transported. Further, the bacteria in the Rifle site were biostimulated by the addition of acetate. Interestingly, several bacterial species, including *Geobacter bemidjiensis* and *Desulfobacter postgateii*, were found to have increased rRNA production, demonstrating their ability to become active and grow when uranium concentrations increased within Rifle site groundwater microcosms (McGuiness et al., 2015).

To characterize the microbial communities of surface soils (reclaimed and thereafter undisturbed) of the Jackpile mine, aseptically collected soil core samples were taken from the reclaimed main mine pit and also from relatively undisturbed nearby sites; and these were metagenomically analyzed. These microbial results were compared to the soil chemistry data provided by a collaborative team which simultaneously characterized fifteen mine sites. This is the first study to characterize the microbial soil communities of a reclaimed uranium mine within a semi-arid desert environment. It is hypothesized that the Jackpile Mine and surrounding areas have soils that contain typical soil microbes, but also show selection for metal-metabolizing bacteria.

CHAPTER 2: MATERIALS AND METHODS

2.1 Site Description

This study was conducted at the Jackpile Mine, which is near the northern border of Laguna Pueblo, 60 km west of Albuquerque, New Mexico. The mine once included three open pits, 32 waste dumps and 23 proto-ore stockpiles, and over 60 acres of buildings and roads (US EPA, 2015). Soils of the Southwest in general, including the Jackpile Mine, are well oxidized. Microbial analysis was performed on only six of the 15 study sites.

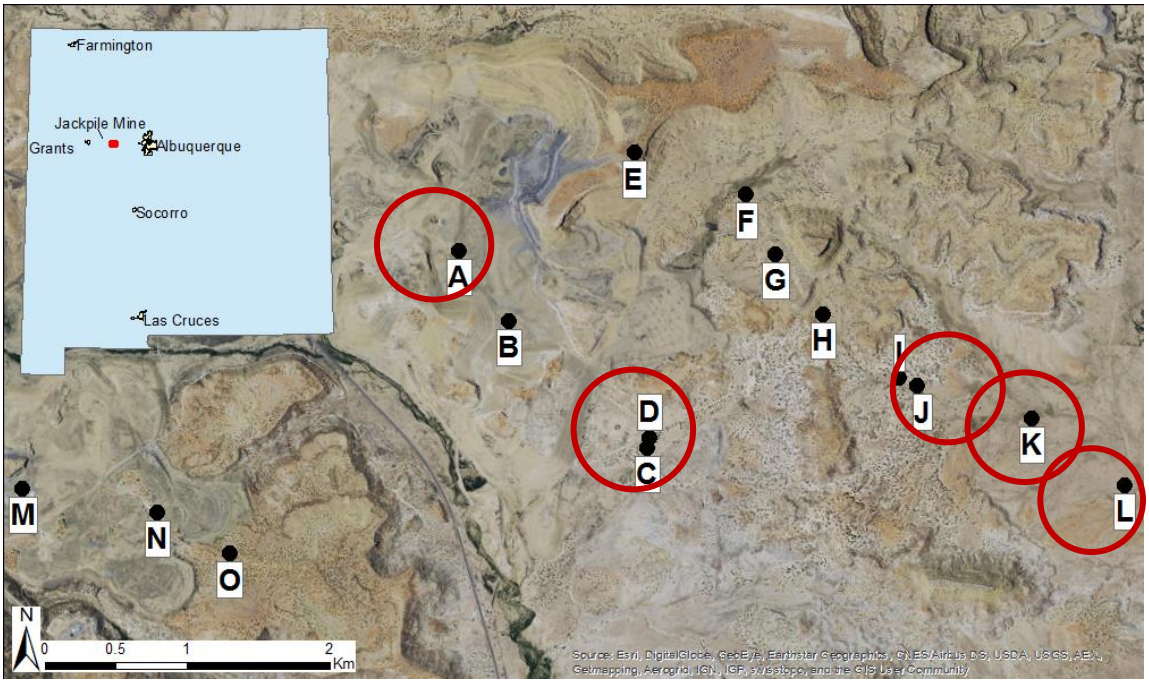

Figure 1- All 15 sites under investigation by the New Mexico Tech uranium team. The six sites chosen for microbial analysis are circled (Sites A, C, D, I, K, L). For coordinates of sites, see Appendix (Table A-3).

2.2 Soil Collection

The six soil cores were collected on November, 11th, 2014 (sites A, C, and D) and on November, 20th, 2014 (sites I, K, and L). In the field, soil was collected from 0-10 cm

depth using a flame-sterilized 2-cm diameter corer and transported on ice to the laboratory where they were stored at -80˚C. Six soil samples were selected from the 15 sites with the aim of obtaining a wide range of uranium concentrations and a diversity of site characteristics (mined vs. unmined and vegetation type). Cores of selected sites were homogenized, and 10-g subsamples were placed into sterile 50-ml tubes and stored at -80˚C until DNA extraction.

In the field, soil radiation counts were taken with a Ludlum Model 19 Micro R Meter. This instrument is a sodium iodide scintillometer that is able to detect radionuclides and low-level (near background) gamma radiation. Radiation counts were taken at ground level, and one meter above the ground (one meter readings are in the Appendix, Table A-4).

2.3 Soil analysis

Elemental analysis of soils was performed by inductively coupled plasma mass spectrometry (ICP-MS). First, soils were weighed at 0.2 +/- 0.01 grams. Soils were prepared as follows: 9 ml of nitric acid and 3 ml hydrofluoric acid were mixed with the pre-weighed soil sample, and microwaved at the EPA 3052 setting (180 ± 5 ˚C for 9.5 minutes) in a Milestone Connect, ETHOS Up microwave. Next, the solution was filtered with Whatman ashless medium-fast filter paper, and brought to 50 ml with reverse osmosis-purified water. The solution was diluted to a 1:50 ratio with 0.24 M nitric acid, then analyzed by an Agilent Technologies, 7900 ICP-MS.

Soils from six sites were also analyzed for moisture and organic matter content. Ten grams of soil were baked for 24 hours at 105˚C to remove moisture content. The weight was recorded. Then, the samples were baked for 3 more hours at 550˚C, and the weight was recorded again. The difference of the weights before and after baking is the percent of organic carbon from the sample.

2.4 DNA Extraction

The DNA from the Jackpile mine soil was extracted using the MoBio PowerMax Soil DNA Isolation Kit, following the manufacturer's instructions (MoBio Laboratories Inc.).

2.5 DNA Sequencing

Extracted DNA from the six sites was sent to the National Center for Genome Resources in Santa Fe, New Mexico to be sequenced. DNA libraries were constructed using the standard Illumina TruSeq protocol, and normalized libraries were sequenced on one lane of an Ilumina flowcell to generate 2x100-bp reads. On average, approximately 24 million pairs or 48 million reads (post-processing, high quality reads) were generated per sample. Paired-end reads generated for each sample were assembled using MEGAHIT (Li et al., 2015), a metagenome assembler. Individual assemblies were loaded onto MG-RAST (Metagenomics Rapid Annotation using Subsystem Technology), "an open-submission data portal for processing, analyzing, sharing and disseminating metagenomics datasets" (Wilke et al., 2016). Comparisons of metagenome and soil analysis was done using JMP Statistical Software.

CHAPTER 3: RESULTS

3.1 Soil Data

Elemental concentrations were very similar for all elements tested among the sites. The data show that uranium is not a key component in the soils tested. Fe, Ba, and Zn had the highest concentrations of the elements tested. The six soils in this study had very different organic matter and vegetation types associated with them. Site K had the highest organic carbon percent and radiation counts (Table 1), as well as the highest Mn, Ni, and Se concentrations (Fig. 3). On the other hand, site D had the highest metal concentrations of many of the metals tested, including Fe, U, V, Cr, Cd, Cu, As, Mo, and Pb (Fig. 3). The unique metal profile of site D is what drives the PCoA plot of metals and sites (Fig. 2.A.), where site D is an outlier from the other sites in terms of principal component 1. Zn is the metal that is most different from the other metal concentrations in terms of principal component 2 (Fig. 2.B.), whereas all of the other metals cluster according to principal component 1. Figures were generated by either one or both Excel or JMP (JMP 2007); details of figure generation can be found in the Appendix.

It is important to note that the sites not only vary in terms of vegetation, organic carbon percent, and radiation, but also vary in reclamation status. Particle size analysis was done by Brown (2015) and results can be found in the appendix (Table A-6). Site A is the only site that has been reclaimed, whereas the other five sites are considered natural soils (US BLM, 1986). The origin of soils at this site are not known. The materials that were used to reclaim areas of the Jackpile Mine site were a mixture of geological materials including Jackpile Sandstone, Tres Hermanos Sandstone, and Mancos Shale (US BLM, 1986). There was also a mixture of Rio Paguate riverbed soils. All of the soils used in reclamation were tested to ensure they did not have high concentrations of uranium. Site A could contain any of the geological materials named above.

Table 1. Soil data. Soil radiation data collected in the field and organic content determined in the lab for the six sites chosen out of 15 being studied at NMT (Brown, 2015; US BLM, 1986). Background radiation reading was averaged at 600 µR/hour. Details of site A's reclamation, radiation counts at 1 meter and coordinates for sites can be found in the appendix.

Site	Location and soil type	Vegetation - identification was by a combination of pictures and BLM document (US BLM, 1986)	Reclaimed ?	Affected by mining activities/ mined?	Ground level radiation (µR/hour)	% Organic Matter
A	Site within the East Pit- shallow rocky soils	one seed juniper, soaptree yucca, and rabbitbush. Grasses include galleta, feathergrass, Indian ricegrass, sideoats and blue gramas, red threeawn and bottlebrush squirreltail	Yes	Yes	650	0.0256
C	Wide alluvial valley, site near grassland	saltbrush, rabbit brush, cholla and broon snakeweed. Grasses: alkali sacation, galleta, feathergrass and red threeawn. Paperflower, daisy, cutleaf primrose	No	No	800	0.0070
D	Wide alluvial valley, site near a pinyon forest	saltbrush, rabbit brush, cholla and broon snakeweed. Grasses: alkali sacation, galleta, feathergrass and red threeawn. Paperflower, daisy, cutleaf primrose	No	No	1,250	0.0081

Table 1- continued

I	Site within a mixed vegetation forest	saltbrush, rabbit brush, cholla and broon snakeweed. Grasses: alkali sacation, galleta, feathergrass and red threeawn. Paperflower, daisy, cutleaf primrose	No	No	650	0.0091
K	Site with mixed vegetation	saltbrush, rabbit brush, cholla and broon snakeweed. Grasses: alkali sacation, galleta, feathergrass and red threeawn. Paperflower, daisy, cutleaf primrose	No	No	850	0.0336
L	Site within grassland	galleta, feathergrass, and red threeawn. Fleabane fireweed, andverbena, paperflower, daisy and cutleaf primrose	No	No	500	0.0203

Fig. 2 presents the differences of variance between sites according to metals, and between metals. For PCoA, one looks for clustering and outliers of data points. There is no significant clustering of sites according to principal component analysis (Fig. 2.A.), but it can be seen that sites C and D were the most dissimilar.

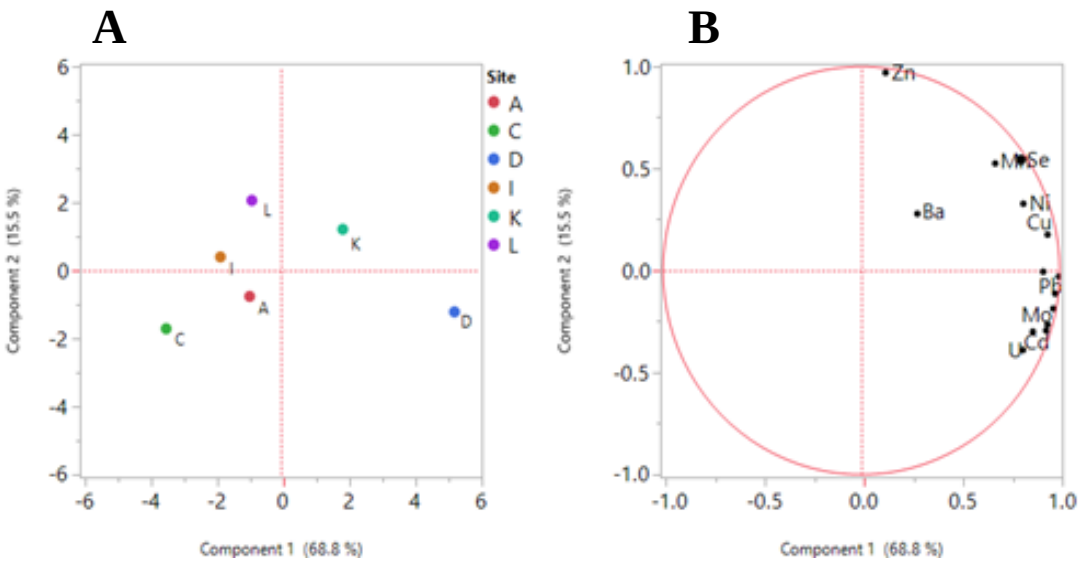

Figure 2: Principal component analysis of metal concentrations for the six sites. (A) Principal component analysis for the six sites. (B) Principal component analysis for metal concentrations measured with ICP-MS. Eigenvalues used for the PCoA plots can be found in the appendix.

Uranium concentrations are low in comparison to most other metals (Fig. 3), ranging from 2.24 mg/kg to 12.9 mg/kg. In fact, half of the sites are below global and New Mexican uranium soil background, which is approximately 3 mg/kg (USGS, 2004; Brown 2015). Because of these low uranium concentrations, the study sites cannot be considered a uranium-contaminated site. Complete ICP-MS data for the six sites are in the Appendix (Table A-5).

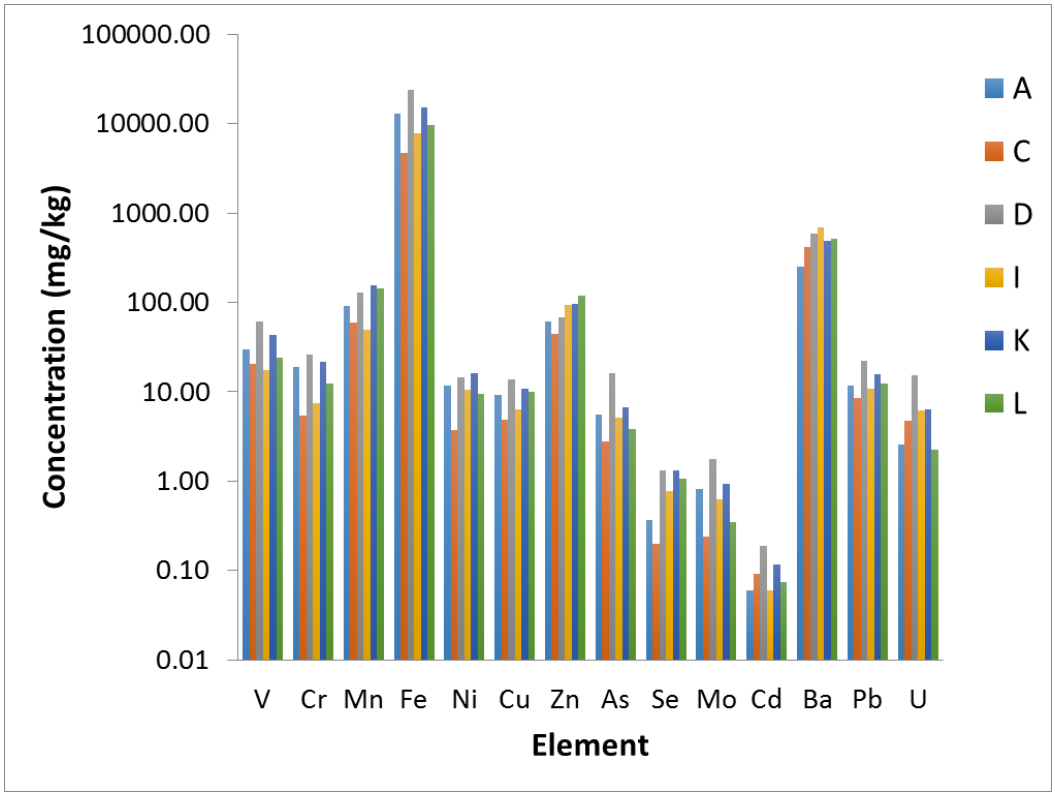

Figure 3: ICP-MS data for all metals measured.

3.2 Metagenomic Analysis

The six sites have unique microbial characteristics associated with each. Included in this analysis is alpha diversity counts (Table 2), rarefaction curve (Fig. 4), relative abundance (Fig. 5), PCoA of functional genes (Fig. 6), and hierarchical clustering of phylogenetic diversity (Fig. 7). After quality check, the base pair count ranged from 17,138,371 bp to 83,320,710 bp (Appendix Fig. A-2). Relative abundance is the percentage of a certain organism in relation to total organisms in a sample. Phylogenetic diversity is a measurement of biodiversity using phylogenetic differences between species. See Appendix (Table A-1) for metagenome identification for MG-RAST.

Alpha diversity, or number of species, ranged from 265 to 380 among the six sites (Table 2). Site D had the highest alpha diversity; site C had the lowest diversity. Sites A, C, and K have species counts below 300, and sites D, I, and L had species counts above 340.

Table 2: Alpha diversity per site (data from MG-RAST). Alpha diversity is "an estimate that summarizes the distribution of species-level annotation in a dataset, using the Shannon index" (MG-RAST), or in other words, a numerical representation of diversity per site.

Site	Alpha Diversity (Species)
A	293
C	265
D	380
I	348
K	294
L	355

The trajectory of site D's rarefaction curve indicated the highest diversity and operational taxonomic unit (OTU) count of all the sites (Fig. 4), with a maximum species count of 3,797. Sites I and L had the largest number of reads, with 162,324 reads and about 147,294 reads, respectively. Sites K, C, and A were all below 73,046 reads, and below 2,616 species. Overall, sites A, C, I, L, and K have very similar diversities.

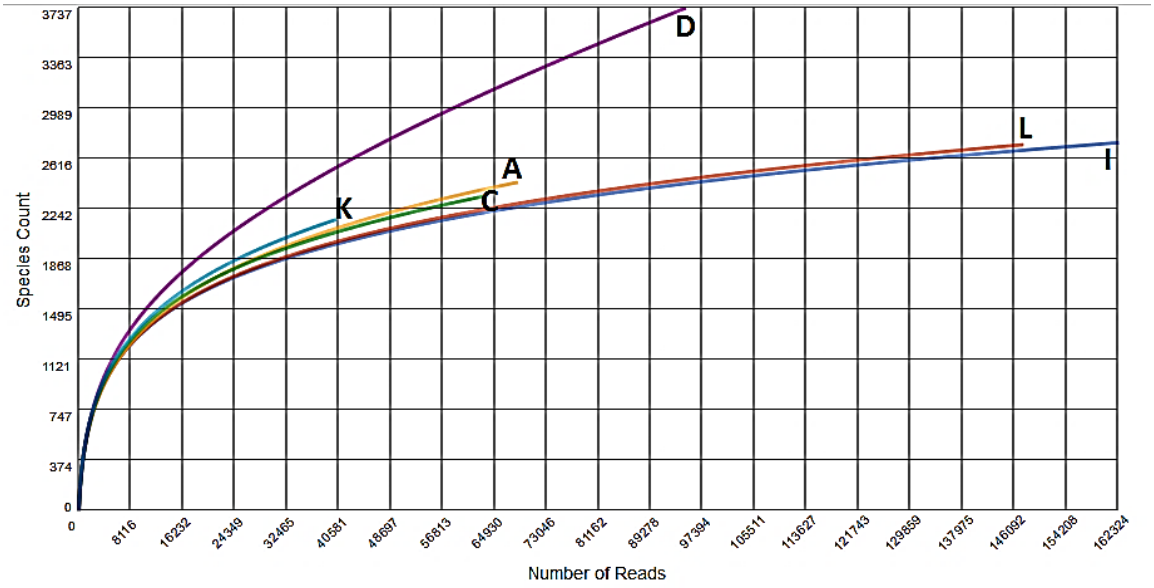

Figure 4: Rarefaction curves for six sites. Number of reads refers to the number of read sequences, but does not refer to the number of hits (or identification of genes).

Actinobacteria dominated the soils in all six sites (Fig. 5). In addition, *Proteobacteria, Firmicutes and Cyanobacteria* were in large relative abundance for all of the sites. Site K had the highest proportion of *Proteobacteria* in comparison to the other five sites, and concomitantly a smaller relative abundance of *Actinobacteria*. *Actinobacteria,* the phylum with the highest abundance in all the six sites, is the phylum that contains a uranium-relevant genus, *Rubrobacter*, which is known to be radiotolerant (Ferreira et al., 1999). Another radiotolerant bacterium that is present, *Deinococcus radiodurans*, from the phylum *Deinococcus-Thermus*, has a minuscule relative abundance

compared to the other phyla within the six sites yet is more or less visible at all sites except site L (Fig. 5). *Proteobacteria*, the phylum with second highest relative abundance, contains other uranium-relevant genera, including *Geobacter*. For relative abundance of uranium relevant species, see the Appendix (Table A-7).

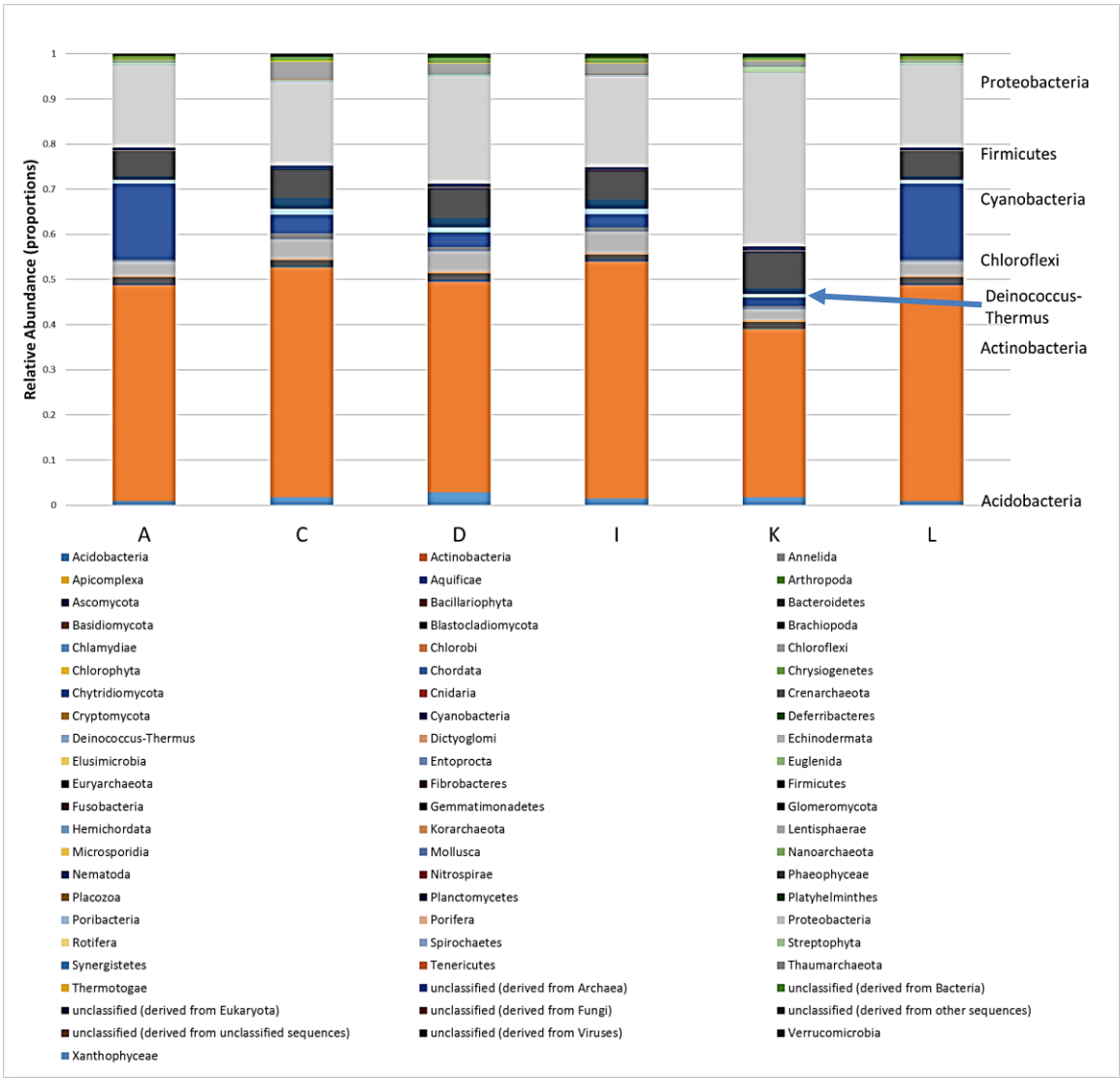

Figure 5- Bar graph of relative abundance of phyla for each site.

Functional gene is a term that includes all genes in an organism, regardless of its a specialized function. In terms of functional genes, there is not much variation along either principal component among the six sites; this was determined by drafting the PCoA graphs (Fig. 6). Sites I and L deviate from the overall trend slightly, driven by principal component 1 (Fig. 6.A). Most functional genes cluster according to both principal components 1 and 2 (Fig. 6.B). The few outliers according to both components include DNA metabolisms, protein secretion systems Types I and II, and the synthesis of proline and 4-hydroxyproline, coenzyme B, lysine, threonine, methionine and cysteine, and protein and nucleoprotein secretions system Type IV. Levels of analysis were determined

using the SEED Subsystem, which organizes functional genes into a hierarchal system at five levels (Overbeek et al., 2014). The five levels range from overall cellular functions at level 1, and focusing to specific genes and functions at level 5. Level 2 is a grouping of the basic cellular functions without specificity of subsystems. This level of analysis was chosen because it was the clearest representation of functional gene differences among sites.

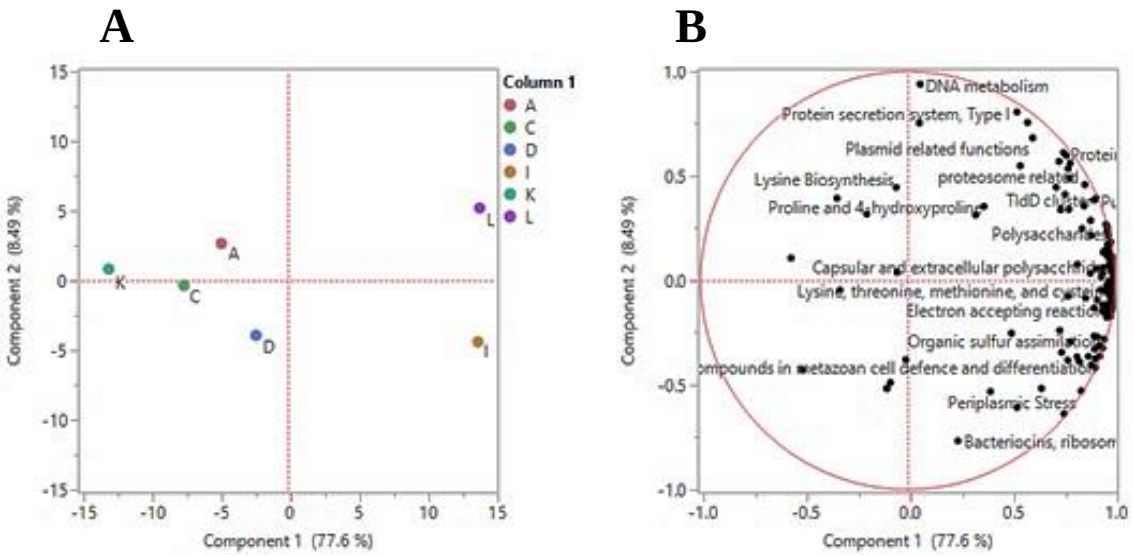

Figure 6- Principal component analysis of level 2 functional genes, taken from MG-RAST, for all six sites. (A) Principal component analysis per site according to functional gene variance. (B). Principal component analysis per functional gene, at level 2.

Hierarchical clustering is a way to show relatedness between data sets. In this case (Fig. 7) relatedness between sites in terms of phylogenetic diversity and functional genes is displayed. Sites A and L are the mostly closely related to each other, according to hierarchical clustering of phylogenetic diversity among sites (Fig. 7.A). In addition, site I is the most distantly related to the other five sites. Sites A, L, C, and K are more related to each other than sites D and I. On the other hand, sites A and D are the most closely related according to hierarchical clustering of functional genes (Fig. 7.B). Again, Site I, this time partnered with site L, is one of the most distantly related sites when compared to the remaining four sites.

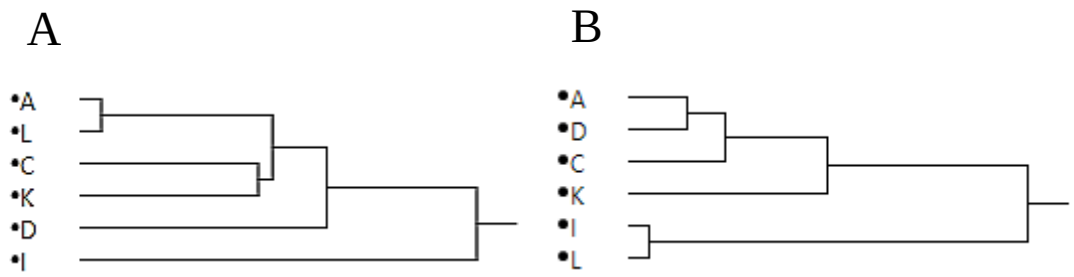

Figure 7- (A) Hierarchical clustering of phylogenetic diversity. (B) Hierarchical clustering of functional gene categories, level 1. Both figures were generated using JMP Cluster analysis, with Ward statistical method (Ward, 1963).

3.3 Metagenomic results compared to soil results

Generic abundances were compared to soil metal concentrations. This was done for each element by taking the site with the highest metal concentration, and determining the top 10 most abundant genera for that site. The abundances of those genera were divided by the abundances of the same genera for different sites, resulting in a ratio of abundance of genera according to metal concentration. All ratios were tested for significance by determining the r value (correlation coefficient), and the p value (calculated probability) of the genus abundance versus metal concentration, or by genus abundance versus organic matter percentage. Two of the comparisons resulted in p values that were very low, indicating that these relationships may not have been due to random sampling error. These two significant correlations were *Bradyrhizobium* and Se (Fig. 8.B) and *Rhodopseudomonas* and Ni (Fig. 9.A), with p values of 0.000363 and 0.000288, respectively. All other p values of the presented relationships were under 0.05 but had an order of magnitude less of a p value than the two relationships mentioned above.

Although there are only six sites for this study, five significant trends between bacteria and metal concentrations were uncovered. Two of the genera, *Bradyrhizobia* and *Rhodopseudomonas,* found in these significant trends are within the same family and are nitrogen fixers. The other genus, *Rubrobacter,* associated with an increase in Zn concentration is radiotolerant. Below are scatterplots of genera relative abundance with corresponding metals whose relationships have a significant r value (Fig. 8., Fig. 9, and Fig. 10). For the abundance of *Rhodopseudomonas* and *Bradyrhizobium,* as Se and Ni concentrations increased, abundance of the genera decreased (Fig. 8 and Fig. 9). The opposite was true for the relationship of the radiotolerant *Rubrobacter* and Zn (Fig. 10); as Zn concentrations increased, abundance of the genus increased as well.

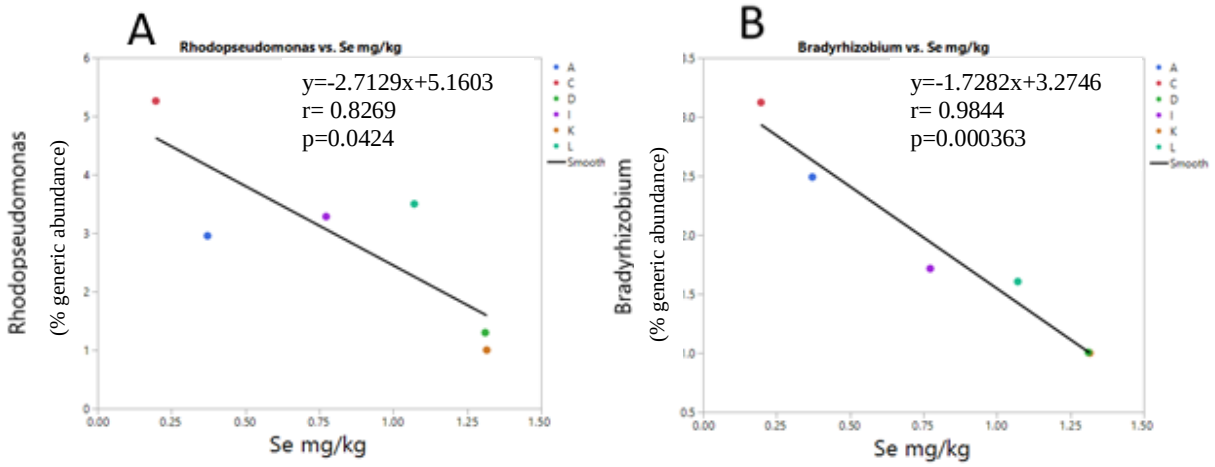

Figure 8- Scatterplots of Se and genera that show significant correlations. (A) Negative relationship of Se soil concentrations vs. abundance of *Rhodopseudomonas*. (B) Negative relationship of Se soil concentrations vs. abundance of *Bradyrhizobium*.

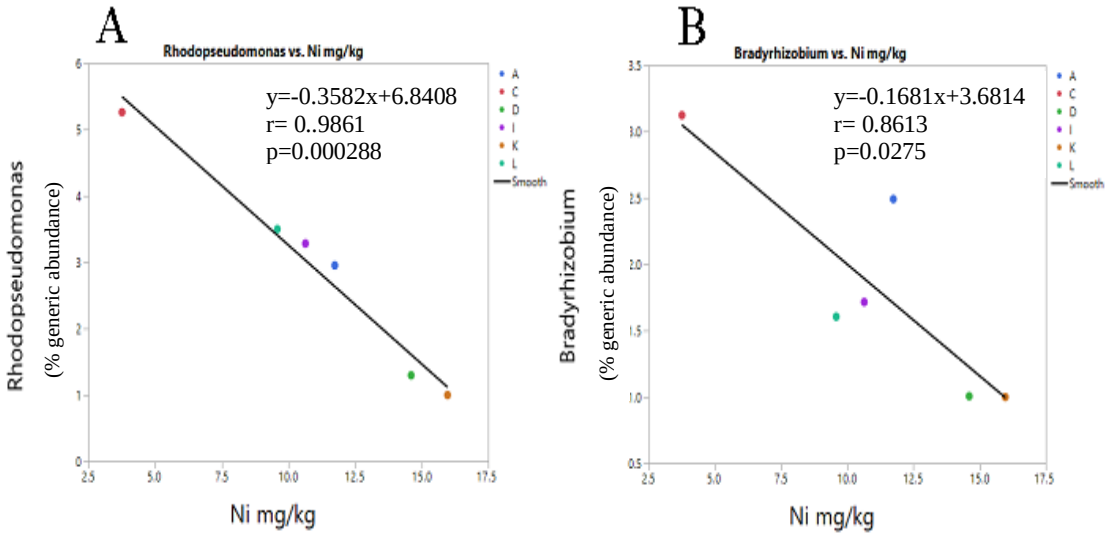

Figure 9- Scatterplots of Ni and genera that are show significant correlations. (A) Negative relationship of Ni soil concentrations vs. abundance of *Rhodopseudomonas.* (B) Negative relationship of Ni soil concentrations vs. abundance of *Bradyrhizobium.*

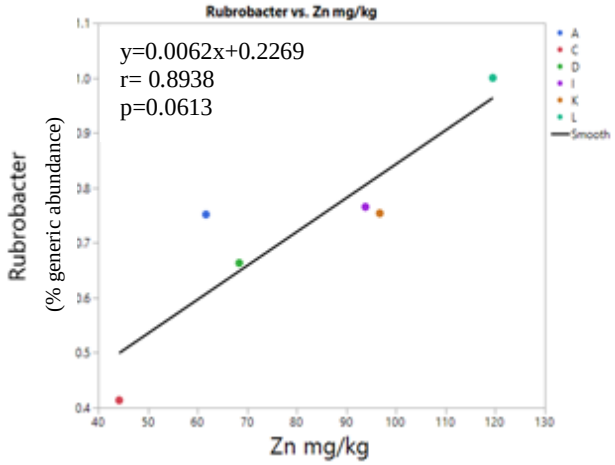

Figure 10- Positive relationship of Zn soil concentrations vs. abundance of *Rubrobacter*.

The same method used to analyze the most abundant genera and metal concentrations was used to analyze the relationship of these same abundant genera and organic matter percent in the soils. Of the genera analyzed, *Ktedonobacter* and *Lactobacillus* had p values less than 0.05 at 0.005344 and 0.0488, respectively (Fig. 11). Although the p values are fairly low, it cannot be definitively ruled out that the relationships happened because of random sampling chance. *Ktedonobacter* had a negative relationship with organic matter percentage, with the opposite relationship shown between organic matter percentage and *Lactobacillus* relative abundance.

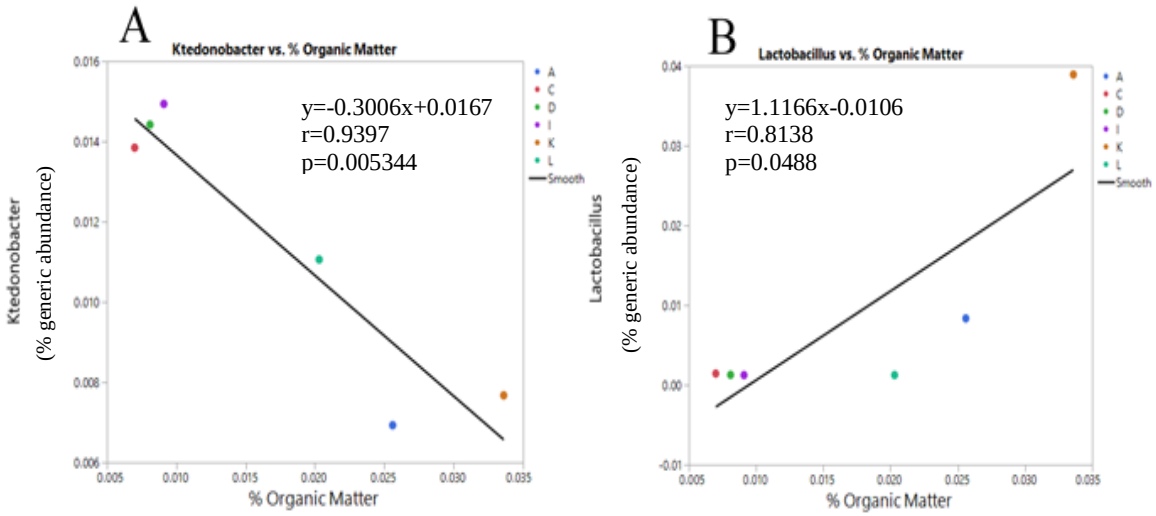

Figure 11- (A) Negative, significant relationship between *Ktedonobacter* and organic matter. (B) Positive, significant relationship between *Lactobacillus* and organic matter.

CHAPTER 4: DISCUSSION

Results showed that, in terms of both soil metal concentrations and phylogenetic differences, the six sites are very similar to each other. It was hypothesized that the Jackpile Mine and surrounding areas have soils that contain typical soil microbes, but also show selection for metal-metabolizing bacteria. This hypothesis was not completely supported; there was no evidence for strong selection of microbial genera/species being driven by heavy metal presence in soil. The exception is that three genera (*Bradyrhizobia, Rhodopseudomonas,* and *Rubrobacter*) showed relative abundance correlations with three heavy metal concentrations. The diversity that is represented is not atypical of other New Mexican or global soils. PCoA and hierarchical cluster analysis supported each other's results (Figs. 6 and 7.A). Sites I and L showed slightly lower diversity according to the rarefaction curve (Fig. 4) and were separated from clustering of other sites in the PCoA (Fig. 6), which may be due to large differences in relative abundance of *Cyanobacteria*. To reiterate, according to the SEED subsystem (Overbeek et al., 2014), a hierarchical categorization of genes, gene levels range from basic cellular function at level 1, to specific gene function at level 5, meaning level 2 is a slightly more detailed basic cellular level organization. Sites A, C, D, and K cluster together in many analyses (Figs. 5 and 6). This is unexpected, as site A has the most distinctive quality from all the other sites: it is a reclaimed soil. This may be indicative of the reclaimed soil moving in the direction of a natural soil ecosystem.

In New Mexican soils that had not been disturbed by human endeavors possess more microbial diversity than the Jackpile Mine soil samples (Fierer et al., 2012). See Appendix (Table A-8) for details of metagenomes used for comparison from Fierer et al. (2012). Their study conducted a metagenomic analysis on natural desert soils in the Chihuahuan Desert in New Mexico, one in the Sevilleta National Wildlife Refuge, and one at Galisteo, NM. The differences between the Jackpile soils and other soils are very obvious in the rarefaction curve (Appendix Fig. 2). In addition to the rarefaction curves, major diversity differences are seen when the alpha diversities of the two studies are compared. The Jackpile soil alpha diversity counts average 322 species, whereas the Fierer et al. (2012) soils had alpha diversities of 879 and 923 species for Sevilleta and Galisteo, respectively. Although there is a large difference in alpha diversity between the present study and Fierer et al. (2012), there is a similar pattern of dominant taxa among the soils of both studies. In both, *Proteobacteria* and *Actinobacteria* dominate the soils' phyla. Major differences between the two studies may be due to Fierer et al. (2012) simply having more data (Appendix Fig. 2 and appendix Table A-8).

Site D's unique high metal concentrations may have affected its unusually high diversity, as seen in the rarefaction curve (Fig. 4). In terms of alpha diversity, sites D and I have relatively high alpha diversity, above 340 species counts (Table 2). Not only phylogenetic differences, but also functional gene differences in site D may be driven by the variety of metals in higher concentrations in the soil, when compared to the other five sites.

In addition to site D, site C may also have been affected by soil metal concentrations. Site C, has the lowest number of reads and species count (Fig. 4), and has a unique metal profile (majority of lowest metal concentrations, Fig. 3) which might indicate that abundance and diversity are dependent on metal concentration, in that soil microbes need a certain concentration of metals to persist.

What is unique to site L is the larger relative abundance of *Cyanobacteria* (Fig. 5). The soil analysis does not uncover a specific reason for this increase in relative abundance. It could pertain to the unique vegetation found at this site, which includes more and thicker grasses than the other sites. *Cyanobacteria*, a photosynthetic phylum, is typical of arid grasslands, occurring as cyanobacterial crusts (Kuske et al., 2002).

Site K, according to most figures (except phylogenetic cluster analysis, Fig. 6), is different from the other five sites. It has the lowest number of reads and species counts but second highest apparent diversity (Fig. 4), the most variable phyla relative abundance (Fig. 5), and the lowest value for functional gene principal component 1 (Fig. 6). This site also has the highest concentration of Mn, Ni, and Se, which may be driving forces for the abundance of *Bradyrhizobium* and *Rhodopseudomonas*. Mn, Ni, or Se may decrease microbial relative abundance as metal concentration increases (Figs. 8 and 9). Without further analysis, it is difficult to determine which of the three metals is actually driving the results that are seen. Notably, these metals are necessary for many metabolic processes as discussed below.

Actinobacteria, Proteobacteria, and *Firmicutes* are the dominant phyla in all six soils; this is not surprising since many soils from very different environments show the same trend (Fierer et al., 2007). The relative abundance of *Cyanobacteria*, which can be nitrogen fixers, is highly variable among the six sites. Members of a different phylum, *Proteobacteria,* some of which are nitrogen fixers, were correlated with concentrations of Ni and Se. However, there is a low representation of nitrogen-fixation genes from the *nif* gene family, so it cannot definitively be known if nitrogen fixers are affected by metal concentration. For functional gene data, view metagenomes in MG-RAST (see metagenome ID's in Appendix, Table A-1).

Two of the genera (*Bradyrhizobium* and *Rhodopseudomonas*) that were found to have a significant relationship with Ni and Se are from the same family: *Bradyrhizobiaceae.* Several studies have found that the two genera correlated with Ni in this study require this metal for uptake of other metals and for the function of certain proteins (Hausinger, 1987; Black et al., 1994; Fu et al., 1995; Olson and Maier, 2000; Liu et al., 2009). These studies relating Ni accumulation to protein function did not find that relative abundance of *Bradyrhizobiaceae* was decreased as Ni concentration increased. Nonetheless, Chodak et al. (2013) also found that the presence of heavy metals reduced diversity and abundance. Metals such as Fe, Mn, and Ba dominate the six sites. Another key point is that Ni, Se, and Zn are the only metals that appear to be correlated with organismal relative abundance in this study. For example, hydrogenase requires Ni to

function; these bacteria are able to store the metal until it is needed (Maier et al., 1990). *Rhodopseudomonas* has a similar relationship to Ni as *Bradyrhizobium.* A certain range of Ni concentration is beneficial for *Rhodopseudomonas* in increasing rates of hydrogen metabolism (Hausinger, 1987; Liu et al., 2009).

Bradyrhizobium interacts with Se differently than with Ni. According to Boursier et al. (1988), Se is necessary for hydrogen metabolism, and its hydrogenase is a seleno-protein. Another study found that this genus has a high resistance to Se and other associated metals (Kinkle et al., 1994). *Rhodopseudomonas,* on the other hand, not only has resistance to higher Se concentrations but is also capable storing elemental Se within the cytoplasm (Kessi et al., 1999; Li et al., 2014). Li et al. (2014), however, also found evidence for growth inhibition as Se concentrations increased in the form of selenite.

Results of the present study suggest that although these metals are necessary for metabolisms within *Bradyrhizobiaceae*, perhaps there is a threshold at which the metal concentration becomes toxic rather than beneficial, and the genera *Bradyrhizobium* and *Rhodopseudomans* within the Jackpile soils may not have a sufficiently high resistance to Ni and Se. Furthermore, Se concentrations were very low overall, with the Se concentrations ranging from 0.197 mg/kg to 1.316 mg/kg. So far, this is the first study to find a negative relationship between genera within *Bradyrhizobiaceae* and Ni soil concentrations.

A significant positive relationship was found between Zn soil concentrations and *Rubrobacter* relative abundance. Other studies have found that in addition to being radiotolerant, *Rubrobacter* is also able to withstand high concentrations of Zn (Li et al., 2014). Although Zn contamination was found to decrease the overall bacterial diversity, *Rubrobacter* was able to thrive (Moffett et al., 2003). In the Jackpile soils, Zn is present in fairly high concentrations, while radioactivity is not. Therefore radioactivity stress may not be a factor interacting with *Rubrobacter* relative abundance in the present study.

Microbes drive the decomposition of organic matter, and it is also essential for microbial growth (Xu et al., 2015). Two significant relationships between genera and organic matter percentage were shown with the opposite trend of each other (Fig. 11). *Ktedonobacter*, a gram-positive bacterial genus, showed a negative relationship with organic matter percentage. To our knowledge, the present study is the first to show evidence that *Ktedonobacter* is negatively affected by high organic content. *Lactobacillus,* on the other hand, showed a positive relationship with organic matter percentage. Organic matter, in addition to other soil variables, affect the ability of microbes to metabolize and thrive (Xu et al., 2015), and this might be the driver of the relationships shown in Fig. 11.

Relationships between the above metals, organic matter percentage, and genera, was considered to be significant when the r value was equal or greater than 0.811 ($p<0.05$). Importantly, these significant findings may be due to random chance, considering that the search for correlation was repeated 150 times and found five. These are significant relationships for relationships to metal concentration, and two for relationships to organic matter percentage. After repeating statistical analysis, when the p value is <0.01, only three patterns are significant: *Bradyrhizobium* vs. Se, *Rhodopseudomonas* vs. Ni, and *Ktedonobacter* vs. organic matter. Because of the high number of comparisons made, a low p value is the necessary criterion used to determine significance. Without a lower p value, the correlations that appear to be significant may be only due to random sampling chance.

CHAPTER 5: CONCLUSIONS

Results showed that the one reclaimed mine site and the other five natural soils have similar diversity to each other, but altogether low diversity in comparison to two other natural New Mexican soils of similar characteristics for which MG-RAST data are available. Further, uranium appeared not to drive microbial differences among the sites. Rather, Se, Ni, and Zn appeared to significantly affect the abundance of three microbial genera. The soils shared microbial characteristics with many other soils, including a large presence of *Actinobacteria* and *Proteobacteria*. Mining and reclamation appears not to have greatly affected the diversity of the soils. With this said, the soils tested in the study were surface soils (0-10 cm). Soils with high uranium concentration, waste-rock, and other driving factors, such as acid from mining operations, would lie well below the reclamation soil layers.

An aspect to keep in mind with this study was the unfortunate lack of a control soil whose location was not on the Jackpile Mine land. All six soils investigated were from the Jackpile mine site and were chosen based on varying uranium concentrations and vegetation types. Not one of the soils was chosen based on lack of uranium presence or human interaction. In addition, the sites tested did not come from the same source; some were natural soils, and only one was a reclaimed soil. This makes it difficult to draw firm conclusions regarding the effects of mining and reclamation.

Future work for this study could involve many aspects. First, increasing the sample size would be beneficial, and might uncover several more significant patterns. It is difficult to find significance with only six sampled soils. Fierer et al. (2012) generated several orders of magnitude more reads in their study in comparison to this one. Differences between the present study and Fierer et al. (2012) may be due to differences in sequencing technology.

Second, testing samples other than the surface soil (deeper than 10 cm) might give insight to the effects of mining on soil diversity and microbial abundance. The soils that were tested in this study cannot be classified as “contaminated” because of low, often-below background uranium concentrations. The contaminated soil, if present, would lie at a deeper level than what was tested. A more thorough analysis would be beneficial not only to biologists but earth scientists researching uranium mines and reclamation. Microbes are confirmed to be key drivers in deposition and solubility of heavy metals, and understanding the unique niches of New Mexican soil microbes in this situation would give acumen to future mining or reclamation of the Jackpile sandstone deposit.

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APPENDIX

Table A-1- MG-RAST Identification for metagenomes

Site	Metagenome Name	Metagenome Identifier
A	1_kieft_metagenome.contigs	256622
C	2_kieft_metagenome.contigs	256685
D	3_kieft_metagenome.contigs	256690
I	4_kieft_metagenome.contigs	256693
K	5_kieft_metagenome.contigs	256694
L	6_kieft_metagenome.contigs	256695

Table A-2 -Base pair count and sequence count for metagenomes. Base pair count is the number of base pairs read per sample. Sequences count is the number of identified sequences per sample.

Site	Base pair count (After quality check)	Sequences count (After quality check)
A	31,383,373	66,231
C	27,241,145	60,388
D	41,573,410	90,146
I	83,320,710	156,441
K	17,138,371	39,443
L	70,362,062	142,722

Table A-3- Coordinates for 6 sites

Site	Latitude	Longitude
A	35° 8'21.95"N	107°20'11.40"W
C	35° 7'41.37"N	107°19'21.82"W
D	35° 7'38.47"N	107°19'22.48"W
I	35° 7'55.66"N	107°18'6.85"W
K	35° 7'47.45"N	107°17'34.28"W
L	35° 7'33.40"N	107°17'9.24"W

Table A-4- 1 m radiation counts for 6 sites.

Site	1 meter level radiation (µR/hour)
A	750
C	700
D	1,200
I	700
K	800
L	400

Table A-5 Complete ICP-MS data for all 6 sites.

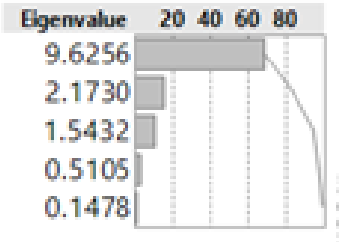
	A	C	D	I	K	L
51 V mg/kg	29.898	20.420	61.008	17.477	42.899	24.094
52 Cr mg/kg	18.740	5.344	26.165	7.533	21.761	12.257
55 Mn mg/kg	91.714	59.985	129.642	49.585	154.537	141.383
56 Fe mg/kg	13061.692	4655.916	24096.989	7868.192	15075.614	9669.145
60 Ni mg/kg	11.740	3.757	14.607	10.641	15.974	9.584
63 Cu mg/kg	9.277	4.909	13.857	6.308	10.843	10.039
66 Zn mg/kg	61.714	44.226	68.417	93.829	96.721	119.499
75 As mg/kg	5.612	2.759	16.074	5.107	6.719	3.812
77 Se mg/kg	0.372	0.197	1.311	0.773	1.316	1.071
95 Mo mg/kg	0.806	0.236	1.767	0.625	0.925	0.346
111 Cd mg/kg	0.059	0.091	0.186	0.059	0.116	0.075
137 Ba mg/kg	250.226	410.287	594.890	691.445	489.302	510.133
208 Pb mg/kg	11.601	8.435	22.120	10.908	15.715	12.374
238 U mg/kg	2.560	4.705	15.191	6.115	6.371	2.245

Table A-6- Particle size analysis (Brown 2015). Data are proportions by weight.

Size Class	Size Description	A	C	D	I	K	L
1	> 2 mm	0.129	0.0214	0.003	0.0171	0.0127	0.0197
2	2-1.4 mm	0.0498	0.00935	0.00501	0.0106	0.0291	0.00228
3	1.4-1.0 mm	0.0494	0.0131	0.0101	0.0136	0.0433	0.0136
4	1.0-0.5 mm	0.127	0.0697	0.0475	0.0815	0.158	0.0500
5	0.5-0.25 mm	0.174	0.368	0.292	0.353	0.271	0.214
6	0.25-0.125	0.232	0.397	0.434	0.295	0.217	0.381
7	0.125-0.09 mm	0.0861	0.0621	0.0964	0.105	0.0824	0.144
8	0.09-0.063 mm	0.0789	0.0333	0.0617	0.0710	0.0721	0.101
9	<0.063 mm	0.0735	0.0265	0.0495	0.0530	0.113	0.0740

Eigenvalues for figures- Eigenvalues are the numeric representation of variance that drive data points in a particular direction. The highest eigenvalue gives the most realistic principal component.

Eigenvalue for Figure 2:



Eigenvalue for Figure 6:

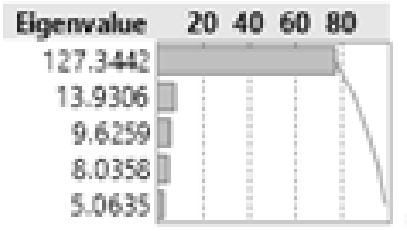


Table A-7- Uranium relevant bacterial relative abundances

Site	*Geobacter uraniireducans*	*Geobacter uraniumreducens*	*Geobacter metallireducans*	*Geobacter bemidjiensis*	*Desulfobacter postgateii*	*Shewanella putrefaciens*
A	4.25E-04	6.86E-04	6.54E-04	3.43E-04	0	2.36E-05
C	6.79E-04	1.12E-03	5.11E-04	3.96E-04	0	2.74E-05
D	7.84E-04	1.23E-03	7.70E-04	5.03E-04	0	6.18E-05
I	6.96E-04	1.05E-03	5.54E-04	4.42E-04	0	3.47E-05
K	4.84E-04	5.22E-04	7.57E-04	4.46E-04	0	3.96E-05
L	5.53E-04	9.20E-04	6.57E-04	5.04E-04	0	3.64E-05

Table A-8- Metagenomes from Fierer et al. (2012) study

Biome type	Location	Sample ID	MG-RAST ID	Alpha Diversity (species)
Hot Desert	Chihuahuan Desert, Galisteo, New Mexico, USA	SF2	4477872.3	923.007
Hot Desert	Chihuahuan Desert, Sevilleta, LTER, New Mexico, USA	SV1	4477873.3	879.211

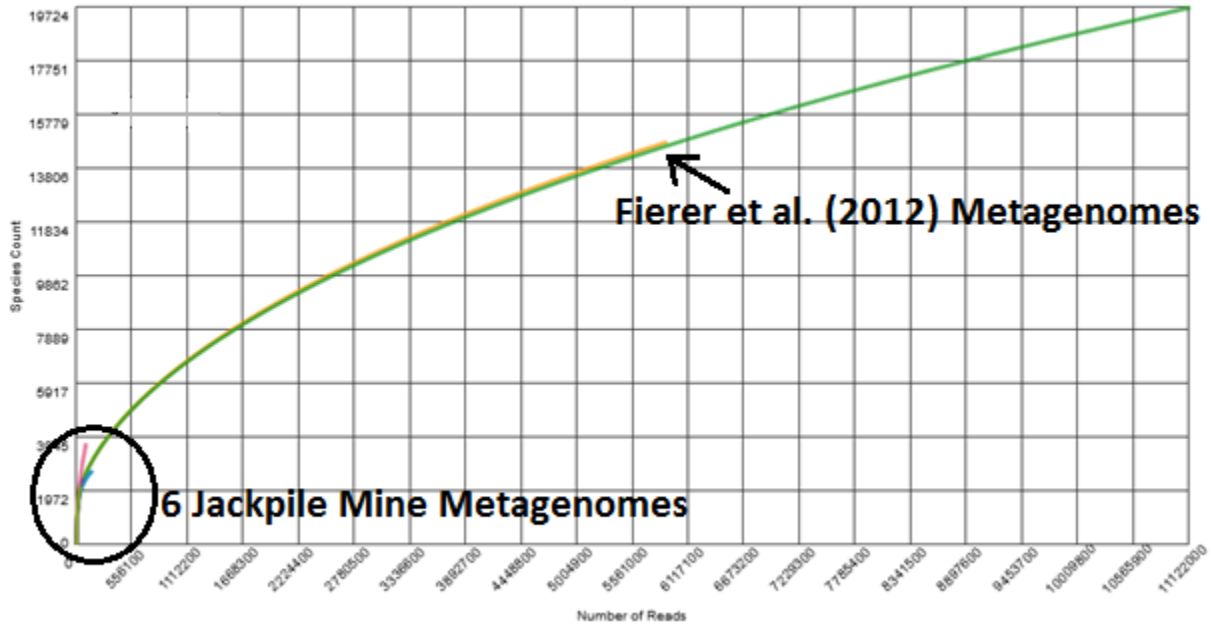


Figure A-1- Rarefaction curve of Fierer et al. (2012) metagenome study, graphed with the Jackpile Mine data. The six sites of this study are in the lower left hand corner, and are not visible when compared to the Fierer et al. study results.

Figure generation using MG-RAST- It should be noted that any results of relative abundance or percentages are not direct counts. MG-RAST presents these data as an average of sequence hits from several metagenomic databases. Depending on the sequence, hits can come from anywhere from one to 11 database sources.

Figure generation using JMP- Figures generated with JMP analysis software were done by first manipulating the data in Microsoft Excel, and then uploading to JMP to produce more clear figures than Excel. This was often done mainly because Excel could not analyze such a large data set such as a metagenome. For metagenomic analysis, data was uploaded directly to JMP from MG-RAST.

MICROBIOLOGY OF A RECLAIMED URANIUM MINE, LAGUNA PUEBLO, NM

By Olivia Raquel Chavez