COMPARISON OF MICROBIAL COMMUNITIES IN SOIL SEDIMENTS IMPACTED BY MERCURY CONTAMINATION ALONG A GRADIENT IN THE TRINITY RIVER, TEXAS

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by

Sujina Manandhar

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DEDICATION

I would like to dedicate this thesis to my father Raju Manandhar, and mother Shreejana Manandhar, and my husband Ajay K Dhakal. I would like to especially thank my mentor, Dr. Madhusudan Choudhary, for his guidance throughout the journey of my master's degree. A special thanks to my husband Ajay who has supported and motivated me in all possible ways throughout the process.

ABSTRACT

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Heavy metal contamination in the freshwater ecosystem has become a serious global issue impacting ecological, environmental, and human health. Over decades, the Trinity River in Texas has become polluted with several toxic heavy metals, including mercury (Hg), due to intensive anthropogenic activities and natural sources. The combustion of coal in power plants to generate electricity and several other Hg sources have led to mercury pollution in this river system. This study aims to i) determine the concentration of mercury in soil sediments along the gradient of the Trinity River, ii) investigate the soil microbial communities in the sites along the Trinity River that are impacted by mercury contamination, iii) examine the soil sediments to find whether the sites closer to coal-fired industries have higher mercury concentrations compared to the sites that are distantly located from coal-fired industries along the Trinity River, and iv) to determine if the soil sediments of the sites contaminated with high mercury concentration were enriched with mercury methylating genera compared to the non-contaminated sites leading to shift in microbial composition and diversity. The findings of the study supported our hypothesis that there is a relatively higher concentration of mercury in the downstream site of the river, but it remained inconclusive that the sites closer to coal power plants have higher mercury concentrations, since most tributaries carries the industrial waste effluents generated by these power plants flow into the main channel of Trinity River farther from the sites where soil sediments were sampled. On the other hand, the result of the microbial diversity analysis showed that statistically, there is

no significant difference in microbial composition (alpha diversity and beta diversity) between the uncontaminated upstream locations and contaminated downstream locations. While certain groups of mercury methylating genera were present in relatively higher abundance in sites with the increased level of mercury. The study suggests that; besides mercury, several other physicochemical factors of river water and soil sediment might contribute to emerging a synergistic microbiome composition at these sites, and while only small microbial differences lead to affect mercury metabolism.
KEY WORDS: Mercury, Methylmercury, Hg, Coal power plant, Microbiome, Soil sediment, Upstream, Downstream, Trinity River.

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CHAPTER I

Introduction

Heavy metal contamination in the aquatic ecosystem has become a global concern, imposing a severe impact on ecological, environmental, and human health. Heavy metals are high molecular weight metals or metalloids having a higher specific density (>5gm/cm^3) than water (Battarbee et al., 1990; Tchounwou et al., 2012). Some of the commonly found heavy metal pollutants in aquatic bodies are Mercury (Hg), Copper (Cu), Cadmium (Cd), Arsenic (As), Zinc (Zn), Lead (Pb), and Chromium (Cr) (Järup, 2003; Zeng & Wu, 2013). Generally, heavy metals are potent environmental pollutants due to their highly pervasive, non-biodegradable, and toxic characteristics (Sterritt & Lester, 1980). Detection of these toxic heavy metals, even at a lower concentration is sufficient to impact the aquatic biota, ultimately leading to a long-term imbalance in the food chain and bio-geochemical recycling (Wang, 2002). It is, therefore, necessary to understand the distribution of these heavy metals in surface water and soil sediments of the aquatic ecosystem.

The sediment microbial communities within the aquatic ecosystem are an indispensable component that serves an important role in regulating the soil ecosystem, biogeochemical cycle, nutrient cycle, and the food chain (Nealson, 1997; Tedersoo et al., 2020). The abundance and ecological function of soil microbial communities are highly influenced by various environmental and physicochemical parameters (pH, temperature, turbidity, nutrients, dissolved oxygen, etc.) (Hammerschmidt & Fitzgerald, 2006). However, little is known about the impact on microbial communities due to changes in environmental variables induced by anthropogenic activities. The soil microorganisms are significantly prone to exposure to various anthropogenic pollutants that the river receives. Among the several toxic

pollutants, heavy metal pollution has become a serious matter of concern (Peng et al., 2009). The soil microbes are highly sensitive to pollutants like heavy metal stress and thus are a good indicator of the changes in the aquatic ecosystem (Pignataro et al., 2012; Vinhal-Freitas et al., 2017). It could potentially result in a shift in microbial community structure and function in an ecosystem (Finlay et al., 1997). Only few numbers of research have been conducted so far in understanding the impact of heavy metals on the soil microbiome composition and function. It is therefore essential to understand the potential effect of heavy metal contamination on the microbial biodiversity of the freshwater ecosystem.

CHAPTER II

Investigation of Mercury Concentration in Soil Sediments of Trinity River, Texas

Introduction

Heavy metal pollution is one of the serious concerns around the world. The sources of heavy metals in the environment include both natural and anthropogenic origins (Giriyan et al., 2021). Generally, heavy metal occurs naturally in the earth's crust at trace concentration and in various forms (Järup, 2003). Some of the natural sources of heavy metals are volcanic eruption, geothermal events (Wang, 2002), weathering of rocks, soil erosion (Pirrone et al., 2010), and biomass burning (Briffa et al., 2020). The potential anthropogenic sources include the burning of fossil fuels, coal power plants (Pacyna et al., 2006), incineration of medical wastes, mining and smelting (Lacerda & Marins, 1997; Musilova et al., 2016), urban runoffs, industrial and agricultural wastewater, landfill leaches, industrial manufacturing (Gautam et al., 2014; Pirrone et al., 2010), and dental amalgams (Tibau & Grube, 2019).

The presence of heavy metals has been frequently documented as a major source of pollutants in the aquatic system (Gautam et al., 2014). The ability of the hazardous heavy metals to readily bioaccumulate and bio-magnify in the food web makes it a potential threat from environmental, ecological, and human health perspectives (Igiri et al., 2018). The input of heavy metals from various point and non-point sources causes the benthic organisms to be most likely impacted by heavy metal pollution in the aquatic ecosystem (Huang et al., 2020; X. Liu et al., 2022; Pejman et al., 2015). The sediment of the aquatic ecosystem serves as both source and sink for heavy metals (Pejman et al., 2015). Moreover, not just the aquatic organisms but also higher animals, including humans, are at major risk due to heavy metal

pollution. It is therefore essential to understand the prime sources of heavy metals, their distribution in the aquatic system, and its harmful impact on the ecosystem.

Heavy Metal Contamination of the Freshwater Aquatic System

Globally, numerous freshwater aquatic systems have been reported to be heavily polluted with hazardous heavy metals such as Pb, Cd, Ni, and Hg (Einax & Geiß, 1994; Meng et al., 2016). The dreadful impact on the ecological entities with these non-biodegradable metals should not be ignored. Numerous researches have been conducted on the risk assessment of heavy metal pollution in several different freshwater river systems across the world to monitor the sources of heavy metals and its distribution in the environment (Duncan et al., 2018; Iordache et al., 2022).

The primary source of elevated heavy metal contamination in aquatic ecosystems is intensive anthropogenic activities (Tchounwou et al., 2012). In addition to that, the long-term impact of the naturally occurring sources of heavy metals also equally contributes to the pollution of the environmental and freshwater aquatic system. There are several studies conducted around the world to study the effect of heavy metals on the freshwater ecosystem. Some studies are focused on determining the effect of heavy metal toxicity in the microbial community of soil sediments in the freshwater ecosystem (Ni et al., 2016). At the same time, some are focused on the bioaccumulation of toxic mercury in aquatic animals and its impact on the aquatic ecosystem (Backstrom et al., 2020). One recent study was conducted in the Chishui River basin located in Southwest China. This study evaluated the concentration of five different heavy metals (Zn, Cu, As, Cd, and Hg) contamination in the sediments with a focus on the ecological protection of the Chishui River. The result indicated that the upstream river basin was mainly contaminated with Hg and Cd and moderate contamination with other heavy metals (Li et al., 2022). The major reason for the

contamination in the upstream sites was primarily due to agricultural farming and the natural weathering of rocks. Apart from that, the continuous discharge of contaminants containing heavy metals directly into the water bodies was another factor responsible for the pollution of the river basin in China (Li et al., 2022).

A study in sediment cores of the Sabine-Neches Estuary, Beaumont, Texas in 1995 examined the concentration of various heavy metals as there was a high input of wastewater effluents from municipal treatments plants and wastewater effluents from more than 160 industries (Ravichandran et al., 1995a). The results depicted that there was a slight increase in the concentration of Pb and Zn while other metals such as Ni, Cu, Cr, and Co were not significantly detected. This study demonstrated that the riverine system has been polluted with heavy metals in Texas for a long period. It can be explained by major oil and chemical industries having their plants in the nearby river and estuary (Ravichandran et al., 1995a).

A study in the Mazaruni River in Guyana, South America examined microbiome composition in river sediments across sites that were impacted and non-impacted by gold mining operations (Obkirchner, 2019). Results from this study showed that there was a higher abundance of mercury methylating bacteria at the mined site indicating that there was a significant difference in the microbiome composition of the soil sediments between the mined and non-mined sites of the Mazaruni River basin (Obkirchner, 2019). This study suggested that gold mining caused an increased level of mercury contamination, thereby altering the microbial community composition of the freshwater ecosystem (Obkirchner, 2019).

Impact of Heavy Metals on Environment and Human Health

Heavy metal pollution in the environment is linked with various environmental, ecological, and health impacts. Once the heavy metals are introduced

into the environment from different sources, it gets mobilized into the atmosphere and ultimately gets deposited into the sediments (Rimondi et al., 2012). These heavy metals have a severe impact on both aquatic and terrestrial ecosystems leading to ecological imbalance. Some heavy metals like Fe, Cu, and Zn tend to have a significant role in the metabolic process as they serve as an important cofactor of several enzymes and are therefore considered to be essential heavy metals (Mildvan, 1970). While other heavy metals, such as Hg and Cd, are categorized as non-essential because these metals do not have any significant role in biological or cellular functions but rather potentially impose toxic impact even at lower concentrations (Esdaile & Chalker, 2018).

Agricultural crops also tend to be affected by the presence of toxic heavy metals in the soil as it causes growth inhibition or damages the cell structure of plants through oxidative stress (Chibuike & Obiora, 2014). Studies have found that Arsenic is responsible for the reduction in seed germination and decrease in seedling height of rice plants (Chibuike & Obiora, 2014). Similarly, cadmium can inhibit the growth of root and shoot, or reduces the uptake of nutrient in maize (Chibuike & Obiora, 2014).

On the other hand, the aquatic ecosystem is highly prone to heavy metal contamination. The industrial discharge of effluents containing metals and metalloids into the freshwater ecosystem without adequate treatment is yet another major problem (Tchounwou et al., 2012). The mobility of heavy metals into the water bodies occurs through the runoff from industries, urbanized cities, and municipalities wastes, ultimately leading to the deposition in the soil sediments of the water bodies (Musilova et al., 2016). The groups of anaerobic bacteria residing in the aquatic environment are responsible for making the bioavailability of these hazardous heavy metals through the process of biotransformation (Gilmour et al., 2013). It then

undergoes bioaccumulation and biomagnification in the food web. The toxic organic form of mercury (methylmercury) is found to be bioaccumulated in the tissues of fish and other aquatic animals (Baldi et al., 1989). The detection of methylmercury in fish is a good indicator of river mercury pollution and represents a potential health concern. The consumption of these mercury-contaminated fish could potentially lead to serious human health impacts as well as affect other higher animals (Hong et al., 2012).

The Minamata disease incidence is a good example of heavy metal poisoning in humans. The intake of mercury-contaminated fish and shellfish (5.61 to 35.7 ppm of Hg) caused the death of thousands of people due to methylmercury poisoning in the 1950s (Harada, 1995; Hong et al., 2012). In addition to that, Lead (Pb) is another detrimental heavy metal imposing a real threat to both humans and animals. Human exposure to Pb could lead to renal or endocrine damage because of its toxicity. While in animals, a higher concentration of Pb can cause reproductive failure (Assi et al., 2016). Hence, the impact of heavy metals is not just limited to the environment, but also on higher plants and animals including humans. It has therefore become a necessity to monitor and control heavy metal contamination worldwide because of the detrimental effect on the environment, ecosystem, and human health.

Heavy Metal Contamination in the Trinity River

There are 12 major freshwater river systems in Texas including the Trinity River. The Trinity River rises from North of Texas in four principal branches: The East Fork, Elm Fork, West Fork, and Clear Fork (DSHS, 2015; Gard, 1976) and flows through 37 counties with 21 reservoirs and covers 17,969 square miles of the total drainage area (DSHS, 2015). Trinity River is one of the longest rivers in Texas, which

is 710 miles long, that flows from North Texas to a few miles south of the Red River (Gard, 1976).

According to the United States Census Bureau (USCB), Dallas-Fort Worth (DFW), Texas is the fourth largest metropolitan city in the United States, with a total area of 9,286 square miles. The Trinity River flows through the DFW metroplex and Greater Houston supplying drinking water to these metroplex cities and serves as a major source of industrial and agricultural water (Atkinson et al., 2007; DSHS, 2015). With the rapid expansion of population, the concentration of business, and increased industrial activities in these highly urbanized metroplexes, the natural condition of the Trinity River basin has been significantly impacted as the river flows through these metroplex cities (Land et al., 1999). Therefore, in the year 1925, the Trinity River was considered a "River of Death" by the Texas Department of Health (Atkinson et al., 2007; Land et al., 1999).

Until the implementation of the Federal Clean Water Act of 1972, the water quality of Trinity River remained highly polluted (Land et al., 1999). In 2012-2013, the Department of State Health Services (DSHS), Seafood and Aquatic Life Group conducted research in Trinity River to examine the various metal concentrations and some pesticides in the fish samples. The result indicated that the concentration of metals such as Zn, Cu, As, Cd, Se, and Pb was detected in fish tissue that was within the DSHS guidelines for the protection of human health (DSHS, 2015). At the same time, Hg concentration exceeded the DSHS guideline indicating that Trinity River has been significantly influenced by increased human activities, industrialization, and population growth. It has also been documented that the Texas Department State of Health Services has been issuing fish advisory and prohibited recreational activities in parts of this river several times in the past few decades due to the increased level of

pollutants that are considered unsafe. This indicates that the Trinity River has been heavily influenced by anthropogenic activities, urbanization, and industrial activities.

The aquatic animals present in this river are found to be contaminated with several hazardous heavy metals (As, Cd, Zn, Hg, Cu, Se) (DSHS, 2015), pesticides, insecticides (Land et al., 1999), and some organochlorine compounds (Martinez, 1990). Among several heavy metals contaminating this river system; detection of mercury is considered to be highly toxic (DSHS, 2015; Jaishankar et al., 2014). Mercury mainly exists in three forms: elemental, inorganic, and organic (Schroeder & Munthe, 1998; Tchounwou et al., 2012). The elemental and inorganic forms are relatively less toxic compared to the organic form, such as methylmercury (Baldi et al., 1989).

Based on one of the studies conducted by Environmental Protection Agency (EPA), coal power plants contribute to almost 50% of the total mercury pollution. Combustion of coal releases fly ash containing various toxic heavy metals including Cd, As, and Hg, as well as a mixture of poisonous gases such as carbon monoxide (CO), sulfur dioxide (SO_2), and nitrogen oxides (NO) (EPA). Generally, the power plants are located beside a large source of water, such as the riverbank, as a vast amount of water is utilized to produce electricity from coal. Every year about 1450 metric tons of mercury are emitted from coal-fired power plants (Driscoll et al., 2007). This can cause a severe impact polluting the surrounding air quality, groundwater, and waterways if not properly managed (Driscoll et al., 2007; Li et al., 2010).

Sources of Mercury Contamination in the Trinity River

Among the 50 States of the United States, Texas is also listed as one of the biggest mercury emitting states, primarily because it is home to 6 out of 10 biggest

and oldest power plants (Madsen et al., 2011). Based on the data from 2010, Texas alone emitted approximately 11,127 pounds of airborne mercury (Madsen et al., 2011). Table 1 depicts the airborne mercury emission from these powerplants in the year 2010 with the national rank. Similarly, a survey from EPA indicated the mercury emission from power plants in Texas was slightly reduced to 8,433 pounds in the year 2014.

Table 1

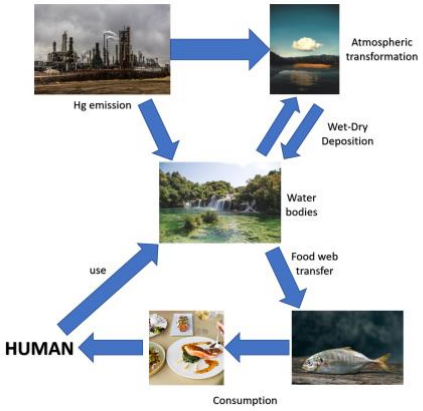
Six Biggest Power Plants in Texas with Their National Ranking for Total Mercury Emission

S.N.	City	Power Plant	National Rank	Annual Mercury Emission (lbs.)
1	Fairfield, TX	Big Brown Steam Electric Station and Lignite Mine	1	1,610
2	Tatum, TX	Martin Lake Steam Electric Station and Lignite Mine	3	1,420
3	Jewett, TX	Limestone Electric Generating Station	4	1,150
4	Hallsville, TX	American Electric Power H.W. Pirkey Power Plant	5	1,070
5	Mount Pleasant, TX	Monticello Steam Electric Station and Lignite Mine	7	1,005
6	Thompsons, TX	W.A. Parish Electric Generating Station	10	820

Note. Texas has 6 out of 10 power plants generating a maximum amount of mercury in the atmosphere (Madsen et al., 2011).

Figure 1

Mercury Cycle in the Environment



Note. A visual representation of the Mercury (Hg) cycle in the environment showing various sources of release and its impacts on environmental events and ecosystem.

Besides power plants, the Texas economy heavily depends on petrochemical plants, oil, and gas refineries. Especially, Houston is considered a major U.S energy source and a leading city in the chemical industry (DeRosa et al., 2019). The operation of these industries produces significant amount of hazardous chemical wastes, heavy metals, and toxic gases, impacting the surrounding atmosphere and the riverine system. In addition to that, urban runoffs and municipal landfill leachates can also potentially contaminate the groundwater in rivers, lakes, and streams (Gworek et al., 2015). Medical waste incineration, Chlor-alkali industry, metal production industry could also equally contribute to the increased mercury pollution (Pacyna et al., 2010). Therefore, mercury emission in the environment is not just limited to coal power plants, in fact, multiple anthropogenic sources are responsible for the elevated mercury pollution.

Mercury, once released into the atmosphere from these points, and non-point sources, makes its way to the ground through rain and snow, consequently contaminating the water bodies (Duncan et al., 2018; Rimondi et al., 2012). One study

suggested that more than half of the pollutants emitted from the power plants ultimately settles down in the nearby waterbodies such as landfills, pond, lake, and river through dry and wet deposition (Ćujić et al., 2016). Once in the soil sediment, Hg gets bio-transformed into highly toxic methylmercury through anaerobic microorganisms present in the aquatic ecosystem (Baldi et al., 1989). Organic material, pH, temperature, salinity, sulfur cycling, nutrient, and microbial community availability might also play a vital role in the biotransformation process for the synthesis of toxic methylmercury in the aquatic system (Hammerschmidt & Fitzgerald, 2006). The methylmercury is readily absorbed and bioaccumulates in the tissue of aquatic organisms passing through different trophic levels, including fish and shellfish. The concentration of methylmercury increases with the increase in trophic level (Montaña et al., 2021; Rimondi et al., 2012). It causes biomagnification in the food web to a level that imposes a severe health threat to humans and animals (Hammerschmidt & Fitzgerald, 2006; Montaña et al., 2021). Because of such condensation mechanisms of mercury pollutants, river systems like the Trinity River are highly prone to mercury pollution. There are at least four coal power plants located within a 145 Km radius in the lower Trinity basin, near Oakwood, Texas (Table 2). Therefore, the toxic pollutants released from these power plants could have a severe impact on the surrounding environment.

Table 2

Power Plants Near Lower Basin of the Trinity River, TX

Location	Name	Capacity (MW)
Trinidad, TX	Trinidad Power Plant	235
Fairfield, TX	Big Brown Steam Electric Station and Lignite Mine	1187
Jewett, TX	NRG Texas	2600
Franklin, TX	Oak Grove Steam Electric Station	1795

Note. Power plants with their capacity located at the proximity of the lower basin of Trinity River, near Oakwood, TX (EIA, 2022).

Apart from that, Texas is also known for seasonal flooding and hurricanes. For instance, Hurricane Harvey in 2017 caused massive and continuous rain that resulted in flash flooding in Texas (Steichen et al., 2020). Such natural calamities can transport the physically trapped contaminated sediment with the accelerated flow of the river during flooding (Lopez et al., 2022). Seasonal flooding, therefore, could potentially lead to the re-distribution of several contaminants and heavy metals, including mercury in nature.

Objectives

This study aims i) to determine the concentration of total mercury in the Trinity River sediment samples along the gradient influenced by different anthropogenic activities, industrialization, and urbanization and ii) to compare the microbial communities in each of the sites along the Trinity River to make inferences about the potential association with mercury contamination. The following hypotheses will be tested:

Hypothesis

Null Hypothesis (H_0): There is no difference in mercury concentration at different sites along the Trinity River.

Alternate Hypothesis (H_{a1}): The sediments of sites close to coal-fired industries have higher mercury concentrations compared to the sites that are distantly located from coal-fired industries along the Trinity River.

Alternate Hypothesis (H_{a2}): The downstream sites have a higher concentration of mercury compared to upstream sites of the Trinity River.

Material and Methods

Sampling Sites of Soil Sediments

Soil sediment samples were collected in July 2019 from five different locations in Northern Texas, along the Trinity River. The sampling sites were namely: Jacksboro (JB), Downtown Dallas (DT), Outside Dallas (DO), Oakwood (OW), and Romayor (RM) (Table 3) (Figure 2).

Table 3

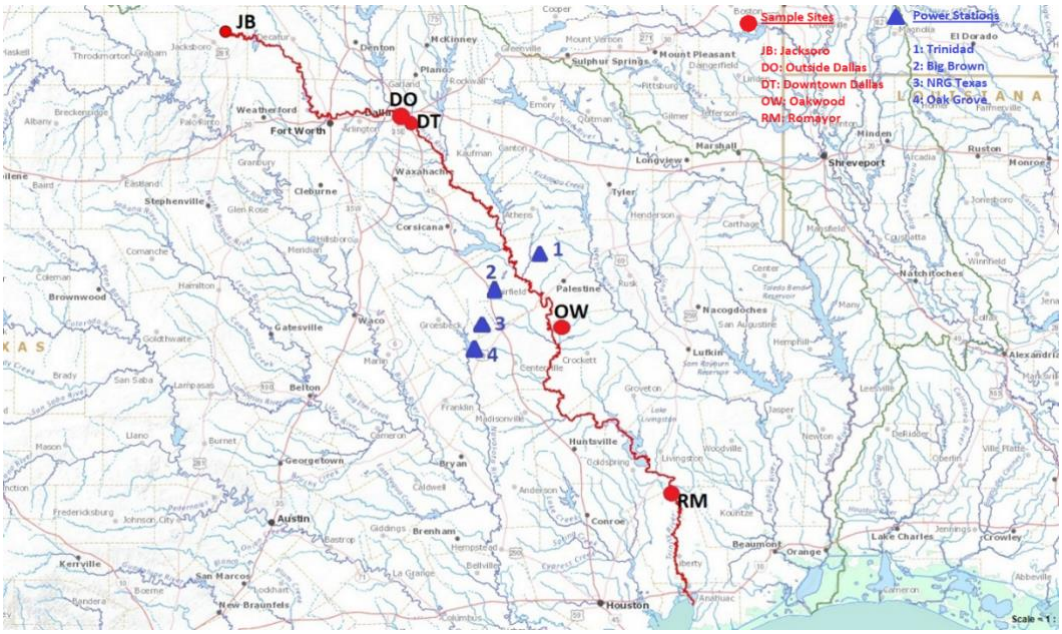
Geographical Co-Ordinates of Sample Locations

Symbol	Location Name	Coordinate	Sample Replicates
JB	Jacksboro	N33.2939, W-98.0791	A1-A5, B1-B5
DT	Dallas Downtown	N32.7768, W-96.8215	A1-A5, B1-B5
DO	Outside Dallas	N32.7072, W-96.7371	A1-A5, B1-B5
OW	Oakwood	N31.6495, W-95.7903	A1-A5, B1-B5
RM	Romayor	N30.4240, W-94.8507	A1-A5, B1-B5

Note. Five sample locations with their approximate geographical longitude and latitude presented as coordinates, N indicates latitude and W indicates longitude. Each location with two replicate samples and each replicate consists of 5 samples. A total of 10 samples from each location.

Figure 2

Sample Locations Along the Gradient of the Trinity River, TX



Note. Map indicating five different selected locations along the gradient of Trinity River in Texas, North America. The red dot indicates the sampling locations, and triangles represent the power plants in proximity. Samples from the five different locations were analyzed for mercury concentration.

The soil samples were collected from the Trinity River. Each sample was collected at ~50 cm depth from the water surface. Sample sites were located 10 meters apart. The samples were collected from those sites in a labeled petri dish. From each site, five replicate samples were collected which were about 1 meter apart. The replicates were designated as A1-A5 and B1-B5. Therefore, a total of 50 samples from all five respective locations were collected for the analysis of mercury concentration along the Trinity River gradient.

Jacksboro (JB) is in the upper basin of Trinity River which is at approximately 135 Km from Dallas. Downtown Dallas (DT), and Outside Dallas (DO) are highly urbanized sites, and Oakwood (OW) is located downstream from Dallas and adjacent to coal power plants. Romayor (RM) is in the lower Trinity River, downstream from OW. However, Romayor was farther from Dallas but was closer to

coal power plants near OW. It is also closer to the coastal areas, which are highly impacted by industrial developments and small factories. The sediment samples collected in the petri dish were then placed in a Ziplock bag separately and stored temporarily in an ice cooler before its transportation to the cold room (4ºC) in the laboratory at Sam Houston State University (SHSU). All 50 samples were analyzed for the total mercury concentration to determine the difference in the level of mercury concentration along the river gradient.

Analysis of Total Mercury Concentration

For the analysis of total mercury concentration of the soil sample of Trinity River, the Cold Vapor Atomic Absorption Spectroscopy (CVAAS) method was used. To determine the total mercury concentration, the Millennium Merlin Analyzer was used. The analysis was performed at the Texas Research Institute for Environmental Studies (TRIES) facility located at SHSU.

Initially, 0.50 gm of each soil sample was measured and transferred to 50 mL digiTUBEs, followed by adding 8 mL of Aqua Regia. The mixture was refluxed for 10 minutes. 2.5 mL of reagent water was then added to each sample tube, followed by another 10 minutes of reflux. The tubes were then allowed to cool at room temperature. Next, samples were filtered using 125 mm of filter paper using a filtration flask. The filtrates thus obtained were diluted by adding 50 mL of reagent water and transferred to 50 mL polypropylene bottles. 2 mL of each diluted sample were taken in the labeled falcon tubes into which 3 mL of $KBrO_3$/ KBr (Potassium bromate/ Potassium Bromide) and 2.5 mL of HCl were added and allowed to rest for 15 minutes. The mixture of each sample solution was further diluted to make the final total volume of 25 mL by adding reagent water. Next, 100 mL of $NH_2OH.HCl$ (Hydroxylamine hydrochloride) was added to the solution and mixed by inverting the

tubes 4-5 times. Finally, the samples were loaded into the Merlin Analyzer racks for the determination of the Hg concentration of each soil sample. The concentration of Hg in each sample is measured by adding 2% stannous chloride ($SnCl_2$) and bypassing the sample into the gas-liquid separator. Argon gas was then added to the mercury vapor. The mercury vapor was then passed onto an atomic adsorption optical cell, where mercury concentration was measured by light adsorption at 253.7 nm. The data of total mercury concentration in each soil sample were thus measured and recorded on the computer.

Statistical Analysis

The descriptive analysis was performed for a total of 50 samples from five different locations. Each location comprised ten samples. For the statistical analysis of total mercury concentration, a non-parametric test Kruskal Wallis rank-sum test was performed using R (version 4.2.0, package name: dplyr). This test is used to test the hypothesis of whether there is a significant difference (p-value <0.05) in at least one of the groups. Hence to determine if there is a significant difference in the mean concentration of mercury in at least one of the locations along the Trinity River, the Wilcoxon signed-rank test which is a post hoc test was performed. This test makes a pairwise comparison between the sample groups to show where exactly the significant difference exists.

Following the Kruskal Wallis test, a pairwise comparison using a Wilcoxon signed-rank test ($p<0.05$) was performed to specifically examine which location has a significant difference in the Hg concentration. This test is also non-parametric, similar to a student's t-test. If the observed p-value between any of the pairwise comparisons obtained from the Wilcoxon test is greater than 0.05, then we accept the null hypothesis, indicating that there is no significant difference in the mean Hg

concentration. On the other hand, if the observed p-value between any of the pairwise comparisons is greater than 0.05, then we accept the alternate hypothesis, indicating that there is a significant difference in the mean Hg concentration.

Results and Discussion

The total mercury concentration varied significantly along the Trinity River gradient comparing the sites from upstream (Jacksboro, Downtown Dallas, Outside Dallas, and Oakwood) locations to downstream (Romayor) location. The average mercury concentrations among these different locations ranged from 0.05 µg/g to 0.44 µg/g. However, there was an observed consistency in the mercury concentration in the upstream locations (JB, DT, DO, and OW) than that of the downstream location (RM) (Table 4).

Table 4

Mercury Concentration of Soil Samples Collected from Five Different Locations Along the Trinity River, TX

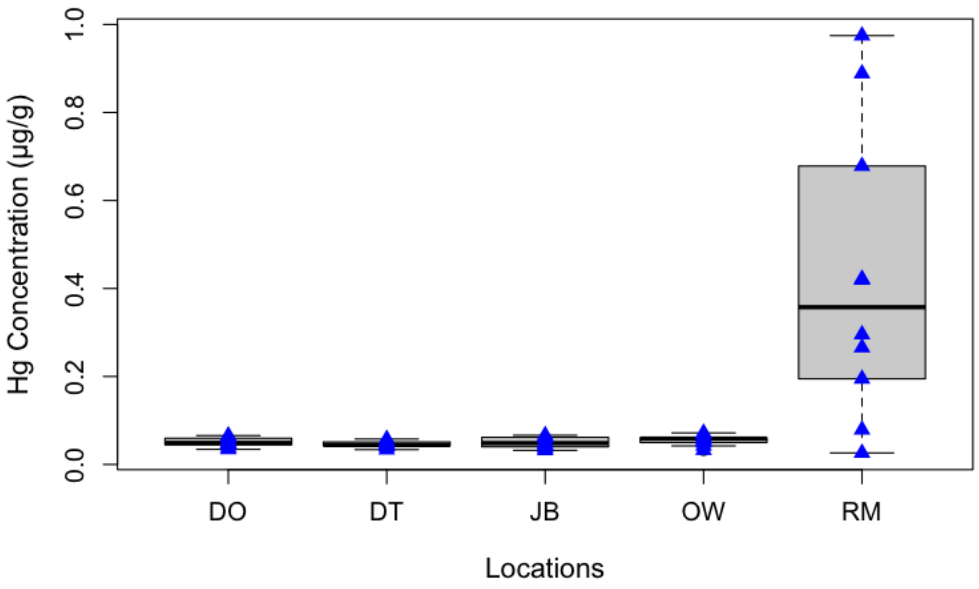
Sampling Location	Number of samples	Mean (µg/g)	Standard Deviation (SD)
Jacksboro (JB)	10	0.050	0.012
Downtown Dallas (DT)	10	0.046	0.007
Outside Dallas (DO)	10	0.051	0.010
Oakwood (OW)	10	0.056	0.012
Romayor (RM)	10	0.424	0.326

Jacksboro (JB) had mercury concentration of 0.050± 0.012 µg/g. Downtown Dallas (DT) had mercury concentration of 0.046± 0.007 µg/g. Outside Dallas (DO) had mercury concentration of 0.051 ± 0.010 µg/g. Oakwood (OW) had mercury concentration of 0.056 ± 0.012 µg/g. Romayor (RM) had mercury concentration of 0.424 ± 0.326 µg/g. There is no significant variation in the average mercury concentration within the upstream locations (JB, DT, DO, and OW). Whereas, in the

extreme downstream location (RM), there tends to be a significant variation in the average mercury concentration even within the location (Figure 3).

Figure 3

Graphical Representation of Hg Concentration at Different Sampling Locations



Note. A graphical representation of mercury concentration of soil sediments collected from five different locations along the Trinity River, TX. Each point in the bar plot represents the concentration of Hg in µg/g. The dark line in the middle of each box represents the median. The extreme points in each box represented by faded lines are the maximum and minimum concentrations of Hg in each location. Box plot was obtained programmatically using R (version: 4.2.0, packages: ggplot, dplyr).

The assumption of normality and variance for the ANOVA test was not met; therefore, a non-parametric test (Kruskal-Wallis rank-sum test, $p<0.05$) was chosen to determine the significant difference. The Kruskal-Wallis test suggested a significant difference in the average mercury concentration in at least one of five different locations ($p=0.001$). At the same time, this outcome did not specifically indicate which location has a significant difference in the mean mercury concentration. The Wilcoxon test provided a better summary of significant differences (Table 5). The result of the pairwise comparison for the four upstream locations (JB, DT, DO, and OW) had an observed p-value greater than 0.05, suggesting that statistically, there is no significant difference in the average Hg concentration between these locations.

Pairwise comparison of four different upstream locations with the extreme downstream location, Romayor had an observed p-value less than 0.05 suggesting that Romayor had a significantly different Hg concentration level compared to upstream locations. The average mercury concentration at Romayor was almost 10-fold higher compared to other locations. The statistical result, therefore, supported the alternate hypothesis (H_{a2}) that the downstream location is highly contaminated with mercury than the upstream locations (Table 5).

Table 5

Pairwise Comparison of Each Location Using the Wilcoxon Test

Locations	JB	DO	DT	OW
JB	1			
DO	0.971	1		
DT	0.437	0.289	1	
OW	0.437	0.473	0.075	1
RM	0.005**	0.005**	0.005**	0.007**

Note. The test was performed using R-language (version: 4.2.0). Significant differences ($p<0.05$) in the average mercury concentration between the locations are indicated by asterisks (**).

On the other hand, the alternate hypothesis (H_{a1}) stating that the sediments of sites close to coal-fired industries have higher mercury concentration compared to the sites that are distantly located from coal-fired industries along the Trinity River was inconclusive. This is because four of the power plants are located closer to Oakwood. However, statistically, the mercury concentration at Oakwood was not significantly different from the three other upstream locations. It might be because the waste effluents from only one power plant (Trinidad power plant) get discharged into the nearby tributaries that ultimately merge in the mainstream before the Trinity River reaches the Oakwood sampling site. While the other three power plants (Big Brown Electric Power Station, NRG, and Oak grove Electric Stations) are located below the Oakwood sampling site suggesting that the waste effluents containing heavy metal

pollutants from these powerplants get merged into the main river which are then carried downstream along with the flow of the river. Alternatively, there could be other potential mercury sources that contribute to increased mercury concentration in Romayor. For instance, the urban runoffs from the Dallas metroplex, medical waste discharge, manufacturing plants, and several other potential industrial activities could contribute to addition of hazardous heavy metals into the waterways downstream the river thereby compromising the water quality. Or it could be that mercury accumulates the farther downstream the river goes. Although the Trinity River flows along the Greater Houston area, however all our sampling sites for the study is located upstream of Houston. Hence, the higher mercury concentration in Romayor might not have significant impact from the Houston area.

Additionally, the influence of various physicochemical characteristics, biological, and natural factors can also equally play a pivotal role in the observed difference in the mercury concentration along the Trinity River gradient. The result corroborates our second alternate hypothesis that higher mercury concentration occurs in the sediments of the downstream site along the Trinity River. However, future work with increasing number of sampling sites will provide a better insight into such observed variation in mercury distribution in the soil sediments along the gradient of Trinity River.

Conclusion

This study quantifies the level of total mercury concentration along the Trinity River. The findings of this study could provide a baseline information on mercury pollution in the downstream location of the Trinity River due to increased anthropogenic activities and industrial sources in the past and present. It was observed that the extreme downstream region represented by Romayor had approximately ten

times higher concentrations of mercury compared to those of the upstream regions. This difference in total mercury concentration could be attributed to the distance of the sampling sites from the point source of contamination. Several coal-operated power plants are present closer to the downstream location of the study area. These power plant could have influenced the concentration of mercury pollution in the site of the Trinity River downstream from the Dallas area which might be one of the several contributing factors to higher mercury concentration in Romayor. According to the EPA, the coal ash containing toxic pollutants generated from the power plants can potentially leak underground and contaminate the surrounding river ecosystem.

Importantly, regardless of the mercury sources, the ever-expanding population in the metroplex cities along the downstream river basin increases the demand for utilization of river water serving as a source of drinking water to collection sites for flood water, discharge of municipal wastewater, and industrial and agricultural waste. The anthropogenic activities in metropolitan cities like Dallas and Fort-Worth could likely add to the increased level of mercury pollution in the river. These metropolitan cities are the major hotspots where urbanization and industrial activities, medical hospitals, and wastewater treatment plants generate a large number of waste effluents directly or indirectly into the water bodies that could impact the river water and sediments (Land et al., 1999). Thus, the high concentration of mercury at Romayor is attributed to the natural flow of contaminated soil sediments from downtown Dallas downstream along the river. On the other hand, the Greater Houston area could also be a potential source of mercury pollution because of the increased anthropogenic activities in the Trinity River. However, the sampling site in Romayor is located upstream of the Greater Houston metroplex and coastal area which suggests that there

is no significant influence of the Houston area on the observed elevated mercury in Romayor.

Apart from that, with the flow direction of the Trinity River from north to south, most of the contaminants could potentially be carried along and get deposited in the river sediment, which might result in an increased level of mercury in the lower basin of the Trinity River. For this study, the soil sediment sample collection was done around July, which is typically a flash flooding season (Ahmadalipour & Moradkhani, 2019). A large amount of floodwater and storm runoffs from the urbanized cities could be factoring in the elevated level of mercury downstream in Romayor. The floodwater results in a highly accelerated and excessive overflow of water that could carry contaminated particles, including heavy metals, along the riverbed (Steichen et al., 2020). This can lead to the re-distribution of the contaminants absorbed with sediment particles which get mobilized with the direction of the river flow (Ahmadalipour & Moradkhani, 2019). As a result, the contaminants could get deposited in the riverbeds registering a relatively higher concentration in the downstream location.

The pattern of the mercury distribution along the river gradient showed a consistent level in the upper basin while there was a noticeable increase in the mercury concentration towards the extreme downstream location. The findings of the study suggested that the distribution pattern of the mercury along the Trinity River gradient could be attributed to past and current human activities, including the power plant, along with the combination of several anthropogenic and natural factors. Thus, our identification of significant mercury concentration in this region also equally imposes a potential threat of Hg poisoning to the population who are residing nearby the Trinity River or consuming contaminated fish from the river. Findings from this

project could be useful in addressing and implementing better alternatives to monitor heavy metal pollution in the freshwater ecosystem in the near future.

CHAPTER III

Microbial Composition in Soil Sediments of the Trinity River, Texas

Introduction

The freshwater aquatic ecosystem is dynamic and complex, harboring rich species biodiversity (Collen et al., 2014). Over the period, due to the increasing stress of industrial and anthropogenic activities worldwide, several freshwater riverine systems have become overexploited and polluted with organic, inorganic chemicals pollutants, and heavy metal contaminants. The soil sediment of waterbodies serves as a prime repository for the accumulation of microbes and deposition of chemical and metal contaminants (Devarajan et al., 2015).

Microbiome Structure in the Freshwater Aquatic System

Most of the previous studies conducted in freshwater ecosystems to date are primarily focused on the impact of heavy metal pollution on aquatic plants (Sethy & Ghosh, 2013) and animals including, the human population. However, less numbers of studies have been conducted to examine its effect on sediment microbial communities. There tends to be a significant role of the soil microbiome in balancing the aquatic ecosystem by regulating the biogeochemical cycles (Lindeman, 1942) and various biological functions such as energy production, degradation of organic matters, mobilization of nutrients, and biosynthesis of essential macromolecules such as protein, lipids, complex carbohydrates (Zhang et al., 2020). However, due to the intensive anthropogenic activities, the level of heavy metal contamination has become prominent in the aquatic ecosystem affecting microbial community diversity. Bacteria are more sensitive to heavy metal stress than the fungal communities (Rajapaksha et al., 2004). In general, the abundance of highly sensitive microbes to heavy metals

decreases while the microbial population that can resist and tolerate the heavy metal in soil has a relatively higher abundance (Zheng et al., 2022).

Studies have found that the combined effect of Cd and Hg contamination in sediment reduces microbial community diversity (Liao et al., 2010; Xie et al., 2011). A recent study was done to assess the long-term impact of mercury mining on bacterial community diversity in paddy soil of Wanshan District, China. The findings from this study demonstrated that the alpha diversity increased in moderately mercury-contaminated soil (Y.-R. Liu, Wang, et al., 2014).

Impact of Heavy Metal Contamination on Microbiome Composition

Heavy metal pollution in the freshwater ecosystem has become a major concern worldwide as a consequence of increased anthropogenic activities (Peng et al., 2009). Generally, the elevated concentration of non-essential heavy metals such as Pb, Cd, Hg, As, and Cr are considered major pollutants in aquatic water bodies (Fosmire, 1990). The presence of these heavy metals in the freshwater ecosystem influences the indigenous microbial community pattern and composition (Ni et al., 2016). Heavy metals can impact the metabolic activities and cellular function of soil microbes due to its toxicity, thereby altering the diversity and structure of the microbial community (Sobolev & Begonia, 2008). Among these heavy metals, mercury is one of the potent neurotoxic heavy metals. Mercury is emitted into the environment through various natural and anthropogenic sources (Schroeder & Munthe, 1998). Primarily, the anthropogenic-derived sources of mercury include coal power plants, incineration of medical waste, discharge of agricultural wastewater containing pesticides and fertilizer, and municipal wastewater (Lacerda & Marins, 1997; Pacyna et al., 2006).

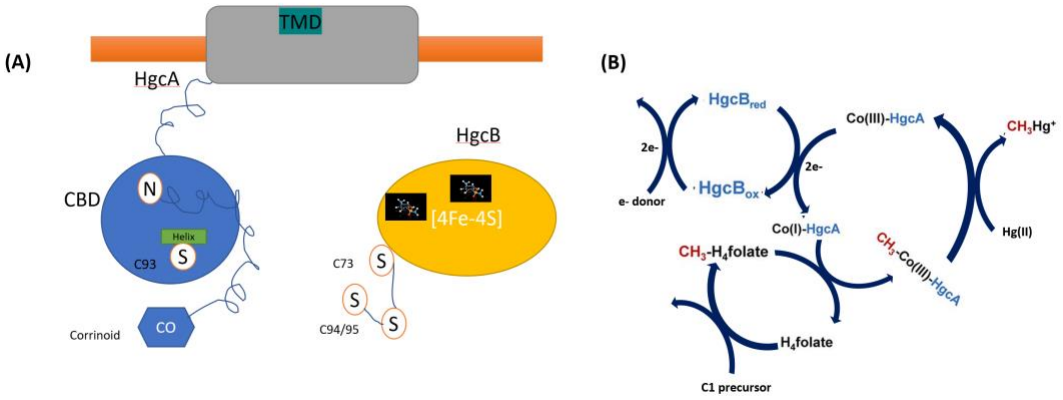
Groups of anaerobic microorganisms present in the soil sediment drive the biotransformation of inorganic mercury under anoxic conditions in aquatic ecosystems (Barkay & Wagner-Döbler, 2005). The mercury biotransformation process involves the conversion of inorganic mercury to highly neurotoxic organic methylmercury (MeHg). Certain groups of microorganisms that coexist in the river sediments are synergistically found to be involved in the mercury biotransformation. Studies have found that some bacteria belonging to Iron-reducing bacteria and Sulfur-reducing bacteria (Compeau & Bartha, 1985), Methanogens (Yu et al., 2013), and Firmicutes are found to be involved in the biotransformation of mercury (Gilmour et al., 2013). However, a better understanding and insight into microbial methylation is still unclear. Some studies have demonstrated that the production of toxic methylmercury is associated with the sulfur cycle and iron cycle as Hg methylation readily occurs in the sulfate and ferric reduction zone by some anaerobic microbes (Gilmour et al., 2011; King et al., 2001). Nevertheless, the characteristic of mercury methylation is uncommon among all sulfur or iron-reducing bacteria. Only a few species have the potential to methylate the mercury, such as order: *Desulfvovibrionales* (Gilmour et al., 2011)

A recent study done by Park et al. in 2013 identified that two clustered genes, *hgcA*, and *hgcB*, are mainly responsible for biotransformation. These genes are not commonly found in all groups of anaerobic microorganisms and therefore account for only about 1.4% of the sequenced genomes (Cooper et al., 2020; Podar et al., 2015). These gene clusters are distributed in microorganisms prevalent in diverse anaerobic settings such as soil sediments, wetlands, rice paddies, or even in the digestive tracts of some invertebrates (Podar et al., 2015). Methylation of mercury is an enzyme-catalyzed reaction that involves the reduction of acetyl Co-enzyme A (CoA) (Choi et

al., 1994). A putative corrinoid protein encoded by the gene *hgcA* functions in transferring a methyl group to inorganic mercury while the *hgcB* gene encodes 2[4Fe-4S] ferredoxin responsible for the reduction of *HgcA* (Poulain & Barkay, 2013). These two-gene clusters are predicted to be involved in the mercury methylation process. (Gilmour et al., 2013; Lin et al., 2021).

Figure 4

The hgcAB Gene Mediated Methylation Process



Note. Visual representation of *hgcA* and *hgcB* structure (A) along with methylation process mediated by *hgcAB* gene (B).

According to the U.S. Energy Information Administration (EIA), Texas is one of the most industrialized states and therefore is a leading state in energy production. The industrial sector in Texas includes petroleum industries, oil and gas refineries, and coal power plants. These industrial activities, along with other anthropogenic activities have been contributing to the heavy metal pollution of several riverine systems throughout history. In general, the issue of heavy metal pollution in the river sediment started in the early 1800s and became more prominent in the late 1900s. Although currently, there is a decreasing trend in heavy metal pollution with the monitoring of the input sources of the contaminants (Ravichandran et al., 1995a). Yet,

the effects of heavy metals can remain for the long-term because of their highly persistent and non-biodegradable characteristic (Wright & Welbourn, 2002).

The bacterial and fungal community structure and diversity were reported to be strongly affected in Hg contaminated soil (Frossard et al., 2018). The study by Frossard et al, 2018 also showed that the major factor responsible for bacterial tolerance to Hg stress in soil was dependent on the solubility of Hg in soil, pH, and organic matter. Many studies have highlighted that long-term heavy metal contamination of soil has a significant effect resulting in alteration in the microbial activity, microbiome composition, diversity, and distribution pattern in the soil due to the sensitivity of several microbes to heavy metal stress (Sobolev & Begonia, 2008). For instance, Cadmium and Chromium can alter the physiological or biological activities of microbes by inducing oxidative damage or inhibiting enzymatic activities (Igiri et al., 2018).

A recent study was done in Galveston Bay estuary, Texas to explore various heavy metals (As, Cu, Hg, Cr, Sb, Ni, Zn, and Pb) concentration distribution in the sediments and their potential toxicity in the estuary influenced by anthropogenic activities. The study depicted that the influx of heavy metals into the bay was dominated by anthropogenic sources such as urban and industrial runoffs, leakage of chemical waste, flood water, and improper discharge from wastewater treatment plants resulting in an increased level of potentially toxic heavy metals like As, Cd, Hg, Cr, Zn, and Pb in the soil sediments (Lopez et al., 2022).

Study of Microbiome Composition in the Trinity River

One of the longest rivers in Texas (DSHS, 2015), the Trinity River, which runs through a highly industrialized metroplex, is significantly influenced by rapidly growing numbers of industries, population, urbanization, and many other human

activities in the metroplex cities and urban and rural areas over the past several years. The presence of a coal power plant at a closer distance from the riverbank makes it an ideal freshwater river system to study the effect of heavy metal pollution, especially mercury pollution, and its impact on microbiome composition in the soil sediments. However, there have not been many studies done to determine the impact on microbial communities with the heavy metal contamination in Trinity River. It is, therefore, crucial to understand how the microbiome community and its diversity are affected in response to mercury contamination as some groups of anaerobic bacteria plays a key role in the mercury reduction and methylation process (Y.-R. Liu, Zheng, et al., 2014; Rothenberg & Feng, 2012).

Limited studies have been conducted on the long-term impact on the microbiome composition of soil sediments with an elevated mercury concentration. However, a study of a similar type was conducted in the freshwater river system, the Mazaruni river in South America. This river has been influenced by mercury pollution due to gold mining suggesting that the soil sediments with higher mercury concentrations are typically enriched with mercury methylating microorganisms (Obkirchner, 2019).

Objectives

In this study, the soil sediment samples from the Trinity River were examined for microbial composition. More specifically, the composition and abundance of microbial communities were compared along the sites of the Trinity River from upstream Dallas to the lower reaches of the Trinity River. Along this gradient, the river is impacted by industrial development, urbanization, and coal power plants, Therefore, the microbial communities in the soil sediment samples were examined to

explore the potential role of mercury methylating genera. The following hypotheses will be tested:

Hypothesis

Null Hypothesis (H_0): There will be no significant difference in the alpha and beta microbiome diversity of soil sediments along the river gradient of the Trinity River.

Alternate Hypothesis (H_a): There will be a significant difference in the alpha and beta microbiome diversity of soil sediments along the river gradient of the Trinity River.

Null Hypothesis (H_0): There will be no significant difference in mercury methylating genera in uncontaminated and contaminated soil sediments along the Trinity River.

Alternate Hypothesis (H_a): The soil sediments of the sites contaminated with higher mercury concentration will be enriched with mercury methylating genera compared to the non-contaminated sites leading to diversity in microbial composition and function.

Material and Methods

For microbiome analysis, four sampling locations and three representative sediment replicate containing Hg were selected and used for analysis due to the cost of each sample for microbiome analysis. The chosen locations were namely, Jacksboro (JB), Downtown Dallas (DT), and Oakwood (OW) representing the upstream sites, a considerably less contaminated region. On the contrary, Romayor (RM) represented the downstream site, a considerably more contaminated region. A total of 12 samples were sent to LC Sciences Houston, Texas for microbiome analysis.

The workflow of Genomic DNA analysis (Figure 5) involves six steps: DNA extraction, PCR amplification, Product purification, Library Preparation, and High throughput sequencing using Illumina NovaSeq platform paired-end reads (2x250 bp),

and Bioinformatics. The first five steps were performed at LC Sciences, Houston, TX, whereas we performed Bioinformatics.

Figure 5

Six Major Steps Involved in Genomic Analysis of 16S rRNA

DNA Extraction

The extraction of DNA from soil sediment samples from four different locations along the Trinity River was performed by LC Sciences following the DNeasy PowerSoil Pro Kit Protocol. Initially, 250 mg of each soil sample was loaded to PowerBead Pro Tube along with the 800 µl of solution CD1. The mixture was then briefly vortexed for 10 min to homogenize and lyse the bacterial cells present in the soil sample. The mixture was then centrifuged at 15,000 x g for 1 min. The supernatant thus obtained was transferred to a clean 2 ml Microcentrifuge tube. Next, 200 µl of solution CD2 was then added and vortexed for 5 sec. The role of Solution CD2 is to precipitate the non-DNA organic and inorganic material and cell debris and proteins in the pellet. It was then centrifuged for about 1 min at 15,000 x g. About 500-600 µl of the supernatant were further transferred into another clean 2 ml microcentrifuge tube taking care to avoid the pellet. Then, 600 µl of solution CD3 was added into the tube and again vortex for 5 sec. The solution CD3 is a salt solution of high concentration that allows the DNA to bind to the silica membrane in the MB spin column filter membrane. Next, 650 µl of lysate was loaded into an MB spin column and allowed to centrifuge at 15,000 g for about 1 min. After centrifugation, the contaminant passes through the filter membrane, leaving the DNA that remains bound to the membrane. This step was repeated until all the lysate was passed through the MB spin column, followed by discarding the flow through each time. The MB

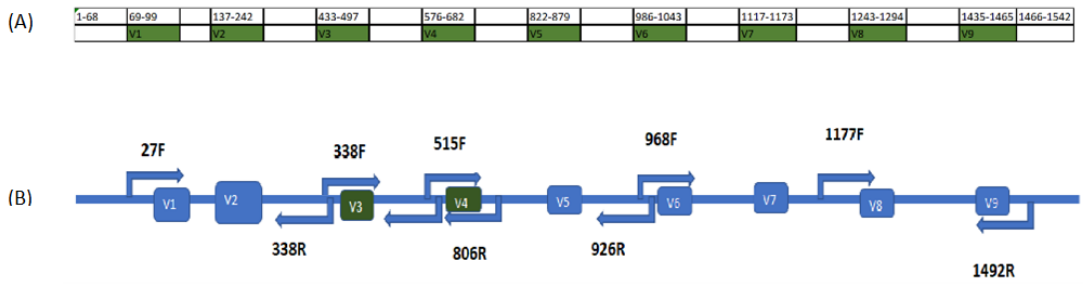
Spin Column was then carefully placed into a clean 2 mL collection tube. Next, 500 µl of EA solution, which is a wash buffer, was added to the MB spin column followed by centrifugation at 15,000 g for 1 min. The EA solution removes the other contaminants and proteins. The flow-through was discarded, and the MB spin column was placed back in the same collection tube into which 500 µl of Solution C5 was added and allowed to centrifuge for 1 min at 15,000 g. The C5 solution is another type of wash solution which is ethanol-based and helps to further clean the DNA that is bound to the silica filter membrane by removing the residual salt and humic acids. Again, the flow-through was discarded and the MB Spin Column was placed into a new collection tube. At this time, the tube was allowed to spin up to 16,000xg for 2 min and then was carefully placed the tube into a new 1.5 ml Elution tube. Then, about 50-100 µl of Solution C6 was added to the center of a white filter membrane which was then centrifuged at 15,000 g for 1 min. The C6 solution placed at the center of the membrane ensures the membrane is wet, which allows for more efficient and complete extraction of DNA. Finally, the DNA thus extracted from the sample was used for the downstream applications. The obtained DNA was stored at -80 ºC until further use.

PCR Amplification

The Polymerase chain reaction (PCR) amplification and purification steps were also performed by LC Sciences, Houston, TX, on the extracted DNA. The forward and reverse primers (341F/805R) were designed to target the variable regions V3 and V4 of the 16S rRNA region. The amplicon reads of approximately 465 bp in length were generated. NovaSeq PE250 platform paired-end reads (2x250) were used for sequencing the amplified library.

Figure 6

Graphical Representation of V3 and V4 Region in 16S rRNA



Note. Figure A show all variables and conserved regions of 16S rRNA. Variable regions are numbered and highlighted in green, whereas conserved regions are left blank. Variable regions are used for group or species-specific applications. Figure B shows the location of the V3 and V4 regions where PCR amplification was conducted. The blue arrow indicates the direction of the forward and the reverse primer.

Initially, the 5′ ends of the primers were tagged in each sample with a specific barcode following the protocol. For PCR amplification, a reaction mixture of a total volume of 25 µl was prepared. The reaction mixture comprised 2.5 ng of each primer, 24 ng of template DNA, and 12.5 µl of PCR premix, to which a PCR grade water was added to adjust the final total volume. The Polymerase chain reaction (PCR) amplification of the 16S fragments was performed by the initial step of denaturation at 98 ºC for 30 seconds. Then, 32 cycles of amplification were performed that comprised each cycle of denaturation at 98 ºC for 10 seconds. It was then followed by an annealing step at 54 ºC for 30 seconds. The final step was the extension which occurs at 72 ºC for 10 minutes. The PCR reaction tubes were then stored at 4 ºC.

PCR Purification

The PCR products thus obtained after completion of the amplification were confirmed with 2% agarose gel electrophoresis. To avoid the chances of false-positive PCR results, ultrapure water was used throughout the process as a negative control instead of the sample solution. The ultrapure water was also used during the DNA

extraction process. For the PCR product purification, AMPure XT beads (Beckman Coulter Genomics, Danvers, MA, USA) were used. It was then further quantified by Qubit (Invitrogen, USA).

Library Preparation

For sequencing, amplicon pools were prepared. Agilent 2100 Bioanalyzer (Agilent, USA) was used to assess the size of the amplicon, whereas Library Quantification Kit Illumina (Kapa Biosciences, Woburn MA, USA) was used to assess the quantity of the amplicon library. The amplified libraries were sequenced using the Illumina NovaSeq PE250 platform.

Illumina NovaSeqPE250

Sequencing outcomes of 12 different samples using the Illumina NovaSeqPE250 platform were obtained in fastq format with a quality score for each nucleotide read encoded in ASCII format. The higher ASCII value indicates higher confidence in nucleotide read accuracy (Bolyen et al., 2019).

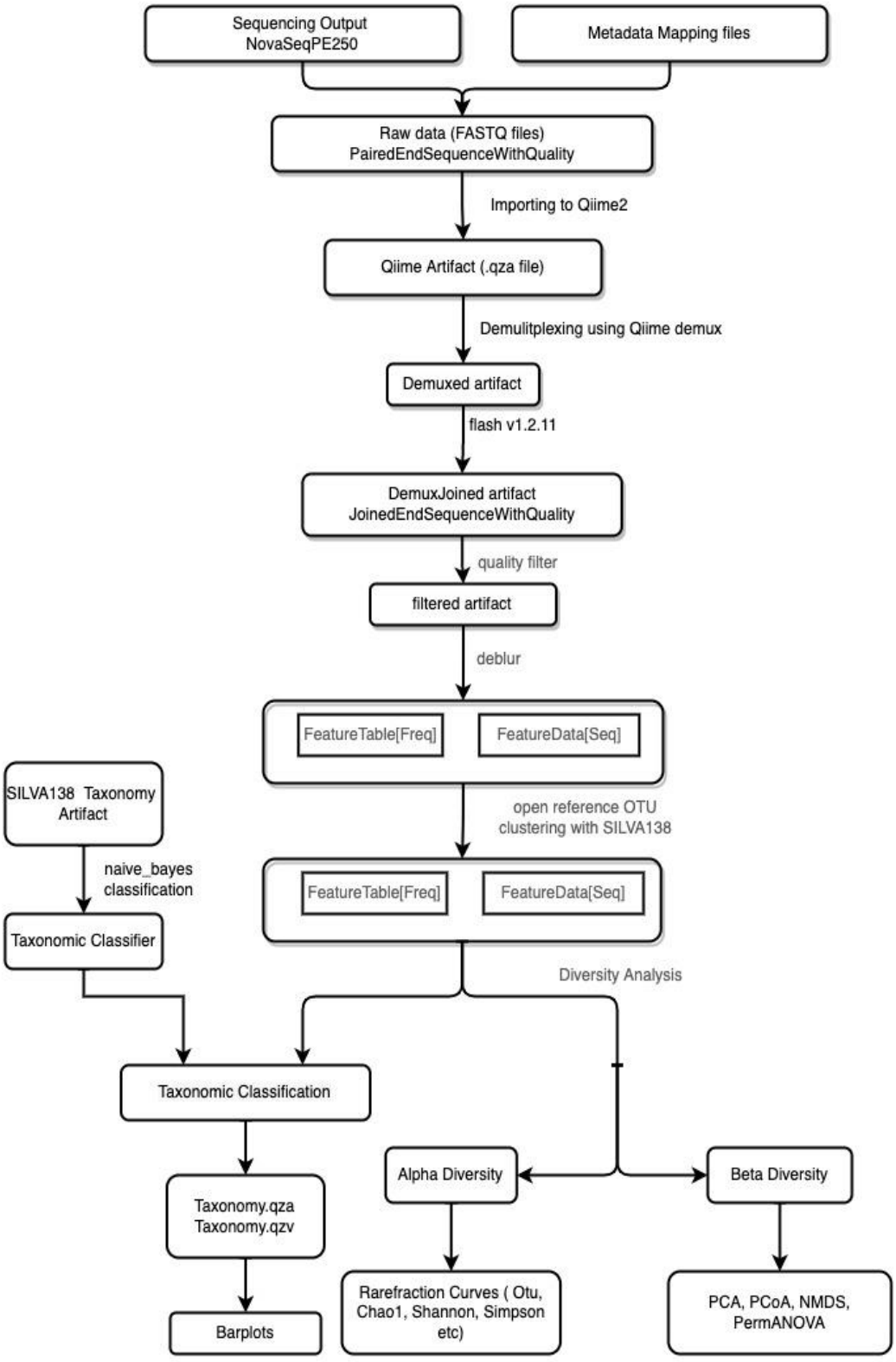
Bioinformatics

Bioinformatics includes three steps: Data analysis, Data Processing, and Statistical analysis.

Data analysis. The analyses of these raw files were carried out using the program QIIME (Quantitative Insights into Microbial Ecology) version 2.0. QIIME is one of the popular bioinformatics software to carry out microbiome analysis (Bolyen et al., 2019; Caporaso et al., 2010; Kuczynski et al., 2011). The overall workflow of 16S rRNA sequencing is shown in Figure 7 below.

Figure 7

Workflow of Microbiome Analysis in QIIME2.0



Note. High-level overview of microbiome analysis for taxonomy and diversity (Alpha and Beta) as performed in QIIME2 (Version: 2022.2).

Data processing. The sequencing output files were NovaSeqPE250 encoded. The semantic type of these sequencing outputs was SampleData [PairEndSequenceWithQuality], and the source format is SingleLanePerSampleEndFastqDirFm. Since they are paired-end sequences, each site had two files, one for a forward read and one for a reverse read. These multiple raw sequencing files were imported into QIIME2.0 to get a single qiime2 artifact. The artifact was demultiplexed using the Qiime-demux command to obtain a demux artifact. The demux artifact still holds reading from the forward end as well as a reverse end. Two reading were merged using the FLASH v1.2.11 to obtain a single continuous sequence tag so that different analyses can be performed. An artifact obtained after using the FLASH software has a data type of JoinedEndSequenceWithQuality. The merged sequences were then filtered based on length distribution and keeping sequence with a Phred quality score greater than 4 (default setting). This was achieved using the quality-filter command within QIIME 2. The QIIME 2.0 Vsearch plugin was used to remove the chimeric sequences, low-quality reads, or ambiguous reads to obtain a clean filtered artifact. In addition to that, the singletons were also discarded to minimize the error.

Further denoising was performed using deblur plugin to remove the noisy reads. At the end of filtering, denoising, and dereplicating, two artifacts were obtained: FeatureTable[Freq] and FeatureData[Seq] (rep-seq). A feature table is a tabular representation of different features obtained from the sample and their frequency in the samples, whereas a feature data is also a table representation of nucleotide sequences. This table and rep-seq were used with the SILVA138 rRNA sequence reference database to perform open reference OTU clustering with 97% sequence identity. Clustered artifacts (feature data and feature table) were then used

for diversity, taxonomical, and phylogenetic analysis. The rarefaction curve illustrates a relationship between the observed OTU and sequencing depth which is a measure of alpha diversity. When the curve is plateaued, it indicates that sequencing depth was sufficient to represent the sequence depths for the optimal OTU predictions for further analysis (Willis, 2019). The alpha diversity was calculated and visualized using different metrices like Observed OTU, Chao1, Simpson, and Shannon. Beta diversity was calculated using the Bray-Curtis method and visualized by the NMDS method.

For the taxonomic analysis, the SILVA138 rRNA taxonomy artifact was trained with a Naïve_bayes classifier to obtain a taxonomic classifier. This classifier was then used to perform a taxonomic classification of the data obtained by clustering. Bar plots at different taxonomical levels (Phylum, Order, Class, Family, and Genus) were obtained using the resulting taxonomic artifact.

Statistical analysis. Statistical analysis was performed using R-programming (version: 4.2.0). All the data obtained after sequencing and clustering at 97% sequencing identity thresholds were statistically analyzed for a significant difference in microbial composition using a non-parametric test (Kruskal-Wallis rank-sum test). To analyze the diversity of the bacterial community within each location, the alpha diversity was calculated (Hollister et al., 2015). Shannon index, Simpson index, and Chao1 were the diversity index measures that were calculated for the study. Goods coverage was calculated to measure sampling coverage for each sample. For statistical analysis of alpha diversity metrices, a non-parametric test and a Kruskal-Wallis rank-sum test (significant difference at $p<0.05$) were performed in the RStudio. For beta diversity between samples, Bray Curtis distance metrices were calculated in QIIME 2.0 to determine the difference in the microbial composition between the different locations. It was then visualized by Non-metric Multidimensional Scaling (NMDS) to

compare the microbial diversity between the communities. The stress coefficient measures the strength of the NMDS analysis result. In general, a stress<0.05 is very representative while stress<0.1 indicates a good ranking. Bray-Curtis distance mainly considers the abundance or the presence/absence of the species (Bray and Curtis, 1957). For statistical analysis of beta diversity, a non-parametric test, Permutational Multivariate Analysis of Variance (PERMANOVA), was performed.

Results and Discussion

Sequence Length Distribution and Sequence Diversity

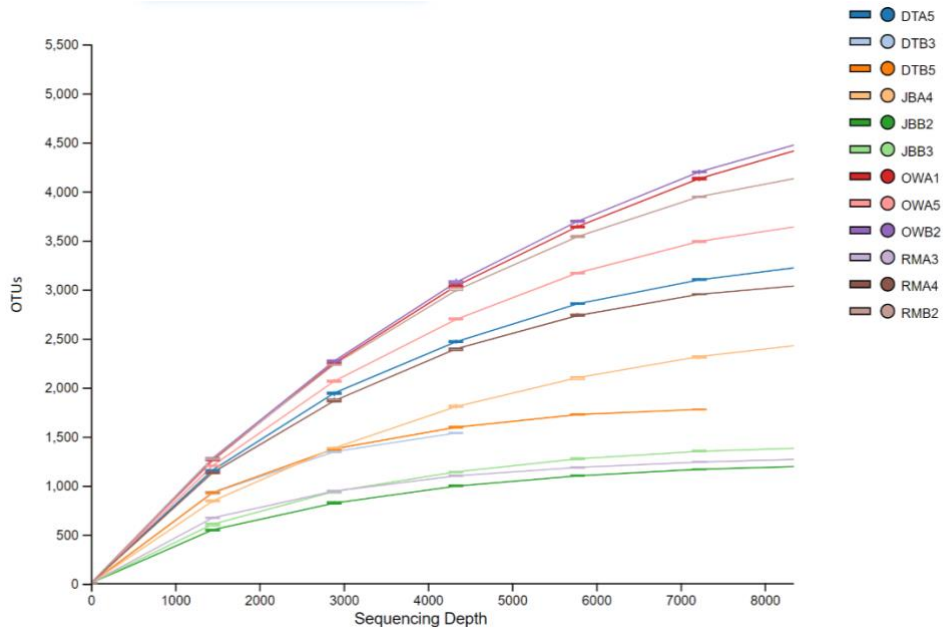
A total of 983,508 sequences of 16S rRNA were generated using the Illumina NovaSeq Paired-End 250 platform. Out of the total sequence obtained, the average number of sequences from each sample was 81,959. The maximum number of sequences observed among the 12 samples was 85,925, while the minimum number of the sequence was 79,383. The 16S rRNA sequence length distribution of each soil sediment sample was 445 nucleotides long.

Rarefaction Curve

The result shown in (Figure 8) demonstrated that Oakwood, site B, replicate 2 (OWB2) exhibited the highest diversity (Shannon index=11.41) whereas the least alpha diversity (Shannon index= 8.40) was observed in most upstream location Jacksboro, site B, replicate 2 (JBB2). The rarefaction curve plateau at a sequencing depth of 8,000 indicated that only a few unique OTUs would be detected with increasing sequencing depth from these samples. This signifies that samples sufficiently represented the original microbial communities from the corresponding sites.

Figure 8

Rarefaction Curve for Observed OTUs vs. Sequencing Depth



Note. Rarefaction curve showing OTUs vs. sequencing depth for twelve different sites. The curve indicated a rapid increase in number of OTUs with the increase in sequencing depth. After reaching the sequencing depth of 8000, the curve started to plateau with no further increase in the number of OTUs.

Table 6

Alpha Diversity Metrices (OTUs, Chao1) and Goods Coverage

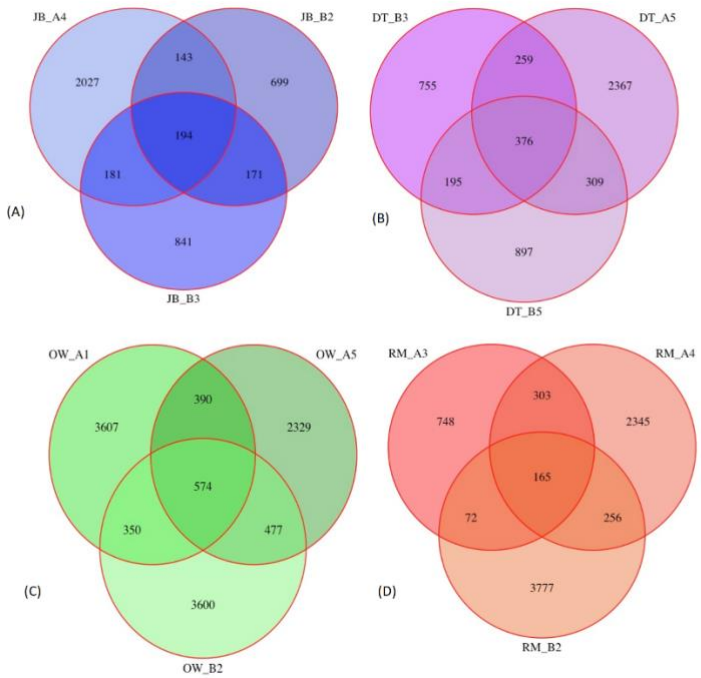
Sites	OTUs	Chao1	Goods Coverage
JBA4	2444	2682	0.91
JBB2	1198	1232	0.97
JBB3	1384	1433	0.96
DTA5	3250	3511	0.92
DTB3	1519	1589	0.96
DTB5	1598	1787	0.90
OWA1	4512	5697	0.80
OWA5	3678	4076	0.90
OWB2	4532	5726	0.79
RMA3	1263	1299	0.97
RMA4	3053	3220	0.94
RMB2	4170	4602	0.91

Note. OTUs, Chao1, and Goods Coverage for twelve sites at a sequencing depth of 8000. The closer the OTUs and Chao1 value, the higher will be the Goods Coverage value (range: 0-1).

A total of 23,095 OTUs were obtained after open reference clustering. The number of OTUs in 12 different samples ranged from 1,198 to 4,532 (Table 6). The result shows that there is a higher number of unique OTUs within the sites of the same location compared to the shared OTUs. Figure 9 shows a large difference in the community between soil samples from the same sites. This tends to support the idea that the beta diversity difference observed in Romayor sites compared to others could be due to soil factors other than Hg.

Figure 9

Number of Shared and Unique OTUs at Different Sites of Each Location Presented in Venn-Diagrams



Note: Figure A, B, C, and D represent the number of shared and unique OTUs in three replicate sites collected from each location of Jacksboro, Downtown Dallas, Oakwood, and Romayor, respectively.

Alpha Diversity

The measures of alpha diversity metrices include Observed OTU, Chao1, Shannon, and Simpson. The result indicates that Observed OTU and Chao1 values for

all 12 sites (Table 6) are close, resulting in a high value for good coverage. The value of goods coverage ranges from 0 to 1, where one represents that the sampling captured the true diversity of the community (Roswell et al., 2021). The majority values for goods coverage for our study are above 0.9 (Table 6), suggesting that the sequencing is complete and representative. The two sites from Oakwood, OWA1, and OWB2, have the lowest coverage of 0.80 and 0.79, respectively, because these sites have a higher number of singletons. Hence these two sites have the highest microbial diversity. The Kruskal-Wallis test showed no significant difference in the alpha diversity metrices within the location (p=0.001). The highest diversity was exhibited in Oakwood site B, replicate 2 (Shannon index=11.41 and Simpson index=1.0), which signifies higher species richness and evenness in this location compared to the other location. (Appendix B – Appendix E)

Beta Diversity

Using the Bray-Curtis dissimilarity in QIIME 2.0, pairwise distance metrics between 12 sample sites were calculated. The statistical analysis of beta diversity using PERMANOVA (p-value=0.001) showed a significant difference in beta diversity. Further, PERMANOVA pairwise comparison (Table 7) indicated no significant difference with p-values greater than 0.05. The non-significant statistical result for the pairwise comparison could be due to the small number of sample size. Moreover, it might be possible that although the number of species present in these four locations is not statistically different, the types of microbial species present in each location might differ, leading to diversity.

The NMDS ordination (Figure 10) showed a cluster of the microbial assemblages within each sample location, indicating that most of the species are shared. While considering the plot between the location, the ordinated for Romayor

appears to be clustered far away from the other three sample locations, suggesting differences in microbial composition. (Appendix F)

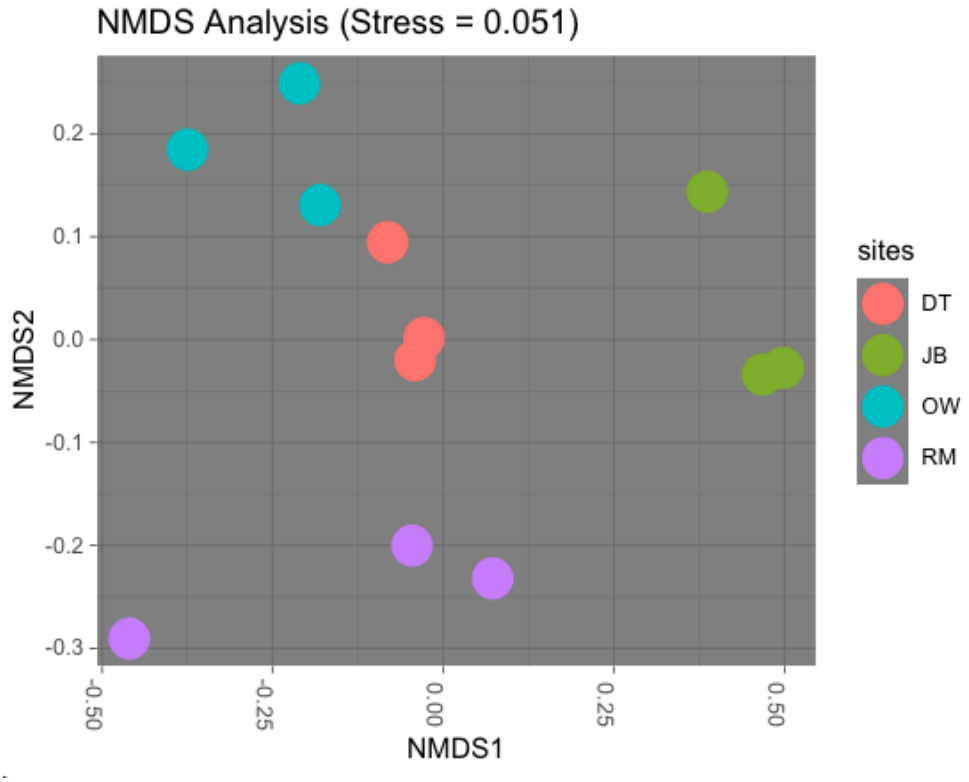
Table 7

PERMANOVA Pairwise Comparison for Beta Diversity Analysis

Locations	JB	DT	OW	RM
JB	1			
DT	0.109	1		
OW	0.112	0.094	1	
RM	0.118	0.383	0.116	1

Figure 10

Beta Diversity Visualized with NMDS Ordination



Note. Beta diversity of microbial communities presented in a non-metric multidimensional scaling (NMDS) ordination (stress=0.051). Different color represents the different location, and the distance between the point represents the degree of difference between the samples.

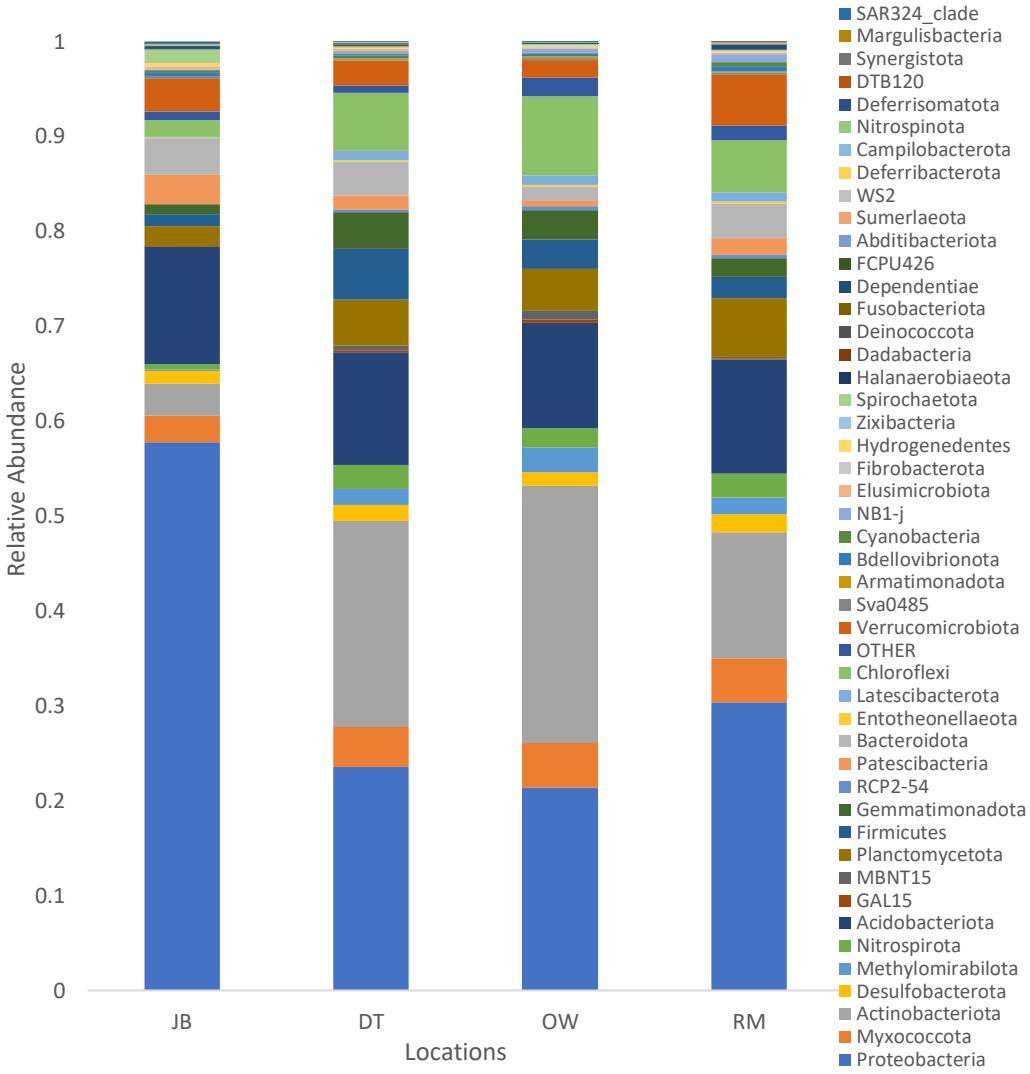
Distribution of Bacterial Phyla Abundance

The taxonomic identification of different phyla was identified (Appendix G). The taxa of each bacterial community in the soil sediment samples comprised of the phyla (Figure 11): *Proteobacteria, Firmicutes, Chloroflexi, Bacteriodetes, Nitrospirae, Spirochetes, Planctomycetes, Actinobacteria,* and *Acidobacteria*. Overall, these phyla contributed more than 90% of the sequences.

The most predominant phyla in Jacksboro were *Proteobacteria* (57.80%), *Acidobacteria* (12.35%), *Bacteroidota* (3.86%), and *Verrucomicrobiota* (3.45%). The phyla *Chloroflexi, Spirochaetota, Firmicutes, Nitrospirota,* and *Desulfobacterota* were 1.70%, 1.42%, 1.23%, 0.59%, and 1.32%, respectively. Similarly, in Downtown Dallas, the *Proteobacteria* was the most dominant phylum, accounting for 23.57% relative abundance, followed by *Actinobacteria* (21.71%), *Acidobacteria* (11.86%), and *Firmicutes* (6.09%). On the other hand, in Oakwood, the phylum with higher abundance was *Actinobacteriota* (27.10%), *Proteobacteria* (21.39%), *Acidobacteriota* (11.12%), *Chloroflexi* (8.37%), and *Myxococcota* (4.71%). The most downstream location Romayor with high mercury concentration included *Proteobacteria* (30.39%), *Actinobacteriota* (13.34%), *Acidobacteriota* (11.99%), *Planctomycetota* (6.22%), *Chloroflexi* (5.53%), and *Verrucomicrobiota* (5.40%).

Figure 11

Relative Abundance of Taxa at Phylum Level for Soil Sediments Collected from Four Different Sampling Locations



Distribution of Bacterial Family Abundance

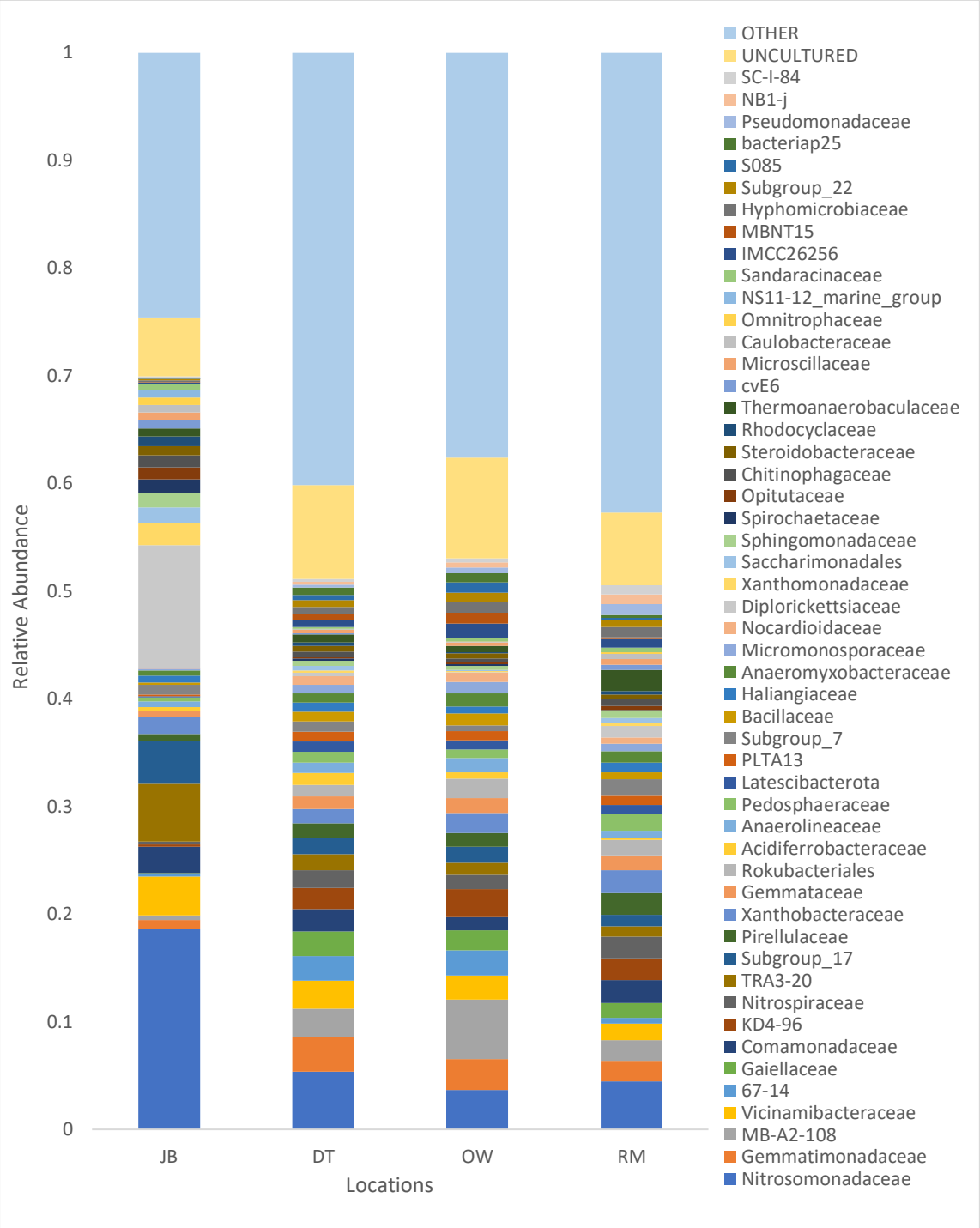
Based on the taxonomic identification, the top 30 abundant families were identified. The microbial distribution at the family level in the upstream location Jacksboro differed from the other three locations (Figure 12). The predominant family in upstream Jacksboro was *Nitrosomonadaceae* (18.66%), followed by *Diplorickettsiaceae* (11.37%), *TRA3_20* (5.37%), and *Vicinamibacteriaceae* (3.59%), respectively. The dominant bacteria in Jacksboro at the family level are more unique

than in the other three locations. The downstream location of Romayor, which contain a relatively higher mercury concentration, was dominated by the family *Nitrosomonadaceae* (4.48%), followed by *Xanthobacteraceae* (2.10%), *Nitrospiraceae* (2.03%). Downtown Dallas and Oakwood showed a similar trend in the taxonomic distribution at the family level with Romayor. However, the major mercury methylating microbes such as *Nitrospiraceae* were in higher proportion at Romayor (2.03%).

Nitrosomonadaceae was the most dominant family in 3 different locations, JB, DT, and RM, which were reported to be 18.66%, 5.35%, and 4.48%, respectively, whereas OW had MB-A2-108 as the most abundant family with 5.55%.

Figure 12

Relative Abundance of Taxa at Family Level for Soil Sediments Collected from Four Different Sampling Locations



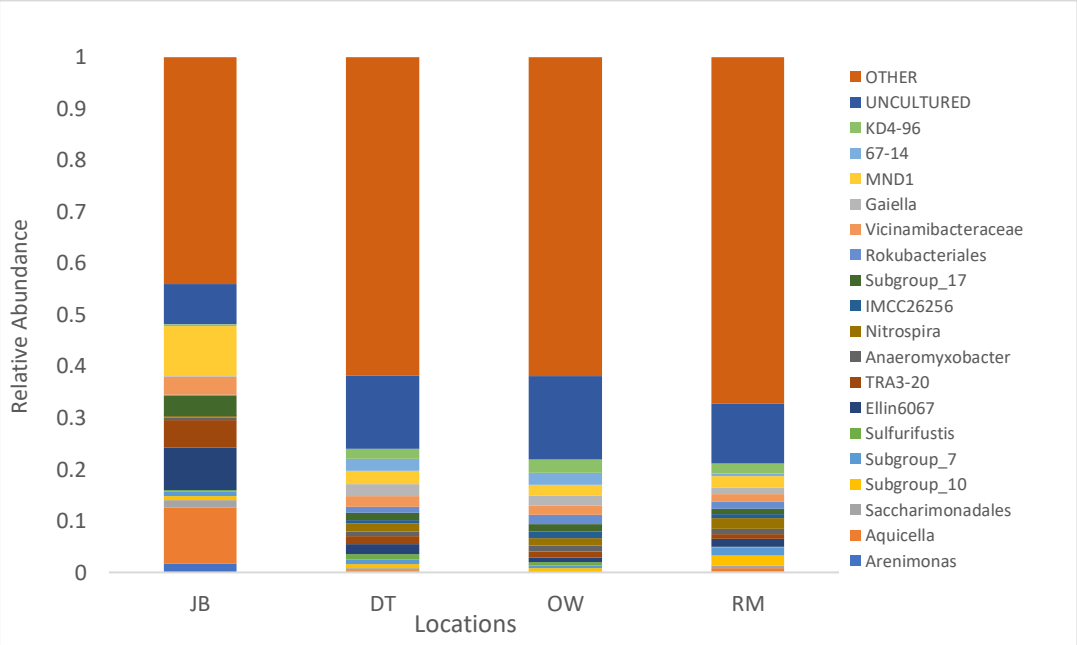
Note. The bar plot was generated using the 30 most abundant taxa at family level from each location. In the plot, OTHER represents cumulative of unidentified taxa at each location.

Distribution of Bacterial Genera Abundance

At the genera level, 18 genera were identified whose relative abundance was more than 1% in the entire study (Figure 13). Jacksboro, which is the most upstream part of the Trinity River, *Aquicella* (10.83%) was predominantly present, followed by MNDN1 (9.76%), belonging to the family *Nitrosomonadaceae*. Other predominant genera were *Ellin6067* (8.20%), *TRA3-20* (5.37%), and subgroup_17(4.00%). The relative abundance of *Spirocheaeta* and *Nitrospira*, known to contain the *hgcA* gene, accounted for 0.75% and 0.25%, respectively. The total of other genera was 31%, making Jacksboro one of the least diverse microbial communities among 4 locations.

Figure 13

Relative Abundance of Taxa at Genera Level for Soil Sediments Collected from Four Different Sampling Locations



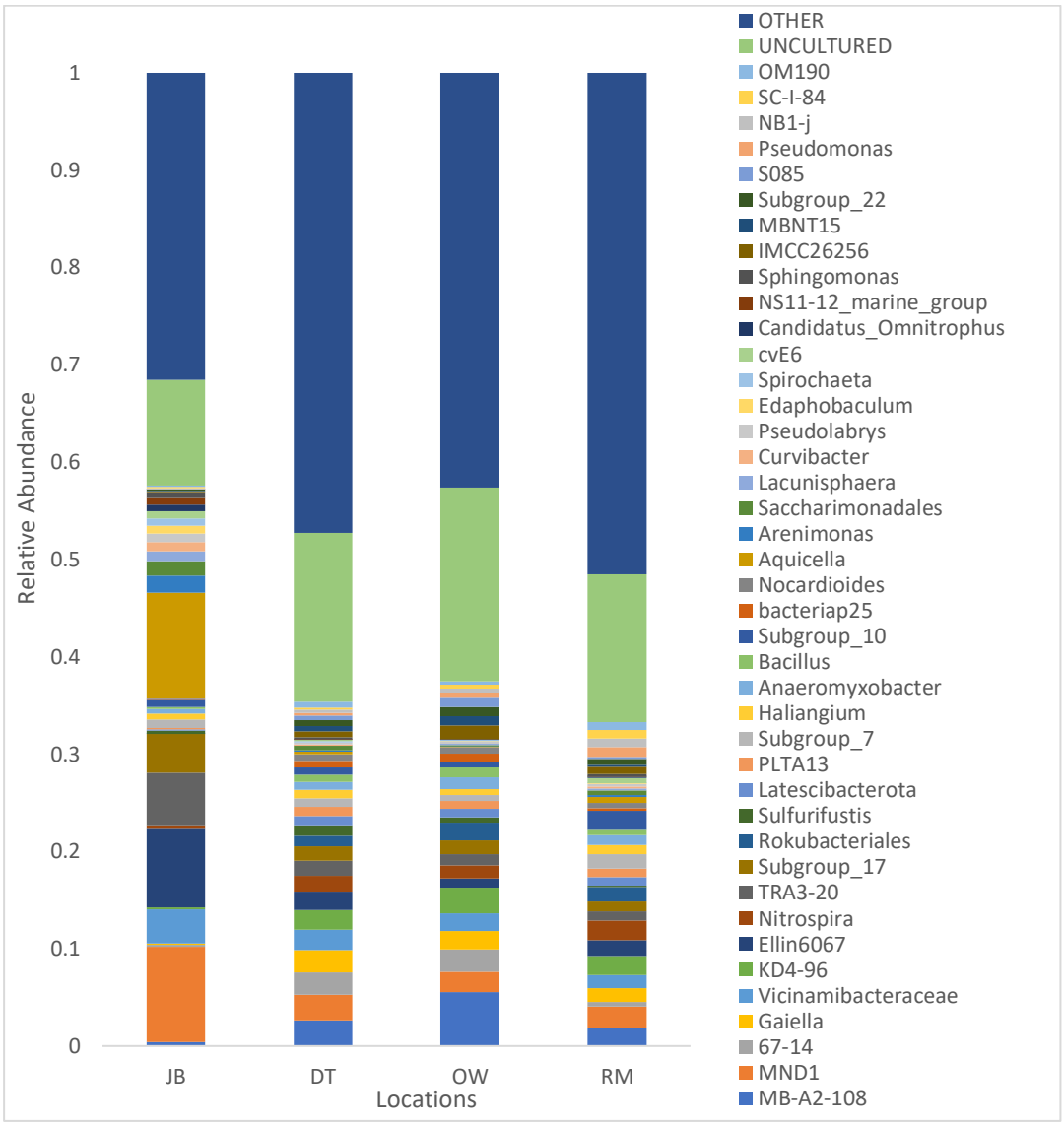
Note. Bar plot was generated using the taxa whose relative abundance is more than 1% at Romayor. OTHER represents cumulative of unidentified taxa at each location.

In Downtown Dallas, the highest relative abundance of *MB-A2-108* (2.66%), belonging to phylum *Actinobacteriota*, followed by MND1, 67-14, *Gaiella*, and

Vicinamibacteraceae with a relative abundance of 2.60%, 2.33%,2.24%, and 2.14%, respectively. Some of the genera that contain the *hgcA* gene involved in mercury methylation found in this location were *Nitrospira* (1.62%), *Spirochaeta* (0.17%), and *Pseudomonas* (0.28%). Oakwood, located at the closest distance from several power plants, also had *MB-A2-108* (5.55%) as the highest occurring genera. Other dominant genera were, *KD4-96* (phylum: *Chloroflexi*), 67-14 (phylum: *Actinobacteriota),* *MND1*(phylum: *Proteobacteria*) and *Gaiella* (phylum: *Actinobacteriota)* were 2.61%, 2.30%, 2.05%, and 1.91%, respectively. *Nitrospira* (1.34%) was comparatively lower than in Downtown Dallas. Romayor, the only downstream location of this study with comparatively high mercury concentration level, comprised *MND1*(2.12%) and *Nitrospira* (2.03%) as two of the most abundant genera. Figure 14 represents the top 30 abundant genera in all four locations. A comprehensive list of all genera identified can be found in Appendix H.

Figure 14

Relative Abundance of Top 30 Genera in the Soil Sediments Collected from Four Different Sampling Locations



Note. Bar plot was generated using the 30 most abundant taxa from each location. OTHER represents cumulative of unidentified taxa at each location.

Distribution and Abundance of hgcAB Gene-Containing Bacterial Genera

Comparison with NCBI database. A list of bacteria containing gene pair *hgcAB* was identified using NCBI protein blast (U.S. National Library of Medicine, 2022). The searching criteria were mainly focused on identifying mercury

methylating bacteria such as Sulfur and Iron-reducing bacteria typically found in soil sediments. A total of 25 blasts were conducted to maximize the match of hgcAB containing genera present in our study data compared to the NCBI reference database. As a result, a total of 2500 microbial genera containing the *hgcAB* gene were obtained after the protein blast.

Upon removal of duplicates, a total of 300 unique genera were identified. These 300 unique genera were compared with the genera found in 12 sites to identify genera with the *hgcAB* gene found in our sample. A match of 100 genera was found which contain the *hgcAB* gene pair (Appendix I). The primary focus of this study was to distinguish such genera in the sites with a high concentration of Hg. The genera at four different locations had the *hgcAB* gene present and had a relative abundance of more than 0.1% at RM (Table 8). A complete table including all 100 genera can be found in the appendix.

Table 8

Relative Abundance of Genera in the Soil Sediments Collected from Trinity River That Possesses hgcAB Gene Pair as Compared with Genera Obtained from NCBI Blasting

hgcAB+ Genera	Relative Abundance (%)			
	DT	JB	OW	RM
Nitrospira	1.62	0.25	1.34	2.03
Candidatus_Protochlamydia	0.3	0.1	0.06	0.68
Flavobacterium	0.05	0.13	0	0.63
Candidatus_Magasanikbacteria	0.12	0.22	0.01	0.35
Mycobacterium	0.17	0.08	0.14	0.32
Candidatus_Nomurabacteria	0.08	0.06	0.14	0.28
Candidatus_Solibacter	0.25	0.01	0.27	0.21
Anaerolinea	0.1	0.07	0.05	0.16
Geothermobacter	0.03	0.04	0.03	0.16
Candidatus_Udaeobacter	0.03	0.03	0.14	0.16
Candidatus_Kaiserbacteria	0.14	0.05	0.1	0.15
Candidatus_Omnitrophus	0.04	0.69	0.05	0.13
Corynebacterium	0.13	0.04	0	0.12
Citrifermentans	0.13	0.02	0.13	0.12
Candidatus_Berkiella	0.05	0.14	0.05	0.11

The result depicted that *Nitrospira* is the most dominant *hgcAB*-containing genus in 3 out of 4 locations, with only Jacksboro having *Candidatus_Omnitrophus* as a highly abundant genus. The high relative abundance of *Candidatus_Omnitrophus* in Jacksboro might suggest that although this genus contains the hgcAB gene, it could be inactive but rather be selective over other metabolic pathways besides mercury methylation. Our data indicate that among four locations, Romayor has the highest overall abundance of mercury methylating genera, possibly due to the higher concentration of mercury in this region. More analysis will be needed in the future to have a better understanding and to justify whether it is just the mercury in the soil sediments or several other factors that are responsible for such findings.

Conclusion

This study provides information about diverse groups of soil microorganisms that were identified in the sediment samples influenced by different concentrations of Hg levels along the Trinity River, Texas. Based on alpha diversity result suggested no significant difference in the microbial community pattern within each location. It might be possibly due to the low sample size. Similarly, the beta diversity, which represents the microbial communities between the sites, was inconclusive because the overall PERMANOVA was significant, but the pairwise was not. Therefore, the findings remained inconclusive for the microbial diversity (alpha and beta diversity) which might be because of several other factors that affect the soil microbiome community, such as temperature, carbon and nitrogen availability, water turbidity, pH, etc. Thus, the Hg could be just one factor among many environmental differences between the soil sites.

It was also hypothesized that the mercury methylating genera would be present in higher frequency at locations with elevated levels of mercury. The result

supported the alternate hypothesis that there is a relatively higher abundance of mercury methylating microbial genera such as Sulfur reducing bacteria (SRB) and Iron reducing bacteria (IRB) in the downstream location than in upstream locations. SRB is classified as a complex, heterogeneous, and anaerobic bacteria commonly found in an anoxic environment. Groups of some SRB and IRB are typically found to be involved in the process of biotransformation of mercury due to the presence of mercury methylating gene *hgcAB* (Compeau & Bartha, 1985; Gilmour et al., 2013). The genera of SRB, such as *Desulfovibrio, Desulfobulbus, Desulfococcus, Desulfobacteria, and Desulfosarcina,* and genera of IRB, such as *Geobacter* were mostly detected in the downstream site, Romayor indicating its abundance might possibly be linked with the presence of elevated mercury in the sediment sample. It can be explained by the fact that IRB and SRB are active mercury methylating groups that have the ability to biotransform the available mercury in the soil sediment and thrive in the higher mercury concentration due to the presence of the gene cluster *hgcAB.*

Based on the taxonomic distribution, the phylum *Proteobacteria* accounted for the highest relative abundance in both the mercury-contaminated and non-contaminated locations was *Proteobacteria*. Similarly, *Bacteroidetes and Actinobacteria* were predominant in sites with a high Hg concentration which might be associated with the presence of the mercury-resistant *merA* gene. The *merA* gene encodes mercuric reductase that enzymatically reduces Hg^{2+} to Hg^{0} (Møller et al., 2014) In addition to that, recent studies have demonstrated that microorganisms belonging to the phylum *Firmicutes, Chloroflexi, Nitrospirae, Spirochaete*, and *Methanogens* are also active mercury methylators and their abundance is higher in soil with elevated mercury concentration (Hur & Park, 2019; Silva-Bedoya et al.,

2016). Based on the taxonomic distribution, our study also corroborates that these phyla were relatively higher in abundance in Romayor compared to other locations.

The observed difference in the microbiome community composition along the Trinity River gradient could be attributed to several factors such as increased anthropogenic activities, environmental parameters of soil sediments as well as the elevated mercury input from the industrial power plants located nearby the river. The intensive anthropogenic activities influence the biodiversity of the soil, which alters the microbial composition and diversity (Fatimawali et al., 2020; Mahbub et al., 2017). Additionally, various physicochemical (temperature, pH, salinity, dissolved oxygen), biological parameters, and nutrient availability in the surrounding environment might also be responsible for such differences in the abundance of certain groups of bacterial phyla. For example, the mobility and bioavailability of heavy metals in soil are highly influenced by several environmental parameters such as presence of organic and inorganic contents in the soil, pH, redox potential, turbidity and temperature of the water (Kelly et al., 1995). Therefore, an improved understanding of the biological and physicochemical changes in the surrounding riverine ecosystem is necessary to understand the changes in the microbial pattern (Steichen et al., 2020).

The microbial diversity reduces due to changes in the natural environment, such as heavy metal stress. Only certain groups of microbes that are physiologically able to adapt to the changing environment are capable of surviving, while those that are vulnerable gets wiped out (Mahbub et al., 2017; Sheik et al., 2012). Therefore, the presence of heavy metals in soil has a greater influence on alteration in microbial diversity (Kamal et al., 2010). The reasons for the observed shift in microbial composition along the river gradient from the upstream Jacksboro to the extreme

downstream Romayor might possibly be due to several other factors contributing to mercury pollution in the river system, of which power plants could potentially be one of them. Therefore, future research is needed to better understand the multiple stressors affecting the microbial communities in the Trinity River by sampling the soil sediments from more sites all the way from upstream to downstream in Galveston Bay.

CHAPTER IV

Future Work

This study was observational and the first in a series, therefore, future research could be more experimental, based on the available data that will be useful in deriving a better conclusion regarding the role of mercury and its impact on microbial composition. Some of the potential future research of interest include:

1. Functional metagenomic prediction using PiCRUST

   Metabolic functions of bacterial communities will be characterized using PiCRUST (Phylogenetic Investigation of Communities by Reconstruction of Unobserved States) (Langille et al., 2013). This tool utilizes the OTU table and the representative sequence for each OTU and uses phylogenetic inference to predict the gene content of a particular OTU. The predicted gene content will then be mapped with databases like KEGG and MetaCyc to give a picture of the pathway abundances of a given sample.

2. PCR amplification and sequencing of *hgcAB* genes

   The *hgcAB* gene cluster will be targeted for PCR amplification using primers. The PCR products will then be purified and cloned using Invitrogen's TOPO TA cloning kit for sequencing and transformed into competent *Escherichia coli*. Approximately, 100 independent colonies will be randomly selected for DNA preparation and sequencing for further study.

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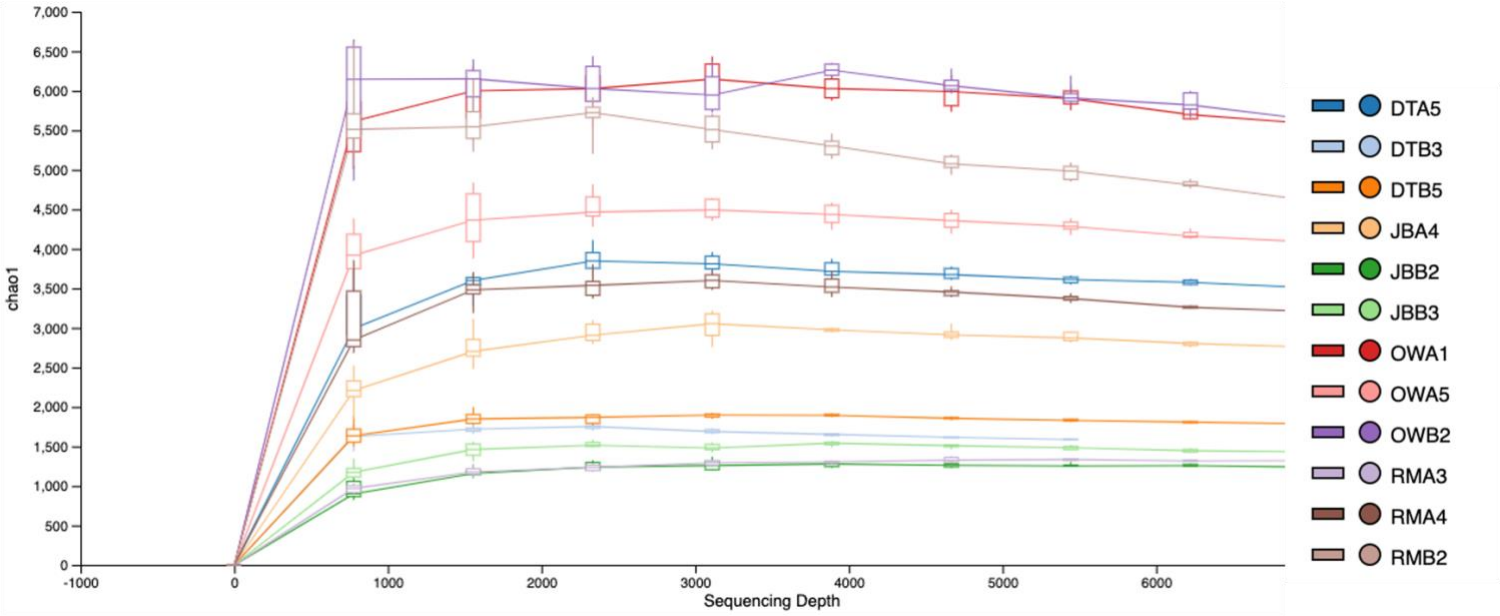
APPENDIX A

Hg concentration of soil sediments collected from different locations in µg per gm of soil

Locations	Hg Conc	Locations	Hg Conc	Locations	Hg Conc	Locations	Hg Conc	Locations	Hg Conc
JBA1	0.0507	DTA1	0.0578	DOA1	0.0623	OWA1	0.0718	RMA1	0.0262
JBA2	0.0628	DTA2	0.0511	DOA2	0.0495	OWA2	0.0596	RMA2	0.4218
JBA3	0.0595	DTA3	0.0441	DOA3	0.0578	OWA3	0.0580	RMA3	0.6783
JBA4	0.0369	DTA4	0.0531	DOA4	0.0596	OWA4	0.0596	RMA4	0.8886
JBA5	0.0463	DTA5	0.0402	DOA5	0.0474	OWA5	0.0547	RMA5	0.4194
JBB1	0.0664	DTB1	0.0442	DOB1	0.0657	OWB1	0.0498	RMB1	0.1947
JBB2	0.0321	DTB2	0.0440	DOB2	0.0391	OWB2	0.0687	RMB2	0.9747
JBB3	0.0401	DTB3	0.0427	DOB3	0.0447	OWB3	0.0327	RMB3	0.0790
JBB4	0.0451	DTB4	0.0454	DOB4	0.0344	OWB4	0.0606	RMB5	0.2955
JBB5	0.0618	DTB5	0.0338	DOB5	0.0484	OWB5	0.0423	RMB5	0.2658

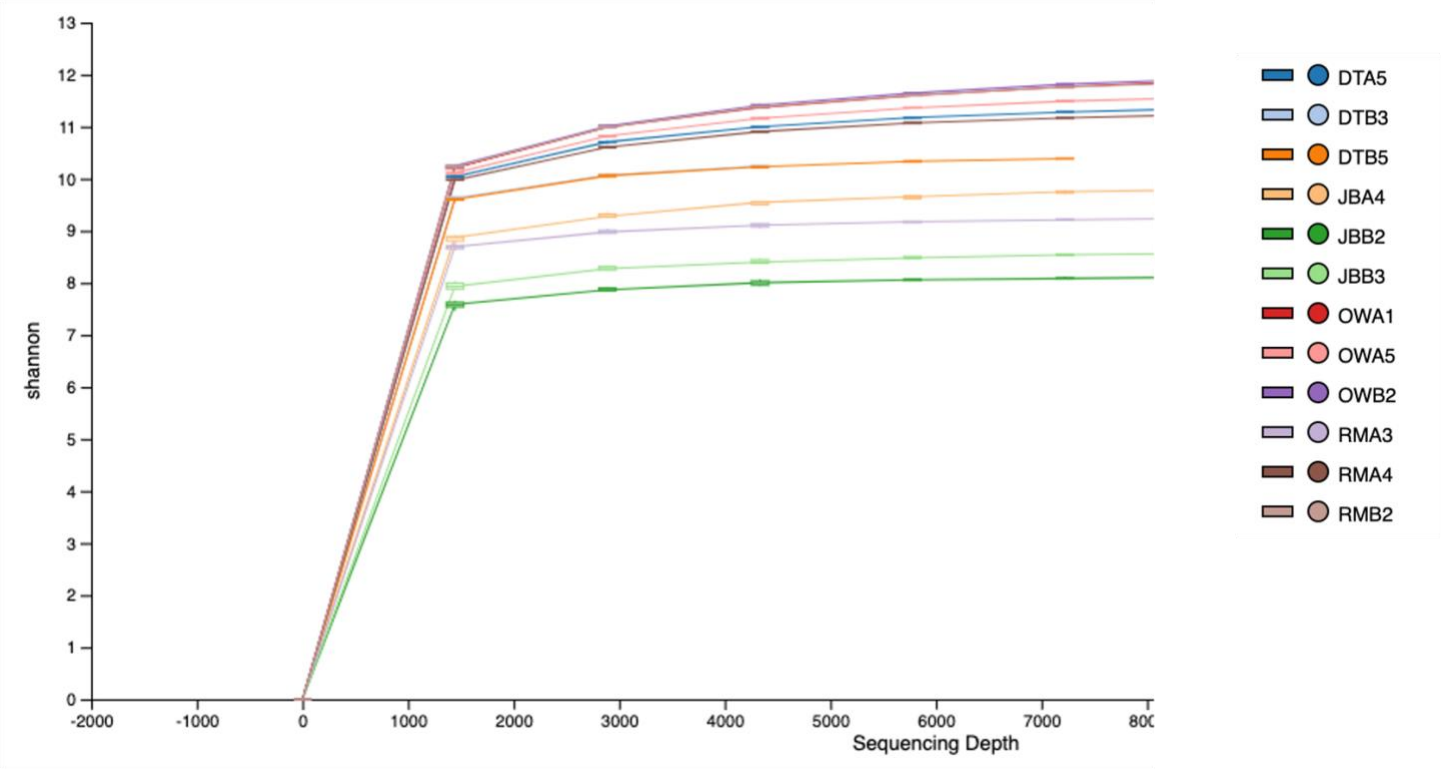
APPENDIX B

Alpha diversity: Chao1



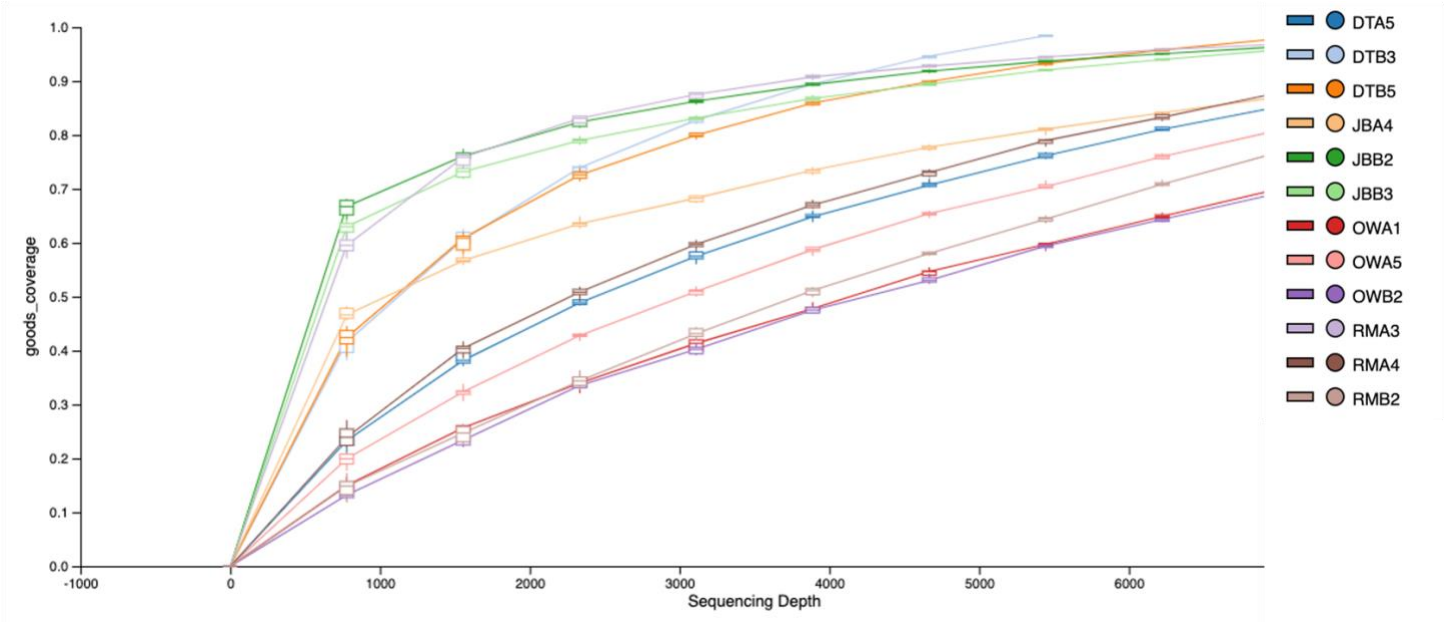
APPENDIX C

Alpha diversity: Shannon



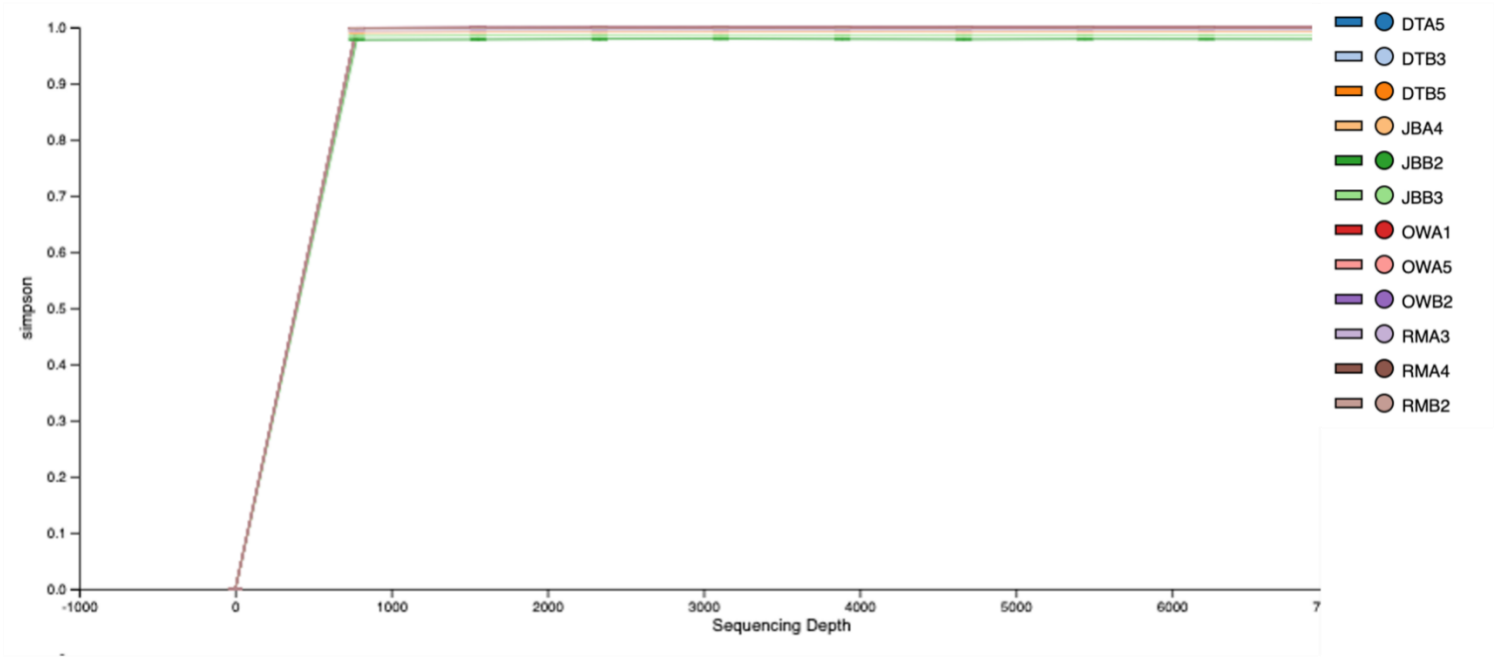
APPENDIX D

Alpha diversity: Goods Coverage



APPENDIX E

Alpha diversity: Simpson



APPENDIX F

Bray-Curtis distance matrix

Sites	JBA4	JBB2	JBB3	DTA5	DTB2	DTB3	OWA1	OWA5	OWB2	RMA3	RMA4	RMB2
JBA4	0	0.67	0.72	0.91	0.93	0.94	0.95	0.93	0.92	0.91	0.92	0.95
JBB2		0	0.61	0.92	0.94	0.94	0.97	0.96	0.95	0.93	0.95	0.97
JBB3			0	0.92	0.92	0.93	0.97	0.95	0.95	0.9	0.95	0.97
DTA5				0	0.74	0.74	0.81	0.77	0.79	0.9	0.86	0.89
DTB2					0	0.68	0.82	0.79	0.82	0.9	0.85	0.91
DTB3						0	0.81	0.79	0.83	0.88	0.83	0.91
OWA1							0	0.76	0.8	0.93	0.87	0.9
OWA5								0	0.74	0.92	0.86	0.89
OWB2									0	0.93	0.87	0.89
RMA3										0	0.81	0.93
RMA4											0	0.89
RMB2												0

APPENDIX G

Relative abundance at phyla level

Phyla	JB	DT	OW	RM	Phyla	JB	DT	OW	RM
Proteobacteria	0.578009788	0.235674368	0.21390535	0.303870259	*NB1-j*	0.000880914	0.002752523	0.004478549	0.009045681
Myxococcota	0.02812398	0.04224706	0.047106693	0.045971441	*Elusimicrobiota*	0.002707993	0.00187672	0.001720418	0.001356852
Actinobacteriota	0.033311582	0.217115689	0.270979546	0.133359178	*Fibrobacterota*	0.000293638	0.000458754	0.000600781	0.000419978
Desulfobacterota	0.01324633	0.016348319	0.014118354	0.018479033	*Hydrogenedentes*	0.003491028	0.000875803	0.000655397	0.001227628
Methylomirabilota	0.001435563	0.017224122	0.026379748	0.017606771	*Zixibacteria*	0.000522023	8.34098E-05	0.000655397	0.000710732
Nitrospirota	0.005905383	0.025398282	0.019962315	0.025554048	*Spirochaetota*	0.014225122	0.001793311	0.001775035	0.000452284
Acidobacteriota	0.123523654	0.118608725	0.111226413	0.119887575	*Halanaerobiaeota*	0	0	5.46165E-05	0
GAL15	0.000228385	0.002168655	0.003331604	0.001324546	*Dadabacteria*	0	8.34098E-05	5.46165E-05	0
MBNT15	0.000783034	0.005213112	0.009967503	0.001550688	*Deinococcota*	0.000522023	0.001251147	0.000218466	0.000775344
Planctomycetota	0.021207178	0.048502794	0.043556624	0.062189055	*Fusobacteriota*	6.52529E-05	0.000333639	0	0
Firmicutes	0.012267537	0.052840103	0.030721756	0.022065	*Dependentiae*	0.00228385	0.001000918	0.000355007	0.00468437
Gemmatimonadota	0.010440457	0.038660439	0.031104072	0.020223558	*FCPU426*	0.000946166	0.000375344	5.46165E-05	0.000193836
RCP2-54	0.000228385	0.002919343	0.003987001	0.00339213	*Abditibacteriota*	0	0	0.000109233	9.6918E-05
Patescibacteria	0.031223491	0.014721828	0.006745132	0.017154487	*Sumerlaeota*	0.000261011	0.00016682	0.000273082	0.001712218
Bacteroidota	0.03862969	0.036241555	0.013736039	0.037054985	*WS2*	6.52529E-05	0.000291934	0.000109233	0.00032306
Entotheonellaeota	0.000195759	0.001000918	0.002375816	0.002035278	*Deferribacterota*	0	8.34098E-05	0	0
Latescibacterota	0.001207178	0.0102177	0.009612496	0.009304129	*Campilobacterota*	0.000717781	0.00016682	0	0
Chloroflexi	0.016998369	0.060930853	0.08367241	0.055340182	*Nitrospinota*	0.000391517	0.000542164	0.000355007	0.000226142
OTHER	0.0091354	0.00792393	0.019470767	0.014957679	*Deferrisomatota*	0	0.00016682	0.000382315	0.000387672
Verrucomicrobiota	0.03451876	0.026107265	0.018569595	0.05398333	*DTB120*	0.000163132	0	5.46165E-05	0.000193836
Sva0485	0.00274062	0.000500459	0.002348508	0.001582994	*Synergistota*	9.78793E-05	0	0	0
Armatimonadota	0.000619902	0.002085245	0.001938884	0.001647606	*Margulisbacteria*	0.000358891	0	0	0
Bdellovibrionota	0.004176183	0.003086162	0.002594282	0.005459714	*SAR324_clade*	0.001990212	0	5.46165E-05	6.4612E-05
Cyanobacteria	0.001859706	0.00196013	0.000628089	0.004135168					

APPENDIX H

Relative abundance at genera level

Genera	DT	JB	OW	RM	Genera	DT	JB	OW	RM
Pseudomonas	0.00283593	0.00019575	0.00543433	0.0098533	*Variovorax*	0	0	0	0.00019383
CCD24	0.00162649	0.00094616	0.00245774	0.0014537	*Plesiomonas*	0	0	0	6.4612E-05
uncultured	0.00141796	0.00538336	0.00270351	0.0039736	*Subgroup_13*	0	0	0.00013654	0.00025844
uncultured	0.04633414	0.00427406	0.04803517	0.0174129	*WCHB1-41*	0.00025022	0.00055464	8.19247E-	0.00106609
uncultured	0.00075068	0.00019575	0.00106502	0.0018091	*Thermoanaerobaculum*	0.00012511	0.00026101	0.00010923	6.4612E-05
Rokubacteriales	0.01088497	0.00039151	0.01788688	0.0143761	*CL500-3*	0	0	0	0.00012922
Gaiella	0.02243723	0.00110929	0.01906114	0.0138592	*Z-35*	0	0	0	6.4612E-05
Anaeromyxobacter	0.00850779	0.0045677	0.01226139	0.0102733	*uncultured*	0	0.00032626	5.46165E-	0.00074303
Ketobacter	0	0.00019575	0.00079193	0	*Dokdonella*	0	0	0	0.00012922
67-14	0.02327133	0.00218597	0.02299352	0.0052335	*Gemmatimonadaceae*	0.00075068	6.52529E-	0.00060078	0.00019383
MND1	0.02602385	0.09758564	0.02050847	0.0212250	*Sulfuritalea*	0.00062557	0.00075040	5.46165E-	0.00083995
Nitrospira	0.0161815	0.00251223	0.01340834	0.0203204	*Acidipila*	0	0	0	0.00022614
Ellin6067	0.01943448	0.08195758	0.00985827	0.0157976	*uncultured*	0	0	0	6.4612E-05
PLTA13	0.00925848	0.00150081	0.00819246	0.0084641	*Sediminibacterium*	0.00029193	0.00022838	0	0.00061381
MB-A2-108	0.02656601	0.00427406	0.05554493	0.0190928	*Rhodobacteraceae*	0	9.78793E-	5.46165E-	0.00012922
Subgroup_17	0.01455500	0.03996737	0.01455528	0.0105640	*Xanthobacteraceae*	0	0	0	0.00032306
Sulfurifustis	0.01084327	0.00309951	0.00576203	0.0015506	*Rubritepida*	0	6.52529E-	0	0.00019383
GAL15	0.00216865	0.00022838	0.00333160	0.0013245	*uncultured*	0.00041704	0	0.00027308	0.00061381
Aetherobacter	0.00045875	0.00071778	0.00030039	0.0002584	*Stenotrophobacter*	0.00016682	0.00013050	5.46165E-	0.00029075
MBNT15	0.00521311	0.00078303	0.00996750	0.0015506	*Paracoccus*	0.00020852	9.78793E-	0	0.00035536
Pir4_lineage	0.00141796	0.00137031	0.00204811	0.0006138	*SM2D12*	0	0.00185970	0	6.4612E-05
Uncultured	0.00358662	0.00016313	0.00718206	0.0019706	*Paucisalibacillus*	0	0	0	6.4612E-05
Bacillus	0.00742347	0.00208809	0.00974903	0.0053304	*Babeliaceae*	0.00037534	0.00026101	0.00016384	0.00067842
Tumebacillus	0.00408708	0.00065252	0.00207542	0.0009368	*Terrabacter*	0	0	0.00013654	0.00019383
uncultured	0.02990241	0.00707993	0.02523280	0.0173483	*Blautia*	0.00025022	0	0	6.4612E-05
Haliangium	0.00859120	0.00652528	0.00644474	0.0091749	*CCM19a*	0.00016682	6.52529E-	0.00021846	0.00032306
Bauldia	0.00070898	0.00058727	0.00120156	0.0011630	*Actinomyces*	0	0	0	0.00029075
Pajaroellobacter	0.00083409	0.00052202	0.00133810	0.0034890	*Peptoniphilus*	0.00020852	0	0	0.00012922
RCP2-54	0.00291934	0.00022838	0.00398700	0.0033921	*Ohtaekwangia*	8.34098E-	0.00048939	0.00010923	0.00019383
BIrii41	0.00166819	0.00345840	0.00477894	0.0031659	*Clostridium_sensu_stricto_5*	0	6.52529E-	0	0.00012922
Candidatus_Kaiserbacteria	0.00141796	0.00045677	0.00103771	0.0014537	*Longivirga*	0	0	0.00016384	0.00067842
Rubrobacter	0.00170990	0.00058727	0.00565280	0.0014860	*JTB255_marine_benthic_group*	0	6.52529E-	0	0.00051689
Chryseolinea	0.00029193	0.00261011	0.00054616	0.0005169	*WS2*	0.00029193	6.52529E-	0.00010923	0.00032306
Subgroup_10	0.00708983	0.00747145	0.00557087	0.0196097	*HSB_OF53-F07*	0	0	0	0.00025844
uncultured	0.00608891	0.00257748	0.00516125	0.0099502	*PHOS-HE36*	0	0.00019575	0.00013654	6.4612E-05

RB41	0.00517140	0.00029363	0.00335891	0.0030367	*Fimbriiglobus*	0	0	5.46165E-	0.00012922
Archangium	0.00070898	0.00042414	0.00027308	0.0004199	*Knoellia*	0	0	0.00016384	0.00058150
Fictibacillus	0.00058386	9.78793E-	0.00071001	0.0006784	*uncultured*	0	0	0	6.4612E-05
Entotheonellaceae	0.00083409	0.00019575	0.00232119	0.0019706	*Methylobacterium-Methylorubrum*	0.00016682	0.00061990	5.46165E-	0.00061381
Vicinamibacteraceae	0.02139461	0.03510603	0.01821458	0.0133100	*Candidatus_Peribacteria*	0	6.52529E-	5.46165E-	0.00012922
uncultured	0.00300275	6.52529E-	0.00316775	0	*uncultured*	0.00020852	0	0.00010923	0.00032306
Sh765B-TzT-35	0.00183501	0.00071778	0.00344083	0.0008076	*Sarcina*	0	6.52529E-	0	0.00019383
Sorangium	0.00058386	6.52529E-	0.00049154	6.4612E-	*Portibacter*	0	6.52529E-	0	0.00016153
Micromonospora	0.00037534	0.00026101	0.00111963	0.0014214	*uncultured*	0.00070898	0.00019575	0.00013654	0.00100148
WX65	0.00362832	6.52529E-	0.00387776	0.0019706	*Uncultured*	0.00025022	0	0.00030039	6.4612E-05
uncultured	0.00066727	0.00081566	0.00038231	0.0003553	*Subgroup_15*	0.00025022	0	0.00090117	0.00064612
Hydrogenophaga	0.00025022	0.00035889	0.00024577	0.0009045	*uncultured*	0.00062557	0	0.00013654	0
uncultured	0.00492117	0.00264274	0.00955788	0.0125347	*Chthonomonadales*	0.00045875	0	0.00054616	0.00025844
211ds20	0	0.00189233	0.00043693	0	*Subgroup_12*	0	0	0.00019115	0.00058150
TRA3-20	0.01522228	0.0537031	0.01116906	0.0095625	*Methylotenera*	0	0.00205546	0.00071001	9.6918E-05
Pla4_lineage	0.00187672	0.00013050	0.00136541	0.0017122	*Planctomicrobium*	0.00033363	6.52529E-	0.00013654	0
Paenibacillus	0.00091750	0.00071778	0.00155656	0.0007430	*NRL2*	8.34098E-	6.52529E-	0.00016384	0
Gallionella	0.00012511	0	0.00032769	9.6918E-	*uncultured*	0	9.78793E-	0.00016384	0
uncultured	0.00863291	0.00172920	0.01471913	0.0046197	*IMCC26207*	0	6.52529E-	5.46165E-	0.00012922
Lutispora	0.00020852	0	0.00038231	0.0001938	*Aneurinibacillus*	8.34098E-	0	5.46165E-	6.4612E-05
uncultured	0.00033363	0.00042414	0.00060078	0.0001938	*BRH-c8a*	8.34098E-	0	5.46165E-	0
Latescibacterota	0.00955042	0.00107667	0.00879324	0.0087872	*Paeniclostridium*	0	0	0.00010923	0.00029075
OC31	0.00025022	6.52529E-	0.00021846	0.0002907	*Domibacillus*	0.00012511	0	5.46165E-	0
uncultured	0.00166819	0.00104404	0.00081924	0.0015829	*uncultured*	0.00033363	0	0.00038231	0.00016153
Kribbella	0.00041704	0	0.00065539	0.0004845	*uncultured*	0	0	0.00010923	0
uncultured	0.00633914	0.00433931	0.00890248	0.0086257	*Asanoa*	0	0	5.46165E-	6.4612E-05
uncultured	0.02723329	0.02411093	0.02140965	0.0147961	*Phaeodactylibacter*	0.00012511	0	0.00046424	0.00048459
Ellin6055	8.34098E-	0.00026101	0.00027308	0	*Microlunatus*	8.34098E-	0.00013050	0.00019115	0
KD4-96	0.01985153	0.00215334	0.02610666	0.0199005	*mle1-8*	0.00050045	0.00013050	0.00060078	0.00012922
KF-JG30-B3	0.00116773	0.00065252	0.00150195	0.0015829	*Humibacillus*	0	0	0.00016384	6.4612E-05
Rubrivivax	0.00196013	0.00013050	0.00065539	0.0015183	*SB-5*	0.00016682	0.00114192	0.00013654	0
Uncultured	0.00100091	0.00091354	0.00491548	0.0064288	*uncultured*	0.00025022	6.52529E-	0.00038231	0
Nordella	0.00112603	0.00013050	0.00117425	0.0008722	*Frankia*	8.34098E-	0	0.00016384	6.4612E-05
Candidatus_Alysiosphaera	0.00041704	0.00019575	0.00060078	0.0001292	*Paenibacillaceae*	0	0	0.00021846	0
uncultured	0.00237717	0.00071778	0.00570742	0.0030044	*Rhizobiales*	8.34098E-	0	5.46165E-	0
Actinomycetospora	8.34098E-	0.00026101	0.00122887	0.0001938	*uncultured*	0.00037534	0.00182708	0.00038231	0.00029075
Ramlibacter	0.00283593	0.00287112	0.00139272	0.0013568	*Amycolatopsis*	0	0	5.46165E-	0
Rhizobacter	0.00125114	0.00329526	0.00073732	0.0011953	*Sandaracinus*	0.00033363	0.00042414	0.00035500	0.00019383
Rhodoplanes	0.00208524	0.00071778	0.00215735	0.0009691	*uncultured*	0.00012511	0.00022838	0.00013654	0.00022614

Nocardioides	0.00654766	0.00088091	0.00641743	0.0052012	*Crenobacter*	0	0	5.46165E-	0.00016153
Singulisphaera	0.00029193	0	0.00021846	0.0001292	*uncultured*	0.00137626	0	0.00147464	0.00019383
bacteriap25	0.00679789	0.00048939	0.00876594	0.0026167	*MSB-5E12*	0	0	5.46165E-	0
Gitt-GS-136	0.00608891	0.00058727	0.00442393	0.0030690	*Tropicibacter*	0	0	5.46165E-	0
uncultured	0.00429560	0.00727569	0.00327698	0.0030367	*Uncultured*	0	0.00022838	0.00016384	0.00019383
SC-I-84	0.00258570	0.00117455	0.00385046	0.0088841	*Aeromonas*	0.00020852	0.00022838	5.46165E-	0.00038767
Bryobacter	0.00383685	0.00048939	0.00387776	0.0029398	*Polyangiaceae*	0	0	5.46165E-	0
Bradyrhizobium	0.00091750	0.00081566	0.00071001	0.0016476	*Truepera*	0.00033363	0	0.00021846	0
Mycobacterium	0.00170990	0.00078303	0.00142002	0.0032306	*Dubosiella*	0.00341980	0	0	0
Burkholderia-Caballeronia-	0.00095921	0.00107667	0.00019115	0.0016153	*Bacteroides*	0.00563016	6.52529E-	0	0
TK10	0.00120944	0	0.00365930	0.0014537	*Akkermansia*	0.00212695	0	0	0
uncultured	0.00062557	0.00048939	0.00109232	0.0003876	*Alistipes*	0.00091750	0	0	0
Terrimonas	0.00191842	0.00065252	0.00109232	0.0006784	*Megamonas*	0.00033363	0	0	0
Acinetobacter	0.00212695	0.00176182	0.00038231	0.0040059	*Candidatus_Entotheonella*	0.00016682	0	5.46165E-	6.4612E-05
Piscinibacter	0.00112603	0.00078303	0.00046424	0.0011630	*Steroidobacteraceae*	0.00016682	6.52529E-	0	0
uncultured	0.00271081	0.00045677	0.00398700	0.0017122	*uncultured*	0.00041704	0	0	0
IS-44	0.00075068	0.00032626	0.00051885	0.0005815	*VHS-B3-70*	0.00016682	0	0.00010923	0
uncultured	0.00271081	0.00306688	0.00207542	0.0030044	*Erysipelatoclostridium*	0.00045875	0	0	0
Uncultured	0.00792393	0.0091354	0.01947076	0.0149576	*Bifidobacterium*	0.00091750	6.52529E-	0	0
Nakamurella	0.00016682	0	0.00060078	0.0001292	*Desulfovibrio*	0.00016682	6.52529E-	5.46165E-	0
Dongia	0.00141796	0.00287112	0.00311313	0.0027783	*mle1-27*	0.00095921	6.52529E-	0.00060078	0.00054920
uncultured	0.00025022	0.00045677	0.00043693	0	*Nitratireductor*	0.00025022	0.00013050	0	0
Geobacteraceae	0.00062557	0.00022838	0.00079193	0.0009368	*Parasutterella*	0.00025022	0	0	0
Leeia	0.00108432	0.00032626	0.00054616	0.0001938	*GCA-900066575*	0.00037534	0	0	0
IMCC26256	0.00642255	0.00107667	0.01357218	0.0079795	*Coprococcus*	0.00012511	0	0	0
mle1-7	0.00271081	0.00042414	0.00229389	0.0041674	*[Ruminococcus]_torques_group*	0.00033363	0	0	0
Reyranella	0.00112603	0.00137031	0.00142002	0.0010661	*Faecalibacterium*	0.00029193	0	0	0
Uncultured	0.00050045	9.78793E-	0.00068270	0.0006461	*Allobaculum*	0.00012511	0	0	0
Pirellula	0.00471265	0.00202283	0.00368661	0.0058473	*Thermoactinomycetaceae*	0.00020852	6.52529E-	0.00013654	0
JG30-KF-CM66	0.00383685	0.00159869	0.00535241	0.0022614	*Lachnospiraceae_NK4A136_group*	0.00200183	6.52529E-	0	0
Candidatus_Udaeobacter	0.00029193	0.00026101	0.00136541	0.0015829	*uncultured*	0.00020852	0	0	0
B1-7BS	0.00196013	0.00101141 9	0.00131079 5	0.0026167 9	*Desulfatirhabdium*	0.00012511 5	0	0	0
uncultured	0.00041704	0	0.00090117	0.0021968	*Uncultured*	0.00045875	6.52529E-	0.00090117	0.00054920
uncultured	0.00212695	0.00185970	0.00188426	0.0025844	*Psychrobacter*	0.00012511	0	0	0
P2-11E	0.00158478	0	0.00111963	0.0007753	*AAP99*	0.00012511	0	0	0
Rhodoferax	0.00041704	0.00084828	0.00035500	0.0004199	*Enterorhabdus*	0.00050045	0	0	0
uncultured	0.00254399	0.00140293	0.00292198	0.0047812	*[Eubacterium]_coprostanoligenes_gro*	0.00050045	0	0	0
Conexibacter	0.00154308	0.00029363	0.00144733	0.0024875	*Butyricicoccus*	0.00100091	0	0	0

Hyphomicrobium	0.00304445	0.00107667	0.00281274	0.0034890	*Negativibacillus*	0.00066727	9.78793E-	0	0
Pedosphaeraceae	0.00546334	0.00088091	0.00357737	0.0062673	*Rikenella*	0.00020852	0	0	0
MA-28-I98C	0.00116773	0.00048939	0.00051885	0.0014537	*Coxiella*	0.00070898	0.00280587	0.00030039	0.00125993
Sva0485	0.00050045	0.00274062	0.00234850	0.0015829	*Chryseobacterium*	0.00012511	0.00022838	0	0.00022614
uncultured	0.00025022	0.00061990	0.00019115	0.0027460	*Tissierella*	0.00012511	0	0	0
Opitutus	0.00037534	0.00078303	0.00054616	0.0001938	*Paenalcaligenes*	0.00012511	0	0	0
Subgroup_7	0.00909166	0.00874388	0.00568011	0.0154422	*uncultured*	0.00045875	0	0	0
uncultured	0	0.00084828	0.00019115	0.0003553	*Lachnoclostridium*	0.00054216	0.00029363	0	0
Thermoflexus	0.00016682	0	0.00046424	0	*uncultured*	0.00095921	0	5.46165E-	0
Streptacidiphilus	8.34098E-	0	8.19247E-	0	*Nitrosospira*	0.00016682	0	0	0
Phenylobacterium	0	0.00176182	0.00019115	0.0004199	*Ferritrophicum*	0.00033363	0.00052202	0	0.00012922
Anaerobacterium	8.34098E-	0	0.00030039	0.0001292	*Nocardia*	8.34098E-	0	0	6.4612E-05
Acidibacter	0.00108432	0.00127243	0.00207542	0.0013891	*Phycicoccus*	8.34098E-	0	0	0.00022614
Ruminiclostridium	0.00062557	0.00019575	0.00120156	0.0005815	*Actinoplanes*	0.00016682	0	5.46165E-	6.4612E-05
uncultured	0.00333639	6.52529E-	0.00363199	6.4612E-	*Woeseia*	8.34098E-	0.00097879	0	0.00064612
WD2101_soil_group	0.00133455	0.00055464	0.00133810	0.0007753	*UCG-005*	0.00025022	0	0	0
Chthoniobacter	0.00233547	0.00019575	0.00191157	0.0021968	*Turicibacter*	0.00016682	6.52529E-	0	0
Verruc-01	0	6.52529E-	8.19247E-	0	*Candidatus_Saccharimonas*	0.00033363	0	0	0
Clostridium_sensu_stricto_1	0.00066727	0.00088091	0.00057347	0.0008399	*[Eubacterium]_eligens_group*	0.00016682	0	0	0
uncultured	0.00062557	0	0.00092848	0.0012922	*Colidextribacter*	0.00041704	0	0	0
Phaselicystis	0.00204354	0.00039151	0.00073732	0.0016799	*Acetivibrio*	0.00016682	0	0	0
GOUTA6	0.00266911	0.00398042	0.00221196	0.0009045	*Angustibacter*	8.34098E-	0	0.00010923	0
Thiobacillus	0.00191842	0.00221859	0.00106502	0.0005169	*Terrisporobacter*	8.34098E-	0	0	0
uncultured	0.00016682	0	0.00087386	6.4612E-	*[Eubacterium]_xylanophilum_group*	0.00041704	0	0	0
Myxococcus	8.34098E-	0	8.19247E-	0	*Pelagibius*	8.34098E-	0	0	0
Nannocystis	0.00112603	0.00075040	0.00073732	0.0001938	*Ruminococcus*	0.00025022	0	0	0
Lacunisphaera	0.00058386	0.00998368	0.00133810	0.0022614	*Subdoligranulum*	0.00050045	0	0	0
Candidatus_Paracaedibacter	0.00029193	0.00045677	0.00035500	0.0003553	*KCM-B-112*	8.34098E-	0	0	0
Pedomicrobium	0.00333639	0.00045677	0.00417815	0.0032629	*Sericytochromatia*	0.00050045	0.00013050	0.00016384	0
Novosphingobium	0.00120944	0.00094616	0.00062808	0.0022291	*Croceicoccus*	8.34098E-	0	5.46165E-	0
OLB12	0	0.00104404	0.00035500	0.0015506	*Labrys*	0.00025022	6.52529E-	5.46165E-	6.4612E-05
Fimbriimonadaceae	0.00104262	0.00045677	0.00073732	0.0008722	*Enterobacter*	0.00016682	0	0	0.00019383
Gemmata	0.00070898	0.00172920	0.00114694	0.0018737	*Candidatus_Arthromitus*	0.00016682	0	0	0
Ilumatobacter	0.00133455	0.00035889	0.00095578	0.0002584	*Cellulosilyticum*	8.34098E-	6.52529E-	0	0
Mesorhizobium	0.00016682	0.00019575	0.00030039	0.0003553	*Methylophilus*	0.00016682	0.00032626	0	0
Solirubrobacter	0.00354491	0.00143556	0.00494278	0.0021645	*NS11-12_marine_group*	0.00016682	0.00668841	5.46165E-	0.00025844
I-8	0	0	0.00013654	6.4612E-	*Candidatus_Accumulibacter*	0.00016682	0	0	0
37010	0.00496288	0.00094616	0.00406892	0.0021968	*Clostridium_sensu_stricto_10*	8.34098E-	0	5.46165E-	0
Uncultured	0.00075068	0.00013050	0.00133810	0.0007430	*Prosthecomicrobium*	0.00016682	0	0	0

Streptomyces	0.00183501	0.00042414	0.00360468	0.0018091	*uncultured*	8.34098E-	0	8.19247E-	0
Luteitalea	0.00362832	0.00035889	0.00188426	0.0008076	*Lachnospiraceae*	0.00016682	0	0	0.00012922
OLB14	0.00100091	0.00081566	0.00087386	0.0020352	*Mucispirillum*	8.34098E-	0	0	0
uncultured	0.00108432	0.00013050	0.00019115	0.0001938	*Helicobacter*	8.34098E-	6.52529E-	0	0
Skermanella	0.00066727	0.00013050	0.00076463	0.0001292	*Litorilinea*	0.00016682	6.52529E-	5.46165E-	6.4612E-05
uncultured	8.34098E-	0.00172920	0.00019115	0.0001938	*Prevotella*	0.00016682	6.52529E-	0	0.00038767
uncultured	0.00145967	0.00137031	0.00076463	0.0027783	*P9X2b3D02*	0.00054216	0.00039151	0.00035500	0.00022614
Desulfuromonas	0	0	0.00030039	0.0002261	*CHKCI001*	8.34098E-	0	0	0
Nannocystaceae	0.00029193	0.00042414	0.00032769	0.0026167	*Sutterella*	8.34098E-	0	0	0
Subgroup_22	0.00621403	0.00084828	0.00925748	0.0063965	*Fluviicola*	0.00016682	0.00022838	0	0.00012922
Methylocystis	0.00025022	6.52529E-	0.00024577	0.0007430	*Candidatus_Stoquefichus*	8.34098E-	0	0	0
Pelotomaculum	0.00033363	6.52529E-	0.00079193	0	*Candidatus_Spechtbacteria*	0.00016682	0	0	0
P3OB-42	0.00033363	0	0.00062808	0.0007430	*uncultured*	8.34098E-	6.52529E-	5.46165E-	6.4612E-05
Spirillospora	8.34098E-	0	8.19247E-	0	*Herbinix*	0.00016682	0	0	0
Desulfosporosinus	0	0	0.00090117	0	*Christensenellaceae_R-7_group*	8.34098E-	0	0	0
uncultured	0.00729835	0.00280587	0.01168792	0.0040382	*Shuttleworthia*	0.00016682	0	0	0
uncultured	0.00066727	0.00182708	0.00046424	0.0002907	*Parvibacter*	0.00016682	0	0	0
uncultured	0.00175160	0.00058727	0.00120156	0.0006461	*Neochlamydia*	0.00037534	6.52529E-	5.46165E-	0.00022614
Iamia	0.00362832	0.00039151	0.00264889	0.0007107	*Arthrobacter*	8.34098E-	6.52529E-	0	0
uncultured	0.00012511	0	0.00021846	0.0001292	*Virgisporangium*	0.00016682	0	5.46165E-	0
Subgroup_2	0.00150137	6.52529E-	0.00185696	0.0026814	*Fournierella*	0.00025022	0	0	0
Uncultured	0.00050045	0.00039151	0.00180234	0.0023583	*[Eubacterium]_ruminantium_group*	0.00016682	0	0	0
Aridibacter	0	0	0.00019115	0.0001938	*SJA-15*	0.00054216	0.00026101	0.00030039	0.00064612
OM27_clade	0.00137626	0.00048939	0.00125617	0.0008399	*Fusicatenibacter*	0.00041704	0	0	0
Pseudolabrys	0.00196013	0.00920065	0.00193888	0.0026490	*Campylobacter*	8.34098E-	0	0	0
AKYG1722	0.00029193	0	0.00038231	0.0001292	*CG2-30-50-142*	0.00050045	0	0.00152926	0.00012922
Flindersiella	8.34098E-	0	8.19247E-	0	*Pseudobacteroides*	8.34098E-	6.52529E-	0	0
AKYH767	0.00045875	6.52529E-	0.00030039	0.0015183	*Uncultured*	0.00066727	6.52529E-	5.46165E-	6.4612E-05
Pseudonocardia	0.00087580	0.00039151	0.00084655	0.0002584	*Coriobacteriaceae_UCG-002*	0.00033363	0	0	0
Sphingobium	0.00037534	0.00029363	0.00030039	0.0002907	*Actinomarinales*	8.34098E-	0	0	0
Luedemannella	0.00183501	0.00019575	0.00281274	0.0020675	*uncultured*	8.34098E-	0	5.46165E-	0
Candidatus_Solibacter	0.00254399	6.52529E-	0.00273082	0.0020675	*Parabacteroides*	8.34098E-	0	0	0
Tolypothrix	0	0.00016313	0.00024577	0.0003553	*Flavihumibacter*	8.34098E-	0	0	6.4612E-05
Hirschia	0.00025022	0.00045677	0.00079193	0.0003230	*Methylobacillus*	8.34098E-	0	0	0
S085	0.00492117	0.00032626	0.00914825	0.0019706	*Uncultured*	0.00016682	0.00016313	0.00010923	0
Clostridium_sensu_stricto_13	8.34098E-	0.00039151	0.00027308	0.0003230	*Ottowia*	8.34098E-	0	0	0
uncultured	0.00487947	0.00045677	0.00161118	0.0025521	*[Eubacterium]_brachy_group*	8.34098E-	0	0	0
Methylomicrobium	0.00166819	0.00013050	0.00054616	0.0001615	*uncultured*	0.00016682	0	0.00024577	0
Hydrogenispora	0.00025022	0.00019575	0.00051885	0.0002584	*Pseudopelobacter*	8.34098E-	0	5.46165E-	0

uncultured	0.00179331	0.00068515	0.00188426	0.0054274	*Lachnospiraceae_UCG-006*	0.00016682	0	0	0
Sphingomonas	0.00187672	0.00580750	0.00057347	0.0024875	*Gastranaerophilales*	8.34098E-	0	0	0
NB1-j	0.00275252	0.00088091	0.00447854	0.0090456	*Alloprevotella*	0.00016682	0	0	0
uncultured	8.34098E-	0	5.46165E-	0	*Odoribacter*	8.34098E-	0	0	0
KF-JG30-C25	0.00083409	0.00104404	0.00136541	0.0011630	*wb1-A12*	0.00020852	9.78793E-	0.00021846	0
Desulfurispora	0.00025022	0	0.00021846	0	*Phascolarctobacterium*	0.00020852	0	0.00010923	0
uncultured	0.01055133	0.00362153	0.01234331	0.0116947	*Phocea*	8.34098E-	0	0	0
uncultured	0.00383685	0.00244698	0.00221196	0.0030367	*Uncultured*	0.00016682	0	0.00062808	0.00029075
Noviherbaspirillum	0.00016682	0.00026101	0.00021846	0.0010337	*Rikenellaceae_RC9_gut_group*	8.34098E-	0	0	0
MVP-88	8.34098E-	0.00016313	0.00021846	0	*Fodinibacter*	8.34098E-	0	0	0
Desulfobulbus	8.34098E-	9.78793E-	0.00016384	0.0001615	*[Desulfobacterium]_catecholicum_gro*	8.34098E-	6.52529E-	0	0
Steroidobacter	0.00033363	0.00153344	0.00049154	6.4612E-	*Isoptericola*	8.34098E-	0	8.19247E-	0
AD3	0	0	0.00049154	0.0006461	*Curtobacterium*	8.34098E-	0	0	0
Fonticella	8.34098E-	0	0.00016384	0.0001292	*Acidaminococcus*	8.34098E-	0	0	0
Azohydromonas	0	0	5.46165E-	0	*uncultured*	8.34098E-	0	0	0
BSV26	0.00546334	0.00091354	0.00273082	0.0025844	*uncultured*	8.34098E-	0.00143556	5.46165E-	0
Pelosinus	8.34098E-	9.78793E-	5.46165E-	0.0001938	*Acrocarpospora*	0	0	0	9.6918E-05
MBMPE27	0.00058386	0.00045677	0.00035500	0.0001292	*Georgfuchsia*	8.34098E-	0	0.00010923	9.6918E-05
CCM11a	0.00100091	0.00032626	0.00166580	0.0015506	*Chthonomonas*	0.00012511	0	0.00010923	0.00016153
Edaphobaculum	0	0.00783034	0.00013654	0.0008076	*uncultured*	0	0	0	0.00016153
27F-1492R	0.00079239	0.00032626	0.00057347	0.0005815	*uncultured*	0	0	0	0.00012922
possible_genus_04	0.00045875	0.00013050	0.00060078	0.0004199	*Phyllobacterium*	0	0	0	6.4612E-05
Latescibacteraceae	0.00066727	0.00013050	0.00081924	0.0003553	*Rummeliibacillus*	0.00020852	0	0	6.4612E-05
MIZ17	0.00066727	0.00016313	0.00065539	0	*Anaerovorax*	0.00016682	6.52529E-	5.46165E-	6.4612E-05
Oikopleura	0.00066727	0	0.00027308	0	*uncultured*	0	6.52529E-	5.46165E-	6.4612E-05
JG30-KF-CM45	0.00421219	0.00081566	0.00619896	0.0021322	*Intrasporangiaceae*	0	0	0	0.00025844
SBR1031	0.00133455	0.00244698	0.00223927	0.0010014	*FFCH5858*	0	0	0	6.4612E-05
uncultured	0.00095921	0	0.00111963	0.0018414	*Alicyclobacillus*	0	0	0	0.00012922
uncultured	0	9.78793E-	0.00024577	0	*Geothrix*	0	0	0	0.00019383
uncultured	0.00025022	6.52529E-	0.00016384	6.4612E-	*SH3-11*	0	0	0	6.4612E-05
SWB02	0.00050045	0.00123980	0.00103771	0.0023906	*Uncultured*	0.00054216	0	0.00101040	0.00074303
uncultured	8.34098E-	0.00052202	5.46165E-	6.4612E-	*Roseiarcus*	0	0	0	6.4612E-05
44889	0.00175160	0.00016313	0.00087386	0.0027783	*Thiothrix*	0	0	0	0.00012922
Geothermobacter	0.00029193	0.00035889	0.00027308	0.0016153	*MIZ14*	0	0	0	0.00019383
Curvibacter	0.00091750	0.00962479	0.00024577	0.0018091	*Lapillicoccus*	0	0	0	0.00012922
Brevibacillus	0.00112603	6.52529E-	0.00030039	0.0012276	*Limnohabitans*	0	6.52529E-	0	6.4612E-05
BIyi10	0	0.00140293	0.00016384	0	*Nostoc_PCC-7524*	0.00033363	0	0	0.00048459
Pontibacter	0	0	0.00016384	0.0002261	*Gemmobacter*	0	0	0	6.4612E-05
Citrifermentans	0.00125114	0.00019575	0.00128348	0.0011630	*Longilinea*	0	0.00019575	0	6.4612E-05

uncultured	0.00020852	6.52529E-	0.00065539	0.0002907	*Zoogloea*	0	0	0	6.4612E-05
Enterococcus	0	0	0.00010923	6.4612E-	*Verrucosispora*	0	0	0	6.4612E-05
Shimazuella	0	0	0.00038231	0	*Lacibacter*	0	0	0	0.00012922
uncultured	0.00250229	0.00071778	0.00040962	0.0001292	*Uncultured*	0	0	0.00010923	0.00045228
Leptospirillum	0.00041704	0	0.00027308	0	*Niastella*	0	0	0	6.4612E-05
Defluviicoccus	0.00125114	0.00026101	0.00172041	0.0002261	*Planosporangium*	0	0	5.46165E-	6.4612E-05
Blastococcus	0.00033363	0	0.00054616	0	*Variibacter*	0	0	0	6.4612E-05
Uncultured	0.00045875	0.00185970	0.00355007	0.0018414	*Microtetraspora*	0	0	0.00030039	0
Aquicella	0.00266911	0.10825448	0.00065539	0.0061058	*PB19*	0.00037534	0.00019575	0.00019115	0
Uncultured	0.00087580	0.00022838	0.00106502	0.0017122	*Haliscomenobacter*	0	9.78793E-	8.19247E-	6.4612E-05
uncultured	0.00020852	0.00045677	0.00010923	6.4612E-	*Uncultured*	0.00016682	0.00013050	8.19247E-	9.6918E-05
uncultured	0.00225206	6.52529E-	0.00303121	0.0005169	*Gracilibacteria*	0	0	0.00016384	6.4612E-05
Desulfitobacterium	8.34098E-	0	0.00021846	0.0002584	*Ammoniphilus*	8.34098E-	0.00013050	0.00013654	0
Cellulomonas	0	0.00032626	5.46165E-	0.0057827	*Deferrisoma*	0.00016682	0	0.00038231	0.00038767
Syntrophobacter	0.00016682	0	0.00027308	0.0001938	*uncultured*	0.00029193	6.52529E-	0.00016384	0
Blfdi19	0.00170990	0.00013050	0.00139272	0.0014860	*Ferruginibacter*	0.00016682	0.00039151	0.00013654	6.4612E-05
Intrasporangium	0.00054216	9.78793E-	0.00021846	0.0001292	*uncultured*	8.34098E-	0	0.00016384	0
A0839	0.00104262	0.00029363	0.00051885	0.0028752	*uncultured*	0	6.52529E-	0.00010923	0
Rhodococcus	0.00045875	0.00013050	0.00021846	0.0002261	*DTB120*	0	0.00016313	5.46165E-	0.00019383
Sporobacter	8.34098E-	0.00029363	0.00032769	0	*Sporosarcina*	0	0	5.46165E-	0
Cystobacter	0	0	5.46165E-	0	*Candidatus_Finniella*	0	0	5.46165E-	0
OPB41	0.00125114	0.00052202	0.00106502	0.0017122	*Undibacterium*	0	0	0.00016384	0.00090456
SJA-28	0.00070898	0.00048939	0.00046424	0.0008722	*Candidatus_Adlerbacteria*	0.00233547	0.00039151	0.00150195	0.00074303
Omnitrophales	0.00129285	6.52529E-	0.00071001	0.0036182	*Aeromicrobium*	0	6.52529E-	5.46165E-	0
JGI_0001001-H03	0.00133455	0	0.00049154	0.0011953	*Planomonospora*	0	0	5.46165E-	0
uncultured	0.00016682	0.00026101	0.00016384	0.0002261	*uncultured*	0	6.52529E-	5.46165E-	0
Altererythrobacter	0.00045875	0.00339314	0.00101040	0.0006138	*Limnobacter*	0.00016682	0	0.00010923	0
OM190	0.00571357	0.00137031	0.00365930	0.0080765	*Actinophytocola*	0	0	5.46165E-	0
Nitrosomonas	0.00016682	6.52529E-	0.00019115	0.0001938	*uncultured*	0	0	5.46165E-	0
GOUTB8	0.00037534	6.52529E-	0.00057347	0.0006138	*Sporacetigenium*	0	0	0.00013654	0
D05-2	0	0.00026101	5.46165E-	0.0001292	*Uncultured*	0.00225206	0.00600326	0.00365930	0.00287523
R7C24	0.00020852	0.00110929	0.00021846	0	*Baia*	0	0	5.46165E-	0
Tabrizicola	0	0.00013050	5.46165E-	0.0002261	*Subgroup_19*	0	0	0.00010923	0
uncultured	0.00020852	0.00022838	0.00057347	0.0007107	*OLB17*	0	0	5.46165E-	0
Xylophilus	0	0.00107667	5.46165E-	0	*TSAC18*	0	0	5.46165E-	0
WWH38	0	0	5.46165E-	0	*Microbispora*	0	0	0.00010923	0
AKYG587	0.00045875	0.00013050	0.00010923	0.0001938	*uncultured*	0	0.00013050	5.46165E-	0
Comamonas	0.00354491	0.00117455	0.00180234	0.0043613	*uncultured*	0	0	5.46165E-	0
Polycladomyces	8.34098E-	0	0.00021846	0	*uncultured*	0	0	5.46165E-	0

uncultured	0.00037534	0.00016313	0.00016384	0.0001292	*uncultured*	0.00012511	6.52529E-	0.00010923	0
Frankiales	0.00054216	0.00045677	0.00054616	0.0023906	*Sulfuricella*	0	0.00055464	0.00010923	0
Clostridium_sensu_stricto_6	8.34098E-	0	0.00010923	9.6918E-	*Streptosporangium*	0	6.52529E-	5.46165E-	6.4612E-05
Caproiciproducens	0	0	5.46165E-	6.4612E-	*Wangella*	0	0	5.46165E-	0
Anaerocolumna	0	0	0.00016384	0	*uncultured*	0	0.00022838	0.00021846	0
Desulfovirga	0.00116773	0.00042414	0.00054616	0.0006138	*Denitratisoma*	0	0.00094616	0	0
Acidothermus	0.00116773	0	0.00215735	0.0029075	*uncultured*	0	0.00107667	0	0.00032306
Escherichia-Shigella	0.00037534	9.78793E-	0.00021846	0.0002261	*Pedobacter*	0.00033363	0.00172920	0	0.00174452
Geodermatophilaceae	0	6.52529E-	5.46165E-	0	*Alterococcus*	0	0.00022838	0.00010923	6.4612E-05
Agromyces	0.00225206	0.00019575	0.00092848	0.0012599	*BBMC-4*	0	0.00016313	0	0
ADurb.Bin063-1	0.00062557	0.00127243	0.00065539	0.0011953	*Porphyrobacter*	0	0.00052202	0	0.00012922
Plantactinospora	0	0	0.00030039	0	*Erysipelothrix*	0	0.00035889	0	0
Crenothrix	0.00037534	9.78793E-	0.00057347	0.0004845	*Tibeticola*	0	0.00013050	0	0
uncultured	8.34098E-	0.00163132	0.00027308	0.0006138	*Caulobacter*	8.34098E-	0.00267536	0	6.4612E-05
Hamadaea	0.00016682	0	5.46165E-	0.0001938	*Nocardioidaceae*	0	0.00013050	0	0
Parviterribacter	0.00037534	0	0.00010923	0	*Stigmatella*	0	0.00013050	0	0
uncultured	0.00141796	0.00013050	0.00259428	0.0034890	*Cryptosporangium*	0	9.78793E-	0	0
OM60(NOR5)_clade	0.00016682	0.00114192	0.00010923	0.0004199	*Sulfuricurvum*	0	0.00065252	0	0
uncultured	0.00079239	0.00032626	0.00128348	0.0014214	*Candidatus_Competibacter*	0	0.00016313	5.46165E-	0
Clostridium_sensu_stricto_8	0.00120944	0	0.00073732	0.0001292	*Trichloromonas*	0	0.00016313	0	0
Hydrogenedensaceae	0.00087580	0.00349102	0.00065539	0.0012276	*Candidatus_Pacebacteria*	8.34098E-	0.00349102	0	0.00025844
Zixibacteria	8.34098E-	0.00052202	0.00065539	0.0007107	*Thermoactinomyces*	0	0.00016313	5.46165E-	0
Tropicimonas	0	0	5.46165E-	0	*JGI-0000079-D21*	0	9.78793E-	0	0
Actinomadura	0.00012511	0	5.46165E-	0.0001938	*uncultured*	0	0.00058727	0.00010923	0.00019383
Ignavibacteriales	0	0.00013050	0.00010923	0	*Craurococcus-Caldovatus*	0	0.00013050	0.00027308	0.00012922
Sporichthya	0.00070898	0	0.00032769	0.0008076	*Lysinimonas*	0	6.52529E-	0	6.4612E-05
Paludibaculum	0.00108432	0.00016313	0.00073732	0.0001292	*1013-28-CG33*	0.00016682	0.00029363	5.46165E-	0
Elev-16S-1166	0	0	0.00046424	6.4612E-	*Arsenicitalea*	0	6.52529E-	0	0
Candidatus_Nomurabacteria	0.00083409	0.00058727	0.00136541	0.0027783	*Planifilum*	0	6.52529E-	0	0
Arenimonas	0.00166819	0.01768352	0.00051885	0.0023906	*Micropepsaceae*	0	6.52529E-	0	0
uncultured	0	0.00234910	0.00038231	0.0007430	*Pelolinea*	0	0.00042414	0	0
Uncultured	0.00133455	0.00013050	0.00406892	0.0015829	*Actibacter*	0	6.52529E-	0	0
uncultured	0.00058386	0.00042414	0.00122887	0.0009691	*Treponema*	0	0.00215334	5.46165E-	0
uncultured	0.00158478	0.00097879	0.00057347	0.0008722	*Bacteriovorax*	0	0.00042414	0	0
Candidatus_Brocadia	0	0	0.00010923	0	*Epulopiscium*	8.34098E-	6.52529E-	0.00010923	6.4612E-05
Devosia	0.00029193	0.00061990	0.00032769	0.0002584	*Quadrisphaera*	0	6.52529E-	0	0
Lineage_IV	0.00075068	0.00172920	0.00081924	0.0009368	*Tychonema_CCAP_1459-11B*	0	6.52529E-	0	0
Sideroxydans	0.00208524	0.00319739	0.00068270	0.0019060	*Caedibacter*	0	0.00016313	0	0
Clostridium_sensu_stricto_12	0.00033363	6.52529E-	0.00076463	0.0002907	*Desulforhabdus*	0	6.52529E-	0	0

Pir3_lineage	0	0	0.00016384	0	*uncultured*	0.00033363	0.00052202	0.00030039	0.00029075
Uncultured	0.00025022	0	0.00016384	0	*Georgenia*	0	6.52529E-	0	0
Neisseria	0.00016682	0.00022838	5.46165E-	0	*UBA12411*	0	6.52529E-	0	0
Subgroup_18	0.00183501	0.00045677	0.00136541	0.0008399	*Ga0077536*	8.34098E-	6.52529E-	5.46165E-	0
Subgroup_5	0.00054216	0	0.00155656	0.0017768	*cvE6*	0.00116773	0.00737357	0	0.00487820
Dolichospermum_NIES41	0	0	5.46165E-	0	*Leptospiraceae*	0	6.52529E-	5.46165E-	0
oc32	0.00095921	6.52529E-	0.00051885	0.0013245	*Unknown_Family*	0	0.00026101	0	0
Candidatus_Jidaibacter	0.00075068	0.00016313	5.46165E-	0.0004522	*CPR2*	0.00016682	0.00133768	0	0
DSSD61	0	0.00029363	5.46165E-	9.6918E-	*Candidatus_Curtissbacteria*	0	6.52529E-	0	0
Spirochaeta	0.00170990	0.00747145	0.00155656	0.0002907	*Parasegetibacter*	0	6.52529E-	0	0
Aminicenantales	0.00029193	0.00133768	0.00021846	6.4612E-	*Cephaloticoccus*	0	0.00029363	0	0.00100148
Clostridium_sensu_stricto_9	8.34098E-	0.00026101	0.00010923	0.0001292	*Uncultured*	0	6.52529E-	0.00016384	0.00022614
Thioalkalispira-Sulfurivermis	0.00054216	0.00035889	0.00060078	0.0001292	*Terrimicrobium*	0	0.00013050	0	0
Dactylosporangium	0.00016682	9.78793E-	0.00024577	0.0003230	*Roseimicrobium*	0	0.00013050	5.46165E-	6.4612E-05
BD2-11_terrestrial_group	0.00275252	0.00097879	0.00117425	0.0011630	*Candidatus_Anammoximicrobium*	0.00050045	6.52529E-	0.00010923	0
Actinocorallia	8.34098E-	0	0.00010923	6.4612E-	*uncultured*	0	0.00075040	0	0
Blastopirellula	0.00116773	0.00016313	0.00155656	0.0037151	*Luteimonas*	0	0.00032626	0	0
Uncultured	0.00025022	0.00045677	0.00027308	0.0003876	*Abiotrophia*	0	6.52529E-	0	0
0319-6G20	0.00095921	0.00146818	0.00092848	0.0025844	*Margulisbacteria*	0	0.00035889	0	0
uncultured	0.00241888	0.00084828	0.00182965	0.0028429	*SAR324_clade(Marine_group_B)*	0	0.00199021	5.46165E-	6.4612E-05
Methylosarcina	0.00050045	0	0.00021846	0	*SZB30*	0	6.52529E-	0	0
Lysinibacillus	0.00016682	0.00032626	0.00035500	6.4612E-	*Caldicoprobacter*	0	6.52529E-	0	0
Anaerolinea	0.00104262	0.00068515	0.00046424	0.0016476	*MSB-4B10*	0	6.52529E-	5.46165E-	0
Lautropia	8.34098E-	6.52529E-	0.00010923	0	*Cytophaga*	0	0.00026101	0	0
Pseudarthrobacter	0.00050045	0.00013050	0.00076463	0.0004199	*Candidatus_Collierbacteria*	0	0.00192495	0	0.00022614
Thermincola	0.00020852	6.52529E-	0.00021846	0.0005815	*Sphingorhabdus*	0.00016682	6.52529E-	0	0
Candidatus_Moranbacteria	0.00104262	0.00013050	0.00038231	0	*Diaminobutyricimonas*	0	6.52529E-	0	0
uncultured	0.00108432	6.52529E-	0.00292198	0.0063965	*Sandaracinobacter*	0	6.52529E-	0	0
Minicystis	0.00020852	9.78793E-	0.00016384	6.4612E-	*uncultured*	0	6.52529E-	0.00010923	0.00035536
uncultured	0.00241888	0.00081566	0.00150195	0.0031336	*Uncultured*	8.34098E-	0.00058727	0.00027308	0.00019383
Romboutsia	0.00037534	0.00013050	0.00016384	0.0004522	*Leptolinea*	0	0.00013050	5.46165E-	6.4612E-05
Geobacter	0.00141796	0.00215334	0.00054616	0.0008076	*uncultured*	0	0.00013050	5.46165E-	0
Azoarcus	0.00066727	0	0.00035500	0.0001938	*37-13*	8.34098E-	0.00039151	0	0.00025844
uncultured	0.00062557	0.00019575	0.00071001	0.0001615	*Alloiococcus*	0.00033363	0	0	0
Geotalea	0.00020852	0	5.46165E-	0	*Incertae_Sedis*	0.00033363	0	5.46165E-	0
Micromonosporaceae	0	0.00022838	0.00021846	0	*Herbaspirillum*	0.00020852	0	0	0.00016153
uncultured	0	0	5.46165E-	0	*Uncultured*	0.00075068	0.00019575	0.00032769	0.00083995
uncultured	8.34098E-	0.00016313	0.00024577	0.0002907	*Beutenbergia*	0.00020852	0	0	0
Vicinamibacterales	0.00104262	6.52529E-	0.00073732	0.0018091	*Thauera*	0.00025022	0	0	0

vadinHA49	0.00129285	0.00016313	0.00051885	0.0016153	*Rhodopirellula*	0.00012511	0	5.46165E-	0
Dadabacteriales	8.34098E-	0	5.46165E-	0	*Gracilibacter*	0.00012511	0	8.19247E-	0
B2M28	8.34098E-	0	5.46165E-	0	*Pantoea*	0.00012511	6.52529E-	0	0
Syntrophus	0.00016682	0.00107667	0.00027308	0.0003553	*Bellilinea*	0.00012511	0	0	0
uncultured	0.00012511	0	5.46165E-	0.0001292	*Azospirillum*	0.00020852	0	0	0
JG36-GS-52	0.00016682	0	0.00035500	0.0004845	*Sinomonas*	0.00012511	0	0	0
Catellatospora	0.00050045	0.00019575	0.00016384	0.0001292	*Ellin517*	0.00029193	9.78793E-	0.00021846	0.00058150
Schlesneria	0	0	0.00043693	0.0002261	*Ralstonia*	0.00012511	0	0	0
Thermoflavimicrobium	0	0	0.00032769	0	*Actinopolymorpha*	0.00070898	0	0.00043693	0
DEV008	0	0.00039151	0.00010923	0.0004522	*Stenotrophomonas*	8.34098E-	6.52529E-	0	0
Prauserella	0	0	5.46165E-	0	*Uncultured*	0.00083409	0.00094616	0.00172041	0.00281062
Saccharothrix	0	0	5.46165E-	0	*Sulfurirhabdus*	8.34098E-	0	0	0
Candidatus_Koribacter	0.00041704	0.00013050	0.00046424	0.0003553	*Salinispora*	0.00016682	0	0	0
uncultured	0.00054216	0.00169657	0.00016384	0	*type_III*	8.34098E-	0	0	0
AKIW659	0.00075068	0	0.00040962	0.0006461	*Yonghaparkia*	8.34098E-	0	0	0
Adhaeribacter	0.00029193	0	5.46165E-	6.4612E-	*Uncultured*	0.00016682	0	8.19247E-	0.00012922
Polyangium	0.00041704	0.00039151	0.00013654	0.0001615	*Panacagrimonas*	0.00041704	0	0.00035500	6.4612E-05
Candidatus_Xiphinematobacte	0.00041704	6.52529E-	0.00079193	0.0003876	*Modestobacter*	8.34098E-	0	0	0
Kouleothrix	0	0	5.46165E-	0.0002261	*Acetobacteroides*	8.34098E-	0	8.19247E-	0
A21b	0.00029193	0	0.00040962	0.0001938	*Pseudorhodobacter*	8.34098E-	0	0	0
Subgroup_21	8.34098E-	0	0.00032769	6.4612E-	*Kinneretia*	8.34098E-	0	0	0
A4b	8.34098E-	0.00019575	0.00090117	0.0008076	*FS118-23B-02*	8.34098E-	0	0.00013654	0
Microvirga	0.00058386	0.00058727	0.00043693	0.0005492	*Morganella*	8.34098E-	0.00029363	0	0
Uncultured	8.34098E-	0.00127243	0.00046424	0.0012276	*Babeliales*	8.34098E-	0	0	0
Desulfuromonadaceae	8.34098E-	0.00013050	0.00010923	6.4612E-	*Promicromonosporaceae*	8.34098E-	0	0	0
PAUC26f	8.34098E-	6.52529E-	0.00049154	0.0001938	*Pseudohongiella*	0	0.00068515	0	0
uncultured	8.34098E-	0.00081566	0.00049154	0.0005815	*Aureispira*	0	0.00055464	0	0
Desulfoprunum	0	0.00048939	5.46165E-	0	*Serratia*	0	0.00035889	0	6.4612E-05
UTCFX1	0.00091750	0.00042414	0.00090117	0.0007107	*Clostridioides*	0	0.00035889	0	0.00012922
Sphingoaurantiacus	0	0	5.46165E-	0	*Thermomonas*	0	0.00022838	0	0.00074303
Lewinella	8.34098E-	0	5.46165E-	0.0002261	*Immundisolibacter*	0	0.00022838	0	0
Candidatus_Berkiella	0.00050045	0.00140293	0.00051885	0.0010984	*Candidatus_Ovatusbacter*	0.00029193	0.00146818	5.46165E-	0.00061381
Pla1_lineage	0.00062557	0.00045677	0.00060078	0.0003230	*Uliginosibacterium*	0	0.00022838	0	0.00080765
Lactobacillus	0.00262740	0	0.00010923	6.4612E-	*uncultured*	0	0.00029363	0	0
uncultured	0.00070898	6.52529E-	0.00024577	0.0001938	*BSV13*	0	0.00016313	0	0
Anaerosolibacter	0	0	5.46165E-	0	*Capnocytophaga*	0	0.00022838	0	0
Gaiellales	0.00070898	0	0.00021846	0	*Sphaerotilus*	0	9.78793E-	0	0
uncultured	0.00667278	0.00071778	0.00532510	0.0009045	*Ornithinibacter*	0	0.00016313	0	0
CL500-29_marine_group	0.00129285	0.00094616	0.00049154	0.0023583	*Williamsia*	0	6.52529E-	0	0

Leptothrix	0.00050045	0.00055464	0.00035500	6.4612E-	*RBG-16-49-21*	0	0.00117455	0	0
Myxococcaceae	0.00037534	0	0.00016384	0	*Veillonella*	0	6.52529E-	0	9.6918E-05
DEV114	0.00037534	6.52529E-	5.46165E-	0.0003230	*WCHB1-81*	0	0.00019575	0	0
Desulfobacca	0.00045875	0.00065252	0.00166580	0.0008399	*TSBb06*	0	6.52529E-	0	0
DS-100	0.00041704	0.00019575	0.00013654	0.0003553	*NK4A214_group*	0	6.52529E-	0	0
EF100-94H03	0	6.52529E-	0.00016384	0.0005492	*pLW-20*	0	0.00045677	0	0
uncultured	0.00025022	0.00065252	0.00117425	0.0004845	*TM7a*	0	0.00013050	0	6.4612E-05
Bacteroidetes_vadinHA17	0.00029193	0.00055464	5.46165E-	9.6918E-	*Rickettsiella*	0	0.00026101	0	0
Subgroup_25	0.00116773	0.00035889	0.00109232	0.0015506	*Demequina*	0	6.52529E-	0.00013654	0
SH-PL14	0.00087580	0.00019575	0.00101040	0.0005492	*Dojkabacteria*	0	0.00323001	0	6.4612E-05
uncultured	0.00075068	0.00048939	0.00106502	0.0005492	*Smithella*	0	0.00016313	0	0
Vicinamibacter	0.00025022	0	0.00071001	0.0003876	*MSBL9*	0	6.52529E-	0	0
Tetrasphaera	8.34098E-	0.00022838	0.00038231	0.0001292	*Micrococcus*	0	6.52529E-	0	0
Allorhizobium-Neorhizobium-	0	0.00035889	0.00038231	0.0008722	*[Aquaspirillum]_arcticum_group*	0	6.52529E-	0	0
RBG-13-54-9	0.00033363	0	0.00095578	0.0018414	*Jeotgalicoccus*	0	0.00013050	0	0
PeM15	0.00025022	0.00032626	0.00010923	6.4612E-	*Chthonobacter*	0	6.52529E-	0	0
Pseudorhodoplanes	0.00079239	0	0.00016384	0.0003230	*GIF9*	0	0.00019575	0	0
env.OPS_17	0.00041704	0.00123980	0.00021846	6.4612E-	*Spirochaeta_2*	0	0.00179445	0	0
Sva0081_sediment_group	0.00062557	0.00068515	0.00027308	0.0003230	*uncultured*	0	6.52529E-	0	6.4612E-05
Marmoricola	8.34098E-	0	0.00016384	0.0001292	*uncultured*	8.34098E-	0.02176182	0.00010923	0.01809136
Methylovirgula	0	0	0.00013654	0	*Roseateles*	0	0	0	0.00219680
Kapabacteriales	0.00037534	0.00045677	0.00010923	0.0007107	*HOC36*	0.00016682	0	0	0.00029075
Pla3_lineage	0.00070898	0.00068515	0.00043693	0.0009368	*uncultured*	0	0	0.00016384	0.00025844
Planctopirus	0.00054216	0.00035889	0.00021846	0.0010014	*Leptospira*	0	0	0	0.00016153
uncultured	0	0	5.46165E-	0	*Deinococcus*	0	0	0	0.00025844
Clostridia_UCG-014	0.00158478	0.00013050	5.46165E-	0	*Lactococcus*	0	0	0	0.00022614
BD1-7_clade	0.00108432	0.00019575	0.00081924	0.0041997	*Gammaproteobacteria*	0	0.00032626	0	0.00167991
uncultured	0.00325298	0.00104404	0.00081924	0.0030690	*Asticcacaulis*	0	0	0	0.00012922
Malikia	0	0	5.46165E-	0	*Rosenbergiella*	0	0	0	0.00012922
Pseudenhygromyxa	0	0	5.46165E-	0	*Flaviaesturariibacter*	8.34098E-	0	0	0.00019383
RBG-16-58-14	0.00016682	0	0.00024577	0	*Methylocapsa*	0	0	0	0.00019383
Stella	0	0.00013050	5.46165E-	0	*Lechevalieria*	0	0	5.46165E-	9.6918E-05
Krasilnikovia	0.00145967	0	0.00027308	0.0001938	*C1-B045*	0	0.00016313	0	9.6918E-05
Tagaea	0	0	0.00010923	0	*Oleiphilus*	0	0.00039151	0	9.6918E-05
Subgroup_11	0.00020852	6.52529E-	0.00057347	0.0005169	*Bacteroidetes_VC2.1_Bac22*	0	6.52529E-	0	9.6918E-05
Ferrovibrio	0	0.00019575	5.46165E-	0	*Dietzia*	0	0	0	9.6918E-05
Flavisolibacter	0.00012511	0.00013050	5.46165E-	0	*Kazania*	0.00016682	0	0.00016384	0.00041997
Sulfurisoma	0	0.00407830	0.00010923	6.4612E-	*Paucibacter*	0	0	0	6.4612E-05
SM1A02	0.00050045	0.00084828	0.00092848	0.0019383	*Clostridium_sensu_stricto_2*	0	0	0	0.00019383

uncultured	8.34098E-	0.00032626	0.00030039	0.0002584	*uncultured*	0	0	0	6.4612E-05
Geodermatophilus	0.00016682	0	5.46165E-	6.4612E-	*Candidatus_Magasanikbacteria*	0.00116773	0.00218597	0.00010923	0.00352135
Haloactinopolyspora	0	0	0.00021846	0	*Klenkia*	0	0	0	6.4612E-05
uncultured	0.00133455	6.52529E-	0.00054616	0.0002584	*Galbitalea*	0	0	0	6.4612E-05
Ideonella	8.34098E-	0	0.00016384	0	*Anaerospora*	0.00025022	0	5.46165E-	0.00016153
S0134_terrestrial_group	0.00200183	0.00146818	0.00090117	6.4612E-	*Microbacterium*	0.00012511	0	0	0.00019383
Fimbriimonadales	0	0	0.00016384	0	*uncultured*	0	0	0	0.00012922
Chungangia	0	9.78793E-	5.46165E-	0	*Cnuella*	0	0	0	6.4612E-05
hgcI_clade	8.34098E-	0	0.00021846	0	*Pseudoclavibacter*	0	0	0	6.4612E-05
Lysobacter	0.00025022	0.00133768	0.00030039	0.0002261	*Peptostreptococcaceae*	0	0	5.46165E-	0.00019383
alphaI_cluster	8.34098E-	0.00013050	0.00021846	0.0004522	*Achromobacter*	8.34098E-	0	0	6.4612E-05
Brevundimonas	0.00020852	0.00081566	0.00016384	0.0032952	*Clostridium_sensu_stricto_11*	0	0	0	6.4612E-05
Gemmatimonas	0.00091750	0.00078303	0.00147464	0.0011953	*Mobilitalea*	0	0	0	6.4612E-05
Nonomuraea	0.00025022	6.52529E-	0.00049154	0	*Porticoccus*	0	0.00035889	0	6.4612E-05
Massilia	0.00075068	0.00013050	0.00038231	0.0013568	*Blvii28_wastewater-sludge_group*	0	0.00019575	0	0
Methylibium	0.00020852	0	5.46165E-	0	*Janthinobacterium*	0	0.00016313	0	0
Ahniella	0.00083409	0.00254486	0.00177503	0.0017445	*uncultured*	0	0.00029363	0	6.4612E-05
Staphylococcus	0.00312786	0.00133768	0	0.0014214	*CM1G08*	0	9.78793E-	5.46165E-	0
Streptococcus	0.00187672	0.00035889	5.46165E-	0.0015183	*1174-901-12*	0	0.00013050	0	0
Anoxybacillus	0.00075068	0	0	0.0001938	*Lentimicrobiaceae*	0	0.00022838	0	6.4612E-05
Lawsonella	0.00125114	0.00035889	0	0.0004522	*Polaromonas*	0	6.52529E-	0	0
Cutibacterium	0.00179331	0.00042414	0	0.0007430	*Turneriella*	0	0.00013050	5.46165E-	0
JG36-TzT-191	0.00041704	0	0.00019115	0	*SG8-4*	0	6.52529E-	0	0
Corynebacterium	0.00125114	0.00039151	0	0.0012276	*WWE3*	8.34098E-	0.00061990	0	6.4612E-05
Comamonadaceae	0.00054216	9.78793E-	5.46165E-	0	*Bradymonadales*	0	6.52529E-	0	0
Cupriavidus	0.00020852	9.78793E-	5.46165E-	0.0001292	*Sterolibacterium*	0	6.52529E-	0	0
Muribaculaceae	0.00375344	0.00013050	0	6.4612E-	*Candidatus_Azambacteria*	0	0.00022838	0.00010923	0.00058150
Clostridia_vadinBB60_group	0.00033363	0	0	0	*Rivibacter*	0	6.52529E-	0	0
Acidovorax	0.00058386	0.00026101	5.46165E-	0.0003876	*Pluralibacter*	0	6.52529E-	0	0
Thermus	0.00083409	0.00045677	0	0.0002584	*Rothia*	0	6.52529E-	0	0
Oceanobacillus	0.00025022	0	5.46165E-	0	*Magnetovibrio*	0	6.52529E-	0	0
uncultured	0.00045875	0.00055464	0.00021846	0.0004522	*Actinotalea*	0	6.52529E-	0	0
Polycyclovorans	0.00145967	6.52529E-	0.00081924	6.4612E-	*Cellulosimicrobium*	0	6.52529E-	0	0
Ensifer	0.00033363	0	0.00010923	0	*SR-FBR-L83*	0	6.52529E-	0	0
Soonwooa	0.00012511	0	0	0	*FukuN18_freshwater_group*	0	6.52529E-	0	0
Kineosporia	0.00066727	6.52529E-	0	0	*Uncultured*	0	6.52529E-	0.00016384	6.4612E-05
Rubellimicrobium	0.00112603	6.52529E-	0.00019115	0.0003230	*Meiothermus*	8.34098E-	6.52529E-	0	0.00025844
Rhizocola	0.00012511	0	0.00010923	0.0001938	*Taonella*	0	0.00013050	0	0
Selenomonas	0.00020852	0	0	0	*Chryseoglobus*	0	6.52529E-	0	0

Vibrionimonas	0.00029193	0.00029363	0	0.0004199	*Uncultured*	0	0	0.00057347	0.00016153
Sphingobacterium	0.00012511	0	0	0	*Uncultured*	0.00079239	0.00058727	0.00385046	0.00151838
Qipengyuania	0.00020852	6.52529E-	0	0	*Uncultured*	0.00120944	0.00026101	0.00120156	0.00113071
Syntrophorhabdus	0.00029193	0.00035889	0.00010923	0.0001292	*Uncultured*	0.00116773	0.00117455	0.00333160	0.00206758
MD2902-B12	0.00012511	0	0	0	*Uncultured*	0	0	8.19247E-	0
AKAU4049	0.00137626	0	0.00049154	0	*Uncultured*	0	0	0.00062808	0.00019383
S-BQ2-57_soil_group	0.00216865	0	0.00128348	0.0019706	*Uncultured*	0.00058386	0	0.00060078	0.00032306
Pelomonas	0.00012511	0.00016313	0	0.0002261	*Uncultured*	0.00054216	0.00022838	0.00071001	0.00054920
Roseomonas	0.00033363	0.00022838	0.00021846	0.0007430	*Uncultured*	0	0	0.00019115	0
Proteus	8.34098E-	0	0	0	*Uncultured*	0.00012511	6.52529E-	0.00185696	0.00116301
Proteiniphilum	0.00016682	0	0	0	*Uncultured*	8.34098E-	0	0.00106502	0.00090456
Ignavibacterium	0.00070898	6.52529E-	0.00016384	0.0001292	*Uncultured*	0.00137626	0.00277324	0.00281274	0.00216450
Oligoflexus	0.00016682	0.00016313	0.00016384	0.0007430	*Uncultured*	0.00012511	0	0.00038231	6.4612E-05
Solirubrobacteraceae	8.34098E-	0	0.00019115	0	*Uncultured*	0	6.52529E-	0.00021846	6.4612E-05
wb1-P19	0.00016682	0	0	0	*Uncultured*	0	0.00026101	0.00021846	0.00022614
Pseudoxanthomonas	8.34098E-	0.00045677	0	0	*Uncultured*	0	0.00019575	0.00016384	0
Jatrophihabitans	0.00045875	0	5.46165E-	0.0002261	*Uncultured*	8.34098E-	6.52529E-	0.00010923	0.00041997
Hymenobacter	0.00016682	0	0	0.0022937	*Uncultured*	0.00016682	0	0.00016384	0
Luteolibacter	8.34098E-	6.52529E-	5.46165E-	0.0004199	*Uncultured*	0.00066727	6.52529E-	0.00106502	0.00025844
Aquisphaera	0.00025022	0.00026101	0.00030039	0.0005815	*Uncultured*	0.00062557	0	0.00062808	0.00041997
Azospira	0.00025022	0	0	6.4612E-	*Uncultured*	0	0	0.00010923	6.4612E-05
Methylobacter	0.00100091	0	0.00016384	9.6918E-	*Uncultured*	0	0.00016313	0.00021846	0.00012922
Flavobacterium	0.00045875	0.00130505	0	0.0062673	*Uncultured*	0.00025022	0.00022838	0.00051885	0.00080765
Flavitalea	0.00016682	6.52529E-	5.46165E-	6.4612E-	*Uncultured*	0.00050045	0	0.00010923	0
Sphaerospermopsis_BCCUSP	0.00016682	0	0	0.0001938	*Uncultured*	8.34098E-	0	0.00010923	0
Caenimonas	0.00016682	0.00029363	0	0.0001292	*Uncultured*	0.00045875	0.00019575	0.00024577	0.00038767
Delftia	0.00037534	0.00022838	0	0.0001938	*Uncultured*	0	6.52529E-	0.00027308	0.00019383
uncultured	0.00016682	0.00016313	0	0.0006138	*Uncultured*	0	0	0.00010923	0
Aquabacterium	0.00058386	0.00022838	5.46165E-	0.0009368	*Uncultured*	0	0	0.00010923	0
Neo-b11	0.00025022	0	5.46165E-	0	*Uncultured*	8.34098E-	0.00061990	0.00101040	0.00332751
Rhodocyclaceae	8.34098E-	0	0	6.4612E-	*Uncultured*	0.00183501	0.00084828	0.00027308	0.00132454
Candidatus_Lloydbacteria	0.00016682	9.78793E-	5.46165E-	0.0003230	*Uncultured*	8.34098E-	0	5.46165E-	6.4612E-05
AKIW781	0.00050045	0	5.46165E-	0.0001292	*Uncultured*	0.00016682	0	0.00016384	6.4612E-05
Aminobacter	0.00020852	0	0	0	*Uncultured*	0	0	0.00016384	0.00012922
Mitochondria	8.34098E-	0	0	0	*Uncultured*	0	0	5.46165E-	0
Candidatus_Nitrotoga	0.00016682	0.00022838	0.00035500	0.0001938	*Uncultured*	0	0	0.00030039	0
Candidatus_Obscuribacter	0.00033363	0.00045677	0	0.0003876	*Uncultured*	0.00050045	0	0.00043693	6.4612E-05
Anaerococcus	8.34098E-	0	0	6.4612E-	*Uncultured*	0.00070898	6.52529E-	5.46165E-	0.00048459
Legionella	0.00158478	0.00172920	0.00101040	0.0017122	*Polymorphospora*	0	0	5.46165E-	0

uncultured	8.34098E-	0	0	0	*Uncultured*	0	0	5.46165E-	0
Promicromonospora	0.00037534	0	0	0	*Uncultured*	0.00033363	0	0.00021846	0.00032306
Subgroup_9	0.00070898	0.00088091	0.00021846	6.4612E-	*Uncultured*	0.00029193	0	0.00016384	0
uncultured	0.00025022	0.00231647	0	0.0004199	*Uncultured*	0	0	5.46165E-	0
Fusobacterium	0.00033363	6.52529E-	0	0	*Uncultured*	0	0	5.46165E-	0
0319-7L14	0.00187672	0	0.00087386	0	*Uncultured*	0.00054216	0.00013050	0.00021846	0.00071073
Vermiphilaceae	0.00020852	0.00169657	0.00010923	0.0030690	*Uncultured*	0	0	5.46165E-	0.00016153
SAR202_clade	0.00016682	0	0	0	*Uncultured*	0	0	0.00030039	6.4612E-05
Bosea	0.00016682	6.52529E-	0	0.0001292	*Uncultured*	0	0	0.00038231	0.00054920
Chloroplast	0.00045875	0.00084828	0.00010923	0.0014860	*uncultured*	0	0	0.00016384	0.00032306
Segetibacter	8.34098E-	0.00013050	0	0.0001938	*Uncultured*	0	0	5.46165E-	0
Dermabacter	8.34098E-	0	0	0	*Uncultured*	0.00016682	0.00026101	0.00024577	0.00058150
uncultured	0.00029193	0.00019575	0	0.0003553	*Uncultured*	8.34098E-	0.00029363	0.00016384	6.4612E-05
Lineage_IIc	0.00037534	0.00048939	0.00019115	6.4612E-	*Uncultured*	8.34098E-	0.00026101	0.00038231	0.00025844
FCPU426	0.00037534	0.00094616	5.46165E-	0.0001938	*Uncultured*	0	0	0.00019115	0
Saccharimonadales	0.00437901	0.01477977	0.00120156	0.0043613	*Uncultured*	8.34098E-	0	5.46165E-	0.00029075
Desulfatiglans	0.00050045	0.00114192	0.00054616	6.4612E-	*Uncultured*	0	0	0.00021846	0.00048459
Rhodobacter	0.00050045	0.00042414	0.00013654	0.0012922	*Uncultured*	0.00012511	0	0	0
Haemophilus	0.00025022	0.00013050	0	0.0001938	*Uncultured*	0.00012511	0	0	0
Dechloromonas	0.00029193	0.00029363	0.00035500	0.0006784	*Uncultured*	8.34098E-	9.78793E-	5.46165E-	0.00019383
Ochrobactrum	8.34098E-	0	0	0	*Uncultured*	0.00016682	6.52529E-	0.00019115	0.00016153
uncultured	8.34098E-	0.00019575	0	0	*Uncultured*	0	0.00026101	0	0.00067842
Cohnella	8.34098E-	0	0.00010923	0.0004522	*Uncultured*	0	0.00039151	0.00010923	0.00019383
Thermicanus	8.34098E-	0	0	0	*Uncultured*	0	0	0	0.00048459
Methylomonas	0.00058386	0.00035889	5.46165E-	0.0004845	*Uncultured*	8.34098E-	0	0.00016384	0.00032306
Actinorectispora	8.34098E-	0	5.46165E-	0	*Uncultured*	8.34098E-	0.00042414	0.00010923	0.00064612
Methylorosula	8.34098E-	0	0	6.4612E-	*Uncultured*	0	0.00013050	0.00010923	0.00035536
Levilinea	0.00016682	0	0	0	*Uncultured*	0	0	5.46165E-	0.00038767
Uncultured	0.00012511	6.52529E-	0	0	*Uncultured*	0	0.00013050	0	0.00025844
LD29	0	0	0	0.0010661	*Uncultured*	0	0	0	6.4612E-05
BD7-11	0.00029193	0.00016313	5.46165E-	0.0004845	*Uncultured*	0	0.00013050	0	0.00012922
uncultured	0.00029193	0.00515497	0.00024577	0.0045874	*Uncultured*	0	0	5.46165E-	6.4612E-05
uncultured	0.00058386	0.00078303	0.00073732	0.0016153	*Uncultured*	8.34098E-	6.52529E-	8.19247E-	0.00041997
Uncultured	8.34098E-	0	0.00136541	0.0010984	*Uncultured*	0.00012511	0	0	0.00012922
Lineage_IIa	0.00066727	0.00026101	0.00043693	0.0003553	*Uncultured*	0	0	5.46165E-	0.00029075
Candidatus_Omnitrophus	0.00037534	0.00685155	0.00046424	0.0012922	*Uncultured*	0	0.00016313	8.19247E-	0.00012922
Desulfurivibrio	0	9.78793E-	0	0.0001615	*Uncultured*	8.34098E-	0	0.00016384	0.00038767
Abditibacterium	0	0	0.00010923	9.6918E-	*Uncultured*	8.34098E-	0.00013050	0.00021846	6.4612E-05
Sumerlaea	0.00016682	0.00013050	0.00021846	0.0017122	*Uncultured*	0	9.78793E-	0.00010923	0

Filomicrobium	0	0	0.00021846	0.0006138	*Uncultured*	0	0	5.46165E-	6.4612E-05
Rhodomicrobium	8.34098E-	0.00045677	0	0.0002261	*Uncultured*	0.00012511	0	5.46165E-	0
Uncultured	0	0.00042414	0.00027308	0.0009368	*Uncultured*	0	0	5.46165E-	0
Candidatus_Nostocoida	0	0	0	9.6918E-	*Uncultured*	0	0	5.46165E-	0
Uncultured	0.00025022	6.52529E-	0.00019115	0.0003230	*Uncultured*	0	0	5.46165E-	0
uncultured	0.00016682	0.00084828	0.00010923	0.0068488	*Uncultured*	0	0	5.46165E-	0
Peredibacter	0	0.00026101	5.46165E-	0.0001615	*Uncultured*	0	0	5.46165E-	0
Blastocatella	0.00041704	0	0.00095578	0.0005492	*Uncultured*	0	6.52529E-	5.46165E-	0
DEV007	0.00050045	0.00032626	0.00027308	0.0008399	*Uncultured*	0	0	5.46165E-	6.4612E-05
Armatimonas	0	0	5.46165E-	0.0001615	*Uncultured*	0	0	0.00010923	0
Vogesella	0	0	0	0.0002261	*Uncultured*	0	0.00032626	5.46165E-	6.4612E-05
KD3-10	0.00016682	9.78793E-	5.46165E-	0.0002261	*Uncultured*	0.00020852	0	0	0
NS9_marine_group	0.00016682	6.52529E-	0.00013654	0.0001615	*Uncultured*	8.34098E-	0	5.46165E-	0.00032306
AT-s3-28	0.00012511	0	0.00016384	0.0005492	*Uncultured*	8.34098E-	0	0	0
Dinghuibacter	8.34098E-	0.00013050	5.46165E-	0.0002584	*Uncultured*	8.34098E-	0	0	0
uncultured	0	0	0	0.0002907	*Corynebacteriaceae*	0.00016682	0	0	0
S-70	0	0	0	0.0001615	*Uncultured*	8.34098E-	0	0	0.00041997
uncultured	0.00020852	0.00332789	0.00013654	0.0002584	*Uncultured*	8.34098E-	0	0	0.00022614
Cyanobium_PCC-6307	0	6.52529E-	5.46165E-	0.0005492	*Uncultured*	8.34098E-	0	0	0
uncultured	0.00075068	0.00032626	0	6.4612E-	*Uncultured*	0	0	0	0.00012922
KCLunmb-38-53	0	0	0	6.4612E-	*Uncultured*	0	0	0	6.4612E-05
Prevotellaceae_UCG-001	0	0	0	6.4612E-	*Uncultured*	0	0	0	6.4612E-05
JG30-KF-AS9	0	0	5.46165E-	0.0014860	*Uncultured*	0	9.78793E-	0	6.4612E-05
uncultured	0.00041704	0.00042414	0.00095578	0.0006784	*Uncultured*	0	0	0	6.4612E-05
KD3-93	0.00025022	0	0.00010923	0.0005815	*Uncultured*	0	0	0	6.4612E-05
Amphiplicatus	8.34098E-	0	5.46165E-	0.0006461	*Uncultured*	0	0	0	6.4612E-05
uncultured	0	0.00032626	5.46165E-	0.0002907	*Uncultured*	0	0	0	6.4612E-05
Candidatus_Methylomirabilis	0	0	0.00019115	0.0002584	*Uncultured*	0	0	0	6.4612E-05
UBA12409	0.00033363	0	8.19247E-	0.0002584	*Uncultured*	0	6.52529E-	5.46165E-	0
Actinoallomurus	8.34098E-	0	0.00027308	0.0003230	*Uncultured*	0	0	5.46165E-	0
uncultured	0	6.52529E-	8.19247E-	6.4612E-	*Uncultured*	0.00016682	6.52529E-	0	0
Uncultured	0.00016682	0	0.00032769	0.0003876	*Uncultured*	0	0.00061990	0	0
Paraclostridium	0	0	5.46165E-	0.0001938	*Lachnospiraceae_NC2004_group*	0	6.52529E-	0	0
Bdellovibrio	0.00058386	0.00130505	0.00019115	0.0005815	*Uncultured*	0	0.00019575	0	0
Prosthecobacter	0	6.52529E-	5.46165E-	6.4612E-	*Uncultured*	0	6.52529E-	0	0
Amb-16S-1323	0.00016682	0.00016313	0.00010923	0.0001938	*Uncultured*	0	0	0	0.00016153
Leifsonia	0	0	0	0.0002584	*Uncultured*	0	6.52529E-	0	0
Candidatus_Protochlamydia	0.00300275	0.00101141	0.00062808	0.0067519	*Uncultured*	0	0.00013050	0	0
Uncultured	8.34098E-	0	0.00032769	6.4612E-	*Afipia*	0	6.52529E-	0	0

Clostridium_sensu_stricto_3	0	0	0	0.0001292	*Uncultured*	0	6.52529E-	0	0
Defluviimonas	0	0.00019575	5.46165E-	0.0003230					

APPENDIX I

Comparison against NCBI data for mercury methylating genera

Taxonomy	Genera Name	DT	JB	OW	RM
d__Bacteria;p__Nitrospirota;c__Nitrospiria;o__Nitrospirales;f__Nitrospiraceae;g__Nitrospira	*Nitrospira*	0.01618	0.00251	0.013408	0.02032
d__Bacteria;p__Patescibacteria;c__Parcubacteria;o__Candidatus_Kaiserbacteria;f__Candidatus_Kaiserbacteri	*Candidatus_Kaiserbacteria*	0.00141	0.00045	0.001037	0.00145
d__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Tistrellales;f__Geminicoccaceae;g__Candidatus_Al	*Candidatus_Alysiosphaera*	0.00041	0.00019	0.000600	0.00012
d__Bacteria;p__Actinobacteriota;c__Actinobacteria;o__Corynebacteriales;f__Mycobacteriaceae;g__Mycobacter	*Mycobacterium*	0.00170	0.00078	0.001420	0.00323
d__Bacteria;p__Desulfobacterota;c__Desulfuromonadia;o__Geobacterales;f__Geobacteraceae;g__Geobacterac	*Geobacteraceae*	0.00062	0.00022	0.000791	0.00093
d__Bacteria;p__Verrucomicrobiota;c__Verrucomicrobiae;o__Chthoniobacterales;f__Chthoniobacteraceae;g__C	*Candidatus_Udaeobacter*	0.00029	0.00026	0.001365	0.00158
d__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Caulobacterales;f__Caulobacteraceae;g__Phenylob	*Phenylobacterium*	0	0.00176	0.000191	0.00041
d__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridia;f__Hungateiclostridiaceae;g__Anaerobacterium	*Anaerobacterium*	8.341E-	0	0.000300	0.00012
d__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Clostridiaceae;g__Clostridium_sensu_stricto_1	*Clostridium_sensu_stricto_1*	0.00066	0.00088	0.000573	0.00083
d__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Paracaedibacterales;f__Paracaedibacteraceae;g__	*Candidatus_Paracaedibacter*	0.00029	0.00045	0.000355	0.00035
d__Bacteria;p__Desulfobacterota;c__Desulfuromonadia;o__Desulfuromonadia;f__Desulfuromonadaceae;g__De	*Desulfuromonas*	0	0	0.000300	0.00022
d__Bacteria;p__Firmicutes;c__Desulfitobacteriia;o__Desulfitobacteriales;f__Desulfitobacteriaceae;g__Desulfos	*Desulfosporosinus*	0	0	0.000901	0
d__Bacteria;p__Acidobacteriota;c__Acidobacteriae;o__Solibacterales;f__Solibacteraceae;g__Candidatus_Solib	*Candidatus_Solibacter*	0.00254	6.5253E	0.002730	0.00206
d__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Clostridiaceae;g__Clostridium_sensu_stricto_13	*Clostridium_sensu_stricto_13*	8.341E-	0.00039	0.000273	0.00032
d__Bacteria;p__Desulfobacterota;c__Desulfobulbia;o__Desulfobulbales;f__Desulfobulbaceae;g__Desulfobulbus	*Desulfobulbus*	8.341E-	9.7879E	0.000163	0.00016
d__Bacteria;p__Desulfobacterota;c__Desulfuromonadia;o__Desulfuromonadia;f__Geothermobacteraceae;g__G	*Geothermobacter*	0.00029	0.00035	0.000273	0.00161
d__Bacteria;p__Desulfobacterota;c__Desulfuromonadia;o__Geobacterales;f__Geobacteraceae;g__Citriferment	*Citrifermentans*	0.00125	0.00019	0.001283	0.00116
d__Bacteria;p__Firmicutes;c__Desulfitobacteriia;o__Desulfitobacteriales;f__Desulfitobacteriaceae;g__Desulfito	*Desulfitobacterium*	8.341E-	0	0.000218	0.00025
d__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Clostridiaceae;g__Clostridium_sensu_stricto_6	*Clostridium_sensu_stricto_6*	8.341E-	0	0.000109	9.6918E
d__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Clostridiaceae;g__Clostridium_sensu_stricto_8	*Clostridium_sensu_stricto_8*	0.00120	0	0.000737	0.00012
d__Bacteria;p__Patescibacteria;c__Parcubacteria;o__Candidatus_Nomurabacteria;f__Candidatus_Nomurabact	*Candidatus_Nomurabacteria*	0.00083	0.00058	0.001365	0.00277
d__Bacteria;p__Planctomycetota;c__Brocadiae;o__Brocadiales;f__Brocadiaceae;g__Candidatus_Brocadia	*Candidatus_Brocadia*	0	0	0.000109	0
d__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Clostridiaceae;g__Clostridium_sensu_stricto_12	*Clostridium_sensu_stricto_12*	0.00033	6.5253E	0.000764	0.00029
d__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rickettsiales;f__Rickettsiales;g__Candidatus_Jidaib	*Candidatus_Jidaibacter*	0.00075	0.00016	5.46165E	0.00045
d__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Clostridiaceae;g__Clostridium_sensu_stricto_9	*Clostridium_sensu_stricto_9*	8.341E-	0.00026	0.000109	0.00012
d__Bacteria;p__Chloroflexi;c__Anaerolineae;o__Anaerolineales;f__Anaerolineaceae;g__Anaerolinea	*Anaerolinea*	0.00104	0.00068	0.000464	0.00164
d__Bacteria;p__Patescibacteria;c__Parcubacteria;o__Candidatus_Moranbacteria;f__Candidatus_Moranbacteri	*Candidatus_Moranbacteria*	0.00104	0.00013	0.000382	0
d__Bacteria;p__Desulfobacterota;c__Desulfuromonadia;o__Geobacterales;f__Geobacteraceae;g__Geobacter	*Geobacter*	0.00141	0.00215	0.000546	0.00080
d__Bacteria;p__Desulfobacterota;c__Desulfuromonadia;o__Geobacterales;f__Geobacteraceae;g__Geotalea	*Geotalea*	0.00020	0	5.46165E	0
d__Bacteria;p__Desulfobacterota;c__Syntrophia;o__Syntrophales;f__Syntrophaceae;g__Syntrophus	*Syntrophus*	0.00016	0.00107	0.000273	0.00035
d__Bacteria;p__Acidobacteriota;c__Acidobacteriae;o__Acidobacteriales;f__Koribacteraceae;g__Candidatus_K	*Candidatus_Koribacter*	0.00041	0.00013	0.000464	0.00035
d__Bacteria;p__Verrucomicrobiota;c__Verrucomicrobiae;o__Chthoniobacterales;f__Xiphinematobacteraceae;g	*Candidatus_Xiphinematobact*	0.00041	6.5253E	0.000791	0.00038
d__Bacteria;p__Desulfobacterota;c__Desulfuromonadia;o__Desulfuromonadia;f__Desulfuromonadaceae;g__De	*Desulfuromonadaceae*	8.341E-	0.00013	0.000109	6.4612E
d__Bacteria;p__Desulfobacterota;c__Desulfobulbia;o__Desulfobulbales;f__Desulfocapsaceae;g__Desulfoprunu	*Desulfoprunum*	0	0.00048	5.46165E	0

d__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Gammaproteobacteria_Incertae_Sedis;f__Unknow	*Candidatus_Berkiella*	0.00050	0.00140	0.000518	0.00109
d__Bacteria;p__Desulfobacterota;c__Desulfobaccia;o__Desulfobaccales;f__Desulfobaccaceae;g__Desulfobacca	*Desulfobacca*	0.00045	0.00065	0.001665	0.00083
*d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidetes_vadinHA17;g__Bacteroidetes_*	*Bacteroidetes_vadinHA17*	0.00029	0.00055	5.46165E	9.6918E
d__Bacteria;p__Actinobacteriota;c__Actinobacteria;o__Propionibacteriales;f__Propionibacteriaceae;g__Cutiba	*Cutibacterium*	0.00179	0.00042	0	0.00074
d__Bacteria;p__Actinobacteriota;c__Actinobacteria;o__Corynebacteriales;f__Corynebacteriaceae;g__Coryneba	*Corynebacterium*	0.00125	0.00039	0	0.00122
d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Sphingobacteriales;f__Sphingobacteriaceae;g__Sphingobacteri	*Sphingobacterium*	0.00012	0	0	0
d__Bacteria;p__Desulfobacterota;c__Syntrophorhabdia;o__Syntrophorhabdales;f__Syntrophorhabdaceae;g__Sy	*Syntrophorhabdus*	0.00029	0.00035	0.000109	0.00012
d__Bacteria;p__Bacteroidota;c__Ignavibacteria;o__Ignavibacteriales;f__Ignavibacteriaceae;g__Ignavibacteriu	*Ignavibacterium*	0.00070	6.5253E	0.000163	0.00012
d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Flavobacteriales;f__Flavobacteriaceae;g__Flavobacterium	*Flavobacterium*	0.00045	0.00130	0	0.00626
d__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Burkholderiales;f__Comamonadaceae;g__Aquaba	*Aquabacterium*	0.00058	0.00022	5.46165E	0.00093
d__Bacteria;p__Patescibacteria;c__Parcubacteria;o__Candidatus_Lloydbacteria;f__Candidatus_Lloydbacteria;	*Candidatus_Lloydbacteria*	0.00016	9.7879E	5.46165E	0.00032
d__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Burkholderiales;f__Gallionellaceae;g__Candidatu	*Candidatus_Nitrotoga*	0.00016	0.00022	0.000355	0.00019
d__Bacteria;p__Cyanobacteria;c__Vampirivibrionia;o__Obscuribacterales;f__Obscuribacteraceae;g__Candidat	*Candidatus_Obscuribacter*	0.00033	0.00045	0	0.00038
d__Bacteria;p__Fusobacteriota;c__Fusobacteriia;o__Fusobacteriales;f__Fusobacteriaceae;g__Fusobacterium	*Fusobacterium*	0.00033	6.5253E	0	0
d__Bacteria;p__Verrucomicrobiota;c__Omnitrophia;o__Omnitrophales;f__Omnitrophaceae;g__Candidatus_Om	*Candidatus_Omnitrophus*	0.00037	0.00685	0.000464	0.00129
d__Bacteria;p__Abditibacteriota;c__Abditibacteria;o__Abditibacteriales;f__Abditibacteriaceae;g__Abditibacteri	*Abditibacterium*	0	0	0.000109	9.6918E
d__Bacteria;p__Planctomycetota;c__Planctomycetes;o__Isosphaerales;f__Isosphaeraceae;g__Candidatus_Nost	*Candidatus_Nostocoida*	0	0	0	9.6918E
d__Bacteria;p__Methylomirabilota;c__Methylomirabilia;o__Methylomirabilales;f__Methylomirabilaceae;g__Ca	*Candidatus_Methylomirabilis*	0	0	0.000191	0.00025
d__Bacteria;p__Verrucomicrobiota;c__Chlamydiae;o__Chlamydiales;f__Parachlamydiaceae;g__Candidatus_Pr	*Candidatus_Protochlamydia*	0.00300	0.00101	0.000628	0.00675
d__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Clostridiaceae;g__Clostridium_sensu_stricto_3	*Clostridium_sensu_stricto_3*	0	0	0	0.00012
d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Chitinophagales;f__Chitinophagaceae;g__Sediminibacterium	*Sediminibacterium*	0.00029	0.00022	0	0.00061
d__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Clostridiaceae;g__Clostridium_sensu_stricto_5	*Clostridium_sensu_stricto_5*	0	6.5253E	0	0.00012
d__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Beijerinckiaceae;g__Methylobacteri	*Methylobacterium-*	0.00016	0.00061	5.46165E	0.00061
d__Bacteria;p__Patescibacteria;c__Gracilibacteria;o__Candidatus_Peribacteria;f__Candidatus_Peribacteria;g	*Candidatus_Peribacteria*	0	6.5253E	5.46165E	0.00012
d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides	*Bacteroides*	0.00563	6.5253E	0	0
d__Bacteria;p__Entotheonellaeota;c__Entotheonellia;o__Entotheonellales;f__Entotheonellaceae;g__Candidatus	*Candidatus_Entotheonella*	0.00016	0	5.46165E	6.4612E
d__Bacteria;p__Actinobacteriota;c__Actinobacteria;o__Bifidobacteriales;f__Bifidobacteriaceae;g__Bifidobacter	*Bifidobacterium*	0.00091	6.5253E	0	0
d__Bacteria;p__Desulfobacterota;c__Desulfovibrionia;o__Desulfovibrionales;f__Desulfovibrionaceae;g__Desul	*Desulfovibrio*	0.00016	6.5253E	5.46165E	0
d__Bacteria;p__Firmicutes;c__Clostridia;o__Oscillospirales;f__Ruminococcaceae;g__Faecalibacterium	*Faecalibacterium*	0.00029	0	0	0
d__Bacteria;p__Firmicutes;c__Clostridia;o__Oscillospirales;f__[Eubacterium]_coprostanoligenes_group;g__[E	*[Eubacterium]_coprostanolig*	0.00050	0	0	0
d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Flavobacteriales;f__Weeksellaceae;g__Chryseobacterium	*Chryseobacterium*	0.00012	0.00022	0	0.00022
d__Bacteria;p__Patescibacteria;c__Saccharimonadia;o__Saccharimonadales;f__Saccharimonadaceae;g__Cand	*Candidatus_Saccharimonas*	0.00033	0	0	0
d__Bacteria;p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g__[Eubacterium]_eligens_gr	*[Eubacterium]_eligens_group*	0.00016	0	0	0
d__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridia;f__Hungateiclostridiaceae;g__Acetivibrio	*Acetivibrio*	0.00016	0	0	0
d__Bacteria;p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g__[Eubacterium]_xylanophil	*[Eubacterium]_xylanophilum*	0.00041	0	0	0
d__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Clostridiaceae;g__Candidatus_Arthromitus	*Candidatus_Arthromitus*	0.00016	0	0	0
d__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Burkholderiales;f__Rhodocyclaceae;g__Candidat	*Candidatus_Accumulibacter*	0.00016	0	0	0
d__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Clostridiaceae;g__Clostridium_sensu_stricto_10	*Clostridium_sensu_stricto_10*	8.341E-	0	5.46165E	0
d__Bacteria;p__Firmicutes;c__Bacilli;o__Erysipelotrichales;f__Erysipelatoclostridiaceae;g__Candidatus_Stoqu	*Candidatus_Stoquefichus*	8.341E-	0	0	0

d__Bacteria;p__Patescibacteria;c__Parcubacteria;o__Candidatus_Spechtbacteria;f__Candidatus_Spechtbacteri	*Candidatus_Spechtbacteria*	0.00016	0	0	0
d__Bacteria;p__Firmicutes;c__Clostridia;o__Lachnospirales;f__Lachnospiraceae;g__[Eubacterium]_ruminanti	*[Eubacterium]_ruminantium_*	0.00016	0	0	0
d__Bacteria;p__Firmicutes;c__Clostridia;o__Peptostreptococcales-	*[Eubacterium]_brachy_group*	8.341E-	0	0	0
d__Bacteria;p__Firmicutes;c__Negativicutes;o__Acidaminococcales;f__Acidaminococcaceae;g__Phascolarctob	*Phascolarctobacterium*	0.00020	0	0.000109	0
d__Bacteria;p__Desulfobacterota;c__Desulfobulbia;o__Desulfobulbales;f__Desulfocapsaceae;g__[Desulfobacte	*[Desulfobacterium]_catecholi*	8.341E-	6.5253E	0	0
d__Bacteria;p__Actinobacteriota;c__Actinobacteria;o__Micrococcales;f__Microbacteriaceae;g__Curtobacteriu	*Curtobacterium*	8.341E-	0	0	0
d__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Rhizobiaceae;g__Phyllobacterium	*Phyllobacterium*	0	0	0	6.4612E
d__Bacteria;p__Deferrisomatota;c__Defferrisomatia;o__Defferrisomatales;f__Defferrisomataceae;g__Deferriso	*Deferrisoma*	0.00016	0	0.000382	0.00038
d__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Paracaedibacterales;f__Paracaedibacteraceae;g__	*Candidatus_Finniella*	0	0	5.46165E	0
d__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Burkholderiales;f__Oxalobacteraceae;g__Undiba	*Undibacterium*	0	0	0.000163	0.00090
d__Bacteria;p__Patescibacteria;c__Parcubacteria;o__Candidatus_Adlerbacteria;f__Candidatus_Adlerbacteria;	*Candidatus_Adlerbacteria*	0.00233	0.00039	0.001501	0.00074
d__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Competibacterales;f__Competibacteraceae;g__Ca	*Candidatus_Competibacter*	0	0.00016	5.46165E	0
d__Bacteria;p__Patescibacteria;c__Microgenomatia;o__Candidatus_Pacebacteria;f__Candidatus_Pacebacteria	*Candidatus_Pacebacteria*	8.341E-	0.00349	0	0.00025
d__Bacteria;p__Patescibacteria;c__Microgenomatia;o__Candidatus_Curtissbacteria;f__Candidatus_Curtissbact	*Candidatus_Curtissbacteria*	0	6.5253E	0	0
d__Bacteria;p__Planctomycetota;c__Planctomycetes;o__Pirellulales;f__Pirellulaceae;g__Candidatus_Anammox	*Candidatus_Anammoximicrob*	0.00050	6.5253E	0.000109	0
d__Bacteria;p__Patescibacteria;c__Microgenomatia;o__Candidatus_Collierbacteria;f__Candidatus_Collierbact	*Candidatus_Collierbacteria*	0	0.00192	0	0.00022
d__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Gammaproteobacteria_Incertae_Sedis;f__Unknow	*Candidatus_Ovatusbacter*	0.00029	0.00146	5.46165E	0.00061
d__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Burkholderiales;f__Rhodocyclaceae;g__Uliginosi	*Uliginosibacterium*	0	0.00022	0	0.00080
d__Bacteria;p__Desulfobacterota;c__Syntrophia;o__Syntrophales;f__Smithellaceae;g__Smithella	*Smithella*	0	0.00016	0	0
d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Bacteroidetes_VC2.1_Bac22;f__Bacteroidetes_VC2.1_Bac22;g	*Bacteroidetes_VC2.1_Bac22*	0	6.5253E	0	9.6918E
d__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Clostridiaceae;g__Clostridium_sensu_stricto_2	*Clostridium_sensu_stricto_2*	0	0	0	0.00019
d__Bacteria;p__Patescibacteria;c__ABY1;o__Candidatus_Magasanikbacteria;f__Candidatus_Magasanikbacteri	*Candidatus_Magasanikbacter*	0.00116	0.00218	0.000109	0.00352
d__Bacteria;p__Actinobacteriota;c__Actinobacteria;o__Micrococcales;f__Microbacteriaceae;g__Microbacteriu	*Microbacterium*	0.00012	0	0	0.00019
d__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Clostridiaceae;g__Clostridium_sensu_stricto_11	*Clostridium_sensu_stricto_11*	0	0	0	6.4612E
d__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Burkholderiales;f__Oxalobacteraceae;g__Janthin	*Janthinobacterium*	0	0.00016	0	0
d__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Burkholderiales;f__Rhodocyclaceae;g__Steroliba	*Sterolibacterium*	0	6.5253E	0	0
d__Bacteria;p__Patescibacteria;c__Parcubacteria;o__Candidatus_Azambacteria;f__Candidatus_Azambacteria;	*Candidatus_Azambacteria*	0	0.00022	0.000109	0.00058

VITA

Sujina Manandhar

EDUCATION

M.S. Biology, Sam Houston State University, Huntsville, Texas
Advisor: Dr. Madhusudan Choudhary
Expected Graduation: August 2022

B.S. Biological Sciences, Southeast Missouri State University, Cape Girardeau, Missouri
Advisor: Dr. James Champine
Graduation: May 2017

B.S. Microbiology, St. Xavier's College, Tribhuvan University, Kathmandu, Nepal
Advisor: Dr. Sudhakar Pant
Graduation: May 2014

PROFESSIONAL EXPERIENCE

EPA Sterility QC Technician, Pharmedium Healthcare Corporation, Sugar Land, Texas
Feb 2019 - April 2020
Perform endotoxin and sterility tests on Pharmedium compounded preparation following standard operation procedures

Laboratory Technician II, Steris Laboratories Inc., Brooklyn Park, Minnesota
Jun 2017 - Dec 2018
Execute microbiological tests (Microbial limit test, Population verification of biological indicators, and Surface disinfectant efficacy tests) in compliance with USP and ISO standards and regulation

Laboratory Assistant, Department of Biology, Microbiology Lab, Southeast Missouri State University, Cape Girardeau, Missouri
Jan 2016 - May 2017

SKILLS

- United State Pharmacopeia (USP) Standards:
  USP <61>, USP <62>, USP <71>, USP <85>, and USP <1072>
- Current Good Manufacturing Practice (cGMP), Good Documentation Practice (GDP)
- Microbiology Testing:
  Endotoxin Test, Sterility Test, Microbial Limit Test, Environmental Monitoring Test, Disinfection Efficacy Testing, Growth Promotion Test, Quality Control
- The R-Language for Statistical Computing

ACTIVITIES

Nov 2021 "The Effects of Mercury on Microbiome Composition of Soil Sediments of the Trinity River, Texas
Poster Presentation at ASM Texas Branch, Online Fall Meeting

HONORS/AWARDS

- The College of Science and Engineering Technology (COSET) Graduate Achievement Scholarship, Sam Houston State University, Spring 2022 $1500
- The College of Science and Engineering Technology (COSET) Graduate Achievement Scholarship, Sam Houston State University, Fall 2021 $1500
- The College of Science and Engineering Technology (COSET) Graduate Recruitment Scholarship, Sam Houston State University, Spring 2021 $1000
- Magna Cum Laude, Southeast Missouri State University, May 2017
- Dean's Honor List, Southeast Missouri State University, Fall 2015, Spring 2016, Spring 2017
- Office of International Education and Services Scholarship, Southeast Missouri State University, Fall 2015 - Fall 2017
- Academic Excellence Award, St. Xavier College, May 2013 - May 2014