BACTERIAL COMMUNITY ANALYSIS OF SEVEN POLLUTED LAKES IN BANGALORE, INDIA: THE FOAM STOPS HERE

AN HONORS THESIS

SUBMITTED ON THE FIFTH DAY OF MAY, 2020

TO THE DEPARTMENT OF PUBLIC HEALTH

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS

OF THE HONORS PROGRAM

OF NEWCOMB-TULANE COLLEGE

TULANE UNIVERSITY

FOR THE DEGREE OF

BACHELOR OF SCIENCE IN PUBLIC HEALTH

WITH HONORS IN PUBLIC HEALTH

BY

Tanya Sarah Isaac

APPROVED:

Dr. Samendra Sherchan
Director of Thesis

Dr. Mark Wilson
Second Reader

Dr. Laurie O’Brien
Third Reader
Abstract

This study analyzes the microbial population of seven highly polluted lakes in the Varthur lake series in Bangalore, Karnataka. Unchecked development made Bangalore the center of India’s IT development, which led to a demographic transition. This resulted in a city populated with thirteen million people whose sewage needs could not be met. Due to increased industrial pollution and poor sewage disposal practices, approximately 60% of Bangalore’s sewage goes into the Varthur lake series, primarily into Varthur and Bellandur lakes causing the proliferation of pathogenic bacteria and opportunistic pathogens. Water samples were collected in December 2019 from seven lakes in the Varthur lake series to determine the level of opportunistic pathogens in these water bodies. Nanopore sequencing of 16S rRNA genes identified several pathogenic bacterial strains such as *Escherichia coli*, *Klebsiella pneumoniae*, and mycobacterium and industrially important species such as *Pseudomonas aeruginosa*. The dominant microorganisms found in the Varthur lake series belonged to the phyla *Proteobacteria*, *Bacteroidetes*, *Firmicutes*, and *Cyanobacteria*. Additionally, all seven samples had fecal indicator organisms indicating fecal contamination. The results of this study indicate the need for drastic and immediate control and remediation measures to reduce neighboring communities’ exposure to these harmful pathogens.
Acknowledgements

I am thankful to Dr. Aidan Smith, Dr. Beth Wee, and Dr. Samendra Sherchan for their leadership in this project. I would also like to thank NCI for the funding and resources provided, which made this project possible.
Table of Contents

Section of Thesis

Page No.

Title Page [i]

Abstract ii

Acknowledgements iii

Table of Contents iv

List of Tables vi

List of Figures vii

Chapter 1: Introduction 1

Chapter 2: Background 4

Causes 4

Urbanization 4

Encroachment 4

Poor Administration 5

Pollution 5

Point source pollution 6

Non-point source pollution 7

Opportunistic Pathogens 8

The Koramangala-Challaghatta lake system: Varthur lake series 10

Agara lake 10

Bellandur lake 10

Varthur Lake 12
List of Tables

Table 1. Barcodes used for metagenome sequencing. ........................................ 16
Table 2. Estimated DNA Concentration. ............................................................ 19
Table 3. Alpha diversity calculated using Shannon index. ............................... 20
Table 4. Classification summary of reads. ......................................................... 20
Table 5. Summary of bacterial community results for Agara lake. ................. 24
Table 6. Summary of bacterial community results for Kaikondrahalli lake. ...... 25
Table 7. Summary of bacterial community results for Kasavanahalli lake. ........ 26
Table 8. Summary of bacterial community results for Bellandur lake. ............. 27
Table 9. Summary of bacterial community results for Bellandur lake. ............. 28
Table 10. Summary of bacterial community results for Doddanekundi lake....... 29
Table 11. Summary of bacterial community results for Varthur lake. .............. 30
List of Figures

Figure 1. Map of Bangalore, India................................................................. 1

Figure 2. The Koramangala-Challaghatta Valley. ....................................... 10

Figure 3. Bellandur Lake. ........................................................................... 11

Figure 4. Sampling Sites in the Koramangala-Challaghatta Valley. .......... 15

Figure 5. Overview of library preparation. .................................................. 17

Figure 6. Agarose gel showing PCR amplification for 16s rRNA gene. ...... 20

Figure 7. (a). Proportion of the 20 most abundant phyla; 1: Agara, 5: Bellandur, 6: Doddanekundi, 7: Varthur, 2: Kaikondrahalli, 3: Kasavanahalli, 4: Haralur lake. ........................................................................................................ 21

(b). Proportion of the 20 most abundant classes; 1: Agara, 5: Bellandur, 6: Doddanekundi, 7: Varthur, 2: Kaikondrahalli, 3: Kasavanahalli, 4: Haralur lake. ........................................................................................................ 22


Chapter 1: Introduction

Water pollution is a burgeoning environmental issue in India and eutrophication of fresh water in urban areas is currently on the rise. Many lakes and freshwater bodies are deteriorating, and their ecosystems destroyed at an alarming rate (Murthy, Kadali, & N, 2017). This is especially prevalent in Bangalore, a city in the South of India, once known as “The city of lakes” primarily due to the sheer number of lakes it housed and also the pivotal role that lakes played for centuries in this region. A major contributor to Bangalore’s water crisis is the rapid and ongoing destruction of the city’s lake ecosystems.

Most of Bangalore’s “lakes” are not lakes in the sense of naturally occurring water bodies, as they were man-made and constructed as reservoirs, locally known as tanks, in the early 17th century for drinking water and recreational use. The tanks are interconnected by various streams in a cascade network creating different lake series.
These are divided into three valleys over an 800 km$^2$ area atop the Deccan plateau, approximately 3034.78 feet above mean sea level (MSL) (Lele & Sengupta, 2018). The Deccan Plateau is completely landlocked making Bangalore’s primary source of water freshwater lakes and stormwater, receiving 900 mm rainfall annually. Therefore, trapping stormwater runoff by constructing earthen bunds across second and third order streams is how many of the beforementioned reservoirs were built, and high densities of reservoirs in the BMA made the landscape resemble a network or cascade system. As one reservoir fills and overflows, the excess water is directed along smaller streams and channels to neighboring reservoirs, which allowed maximized storage of rainwater. This created large interconnected systems of lakes divided into three valleys: i) The Hebbal Valley; (ii) the Koramangala-Challaghatta Valley, and (iii) the Vrishabhavati Valley, which has evolved into Bangalore’s modern-day lake system (Namma Bengaluru Foundation, 2015).

Unchecked development made Bangalore, a city in the South of India, the center of the information technology (IT) industry and led to a demographic transition resulting in a city populated with thirteen million people whose sewage needs could not be met (Jain, Kumar & Saharia, 2012). Land-grabbing for commercial purposes has resulted in a 79% decline in the geographic area of lakes in the Bangalore Metropolitan Area (BMA). Additionally, Industrial pollution and poor sewage disposal practices have resulted in millions of liters of pollutants going into these lakes everyday including untreated sewage, treated sewage discharge, industrial effluents, heavy metals, and solid waste. Approximately 60% of Bangalore’s sewage goes into two lakes in the Koramangala Challaghatta Lake System, i.e., Bellandur and Varthur lake, making them some of the world’s most polluted lakes (Jain, Kumar & Saharia, 2012). The chemicals polluting these lakes create an environment conducive
to the growth of harmful opportunistic pathogens, many of which are unknown to the public due to the lack of testing, that pose several health risks to the lakes’ flora, fauna and surrounding communities. Opportunistic pathogens are defined as organisms that can become pathogenic in individuals with pre-disposing conditions such as age (>70 years), cancer or immunodeficiency (CDC, 2018). These include *e.coli, legionella, mycobacterium* spp, and *C. ochracea*. Pollution and subsequent contamination have also caused the disappearance of native species, dominance of invasive species, such as water hyacinth, and the breeding of disease vectors and pathogens (Ramachandra et al., 2015).

Water samples were collected from 7 of 11 lakes in the Koramangala-Challaghatta lake system and underwent next generation sequencing processes to determine their bacterial communities and the identification of pathogenic species. Aim: The aim of this study is to determine the bacterial communities of seven lakes in the Koramangala-Challaghatta lake system and identify opportunistic pathogens and other pathogenic species.
Chapter 2: Background

As rural landscapes undergo the rapid process of urbanization in South Asia, several water bodies originally created for irrigation or drinking water purposes are undergoing a transition for industrial or residential purposes. In the context of India, this is happening at a very rapid rate and such urbanization demands new forms of governance, and stricter administration and management (Nagendra & Ostrom, 2014). However, Bangalore’s governing bodies have not kept up with its wetland’s needs resulting in them being encroached upon, polluted, drained, or built on. The number of lakes in Bangalore has decreased from 262 in 1960 to 81 in 2010, of which only 34 were biologically live lakes.

Causes

Urbanization. A temporal land analysis demonstrated that Bangalore underwent a 925% increase in the built-up area that resulted in a 79% decrease in the area of water bodies in the Bangalore metropolitan area from 1973 to 2013, (Ramachandra et al., 2015). In 2001, the water level needed to be increased as the water demand increased to 750 million liters per day and the actual supply was 570 million liters per day, which was the start of Bangalore’s water crisis (Environmental Management & Policy Research Institute, 2018). This rapid development not only had detrimental effects on Bangalore’s lakes, but also resulted in the loss of agricultural and eco-sensitive zones, such as forests and wetlands, and increased greenhouse gas emissions and energy consumption (Ramachandra & Shwetmala, 2009).

Encroachment. Encroachment is defined as the placement of any material or structure in any lakes and ponds and to alter, or cause to be altered, the lands underlying any public waters (Agency of Natural Resources, 2019). 98% of Bangalore’s lakes are encroached, half of which were for illegal constructions.
Multi-storied buildings have been constructed atop lake beds causing a sharp decline in the natural flow of water into catchments resulting in a rapid deterioration of water bodies.

**Poor administration.** The official institutional arrangements that oversee the management and governance of lakes is constantly changing. Originally, the reservoirs were managed by local communities until the early 20th century, until the colonial government changed policies and instated the Minor Irrigation Department (MID) and Revenue Department as their owners. After which, the Fisheries Department auctioned off the fishing rights to these water bodies. In the late 1980s, coinciding with when the number of lakes in Bangalore started disappearing, the BDA was given custody of majority of these water bodies, while the remaining were transferred to the Karnataka Forest Department (KFD). In an effort to consolidate lake management, and protect and oversee water bodies, the Lake Development Authority (LDA) was formed in 2002. The LDA was also supposed to behave as an oversight committee to stop corruption and malpractice from destroying these water bodies and to prevent encroachment, however, in 2004 the LDA was found to have been leasing lakes out to private companies for “monetary consideration”. I.e., the LDA were taking bribes to allow encroachment, development, and sewage disposal on these water bodies’ sites, which led to the loss of almost 20 lakes (Lele & Sengupta, 2018).

Currently, all lakes in the Bangalore metropolitan area are under the jurisdiction of Bruhat Bengaluru Mahanagara Palike (BBMP) for administration and Bangalore Development Authority (BDA) for city planning but moves back and forth between the two agencies annually. As of 2019, the BBMP oversees 109 water bodies, the BDA oversees 92, KFD controls 5 and the Fisheries Department continues to control fishing rights.
**Pollution.** Pollution of these lakes is due to pollutants entering from point sources and non-point sources. Point sources are direct sources of pollutants, including nutrients from wastewater through municipal and domestic effluents, organic and inorganic toxic substances from industrial effluents, and fertilizers and toxic pesticides from agriculture runoff. Non-point source pollution is a combination of pollutants from a large area rather than from singular sources including storm water runoff, atmospheric pollutants causing acid rain, and pollution from human settlements (EPA, 2018; Ramachandra et al., 2015).

**Point source pollution.**

*Sustained discharge of untreated domestic sewage.* As of 2015, 90% of Bangalore’s lakes contained sewage and 72% showed a loss in catchment area (Ramachandra et al., 2015). Bangalore Water Supply and Sewage Boards (BWSSB) is not able to treat the generated sewage and has resulted in a gap between the generation and treatment of domestic wastewater. Therefore, a large percentage of Bangalore’s sewage and wastewater is discharged into its lakes.

Untreated sewage discharge into these urban waterbodies has led to eutrophication causing algal blooms, the spread of water hyacinth, and hypoxic conditions (Mahapatra et al., 2011). The largest consequence of sustained discharge of untreated domestic sewage into lakes in Bangalore is the formation of a thick water hyacinth layer on the surface. *Eichhornia crassipes* or water hyacinth is an invasive weed species that derives its nutrients directly from water, draining the lake ecosystems of nutrients that other flora and fauna need (Mahapatra et al., 2011). Sewage provides the necessary nutrients, such as phosphates, nitrates, and potassium, that lead to overgrowth of this plant, allowing water hyacinth to double its population in 15-18 days (Mujere, 2015, p. 343). Additionally, hyacinth mats block sunlight from
entering water bodies and create conditions that limit photosynthesis in other aquatic plant species and algae further reducing the lake’s dissolved oxygen levels (DO) (Mujere, 2015, p. 343).

In its matured stages, water hyacinth frequently succumbs to weevil infestations that makes them sink. In doing so, the macrophytes leach plant nutrients and undergo bacterial degradation that drastically reduces DO levels in the lake thereby increasing the biological oxygen demand (BOD). The widescale depletion of DO in lakes created hypoxic conditions, meaning that the concentration of oxygen was so low that it was detrimental to organisms and very few survived in those conditions causing “Fish Kills”, i.e., the mass mortality of fish over a short period of time (Kitch, n.d. & Mahapatra et al., 2011). Ultimately, the death of such a large number of fish will contribute to a further increase in sediments and nutrients in the water body, further perpetuating the cycle of eutrophication. Once fish kills start to occur more frequently and to a greater extent, the brood stock of fish in these lakes will reduce drastically (Mujere, 2015, p. 356).

*Industrial effluents.* The primary types of industrial waste entering these lakes are synthetic fibers and plastic (Environmental Management & Policy Research Institute, 2018). Fiberglass industries dump several synthetic components, such as polyamide, acrylic, and polyester polypropylene, into these lakes. Additionally, asbestos fibers found in fiberglass was also found in Bellandur lake (Ramachandra et al., 2015). Plastic refers to all items made of polypropylene and compounds containing polypropylene, poly vinyl chloride (PVC) and poly amides (nylon) (Environmental Management & Policy Research Institute, 2018). These substances choke drains and release harmful chemicals such as Bisphenol A, a toxic chemical that damages the reproductive system of animals. Other chemicals present include
ethylene oxide, xylene, and benzene and even alkyl phenols which have been identified as carcinogenic (Gopal et al., 2014).

Heavy metals present in industrial effluents include lead, zinc, copper, arsenic, mercury and cadmium plastic (Environmental Management & Policy Research Institute, 2018). Lead, copper and zinc are extremely persistent, toxic, and have great protentional for bioaccumulation. Mercury and arsenic cause renal and nervous failure in aquatic organisms and via bioaccumulation, if consumed by humans, can have seriously adverse effects on human health (Xu, Wu & Li, 2017).

Non-point source pollution.

Agricultural runoff. The agricultural runoff from neighboring fields consists of fertilizers and pesticides containing nitrates, pesticides, phosphates, salts, and sodium (Energy and Wetland research Group, Centre for Ecological Sciences, Indian Institute of Science, 2007). Nitrates and phosphates contribute to the eutrophication of lakes, and pesticides can cause birth defects, genetic mutations, central nervous system damage and more in organisms they come in contact with (Anderson et al., 2013).

Opportunistic Pathogens

Opportunistic pathogens are microorganisms that cause diseases in individuals with predisposing conditions such as age (>70 years), cancer, or immunodeficiency, which is detrimental to communities surrounding these water bodies and are capable of causing a lot of disease and adverse health outcomes (Byappanahalli et al., 2012). Opportunistic pathogens enter the Varthur lake series through the discharge of treated and untreated sewage. There are four major opportunistic pathogens in the Varthur lake series: *Legionella spp.* had the highest prevalence followed by total coliforms, *mycobacterium spp.* and *Enterobacteriaceae*. These are associated with increased rates of typhoid, dysentery, pneumonia, legionnaire’s syndrome, cholera and bacterial
meningitis in neighboring villages (Byappanahalli et al., 2012). The prevalence of opportunistic pathogens in lakes vary by season due to the amount of rainfall received.

*Legionella* spp. has been found to cause Legionellosis, and is the leading water associated pathogen to cause pneumonia and even death in immunocompromised individuals with an increasing incidence. Salavec et al. (2014) conducted a literature review of pathogenic representatives of *Legionella* bacteria and concluded that it is a febrile respiratory disease-causing pathogen that poses the highest risk to immunosuppressed patients, smokers, people with chronic lung disease and people above seventy years of age. *Legionella* favor the aquatic environment but can also be found in damp soils. In addition to natural reservoirs (surface water, groundwater, fresh and saltwater), they are found in a wide range of water-based technical installations - water mains, showers, swimming pools, spa facilities, fog-towers, aerators, spraying garden irrigation systems, cooling towers, etc. Legionella has been found to have the highest prevalence in Bangalore lakes and poses several health risks (Salavec et al., 2014).

Another bacterium that has been frequently found in Bangalore’s lakes is *Enterococci*. *Enterococci* are common members of gut communities in mammals and birds but are also opportunistic pathogens that cause millions of infections annually. Byappanahalli, Nevers, Korajkic, Staley & Harwood (2012) explored the factors that influence the proliferation of *Enterococci* in the natural environment. They examined how *Enterococci* served as fecal bacteria indicators and are used as measures of water quality. Since these cells are usually shed in human and animal feces, they are readily culturable and are used to predict human health risks from exposure to polluted recreational waters.
The Koramangala-Challaghatta lake system: Varthur lake series

The Varthur lake series in the Koramangala-Challaghatta Valley is composed of eleven lakes, and this study focuses on the following seven—Agara, Bellandur, Doddanekundi, Haralur, Kasavanahalli, Kaikondrahalli, and Varthur lakes. All seven are group c lakes, meaning they are highly polluted and characterized by high ionic contents, nutrient levels, BOD, and organic contents due to anthropogenic activities.

**Agara lake.** Agara lake is located in the southeast of Bangalore spanning 48.38 hectares acres in size (D’Souza & Nagendra, 2011). The surrounding residential areas include Agara, HSR layout, Venkateshwara Layout and some parts of Koramangala. Water from this lake drains into the neighboring Bellandur lake in the north, and eventually into Varthur lake.
Bellandur lake. Bellandur lake is the largest water body in the BMA, spanning an area of 370 hectares, making it one of the largest manmade lakes in Southeast Asia (Lele & Sengupta, 2018). Bellandur lake is located in south-east Bangalore city, a very important ecological zone as it was one of the major sources of water to various fauna and migratory birds from different parts of the country up until 15 years ago. It also provided drinking water to half the city’s population. Since Bangalore does not have a perennial river, it depended on Bellandur the harvestation? of rainwater to sustain livelihood activities such as agriculture, irrigation, and cleaning. Therefore, this lake played a crucial role in recharging groundwater and sustaining flora, fauna and human life through this region of Bangalore.

Until 1970, there were three channels that fed the lake with storm water, allowing it to be a constant source of freshwater to the city. The three channels are part of the Bellandur drainage system, which drained the southern and southeastern parts of the city, however, urbanization in the 1980s blocked those chains resulting in insufficient rainwater reaching the lake. Since the lake wasn’t able to drain itself
anymore, excess untreated sewage and effluents that entered settled in the lakebed, which further led to a decrease in aquatic life.

With decades of indiscriminate waste disposal into this lake and a lack of drainage options, the lake has become heavily polluted. Bellandur lake receives 550 million liters per day (MLD) of sewage, 200 MLD of which is treated effluent from the sewage treatment plant (STP) located upstream and remaining is untreated sewage that comes in through stormwater inlets and official BWSSB sewers. Bellandur lake is also in a very densely populated area. It is located very close to the old airport in Bangalore and surrounded by apartment complexes, settlements of Bellandur and Iblur village, and military lands. These surrounding residents have faced the brunt of Bellandur’s pollution and have filed cases with the Karnataka High Court going back as far as 1998 to augment the capacity of STP.

In April 2015, Bellandur lake reached its peak pollution level. The biosurfactants and other compounds that entered the lake through sewage caused the formation of froth and foam over its surface. Eventually, the froth overflowed onto the roads and started being carried away by the winds, endangering road traffic, especially bicyclists and motorcyclists. In May that year, the froth caught fire and burned for three days. This was repeated again in later November after which, the state government was forced to address the issue and formed an expert committee to go about the rejuvenation of this lake. The Environmental Protection Agency (EPA) characterizes ignitability as one of the four primary characteristics required to designate something as hazardous waste (EPA, 2020).

Varthur Lake. Varthur lake is the second largest lake in the Koramangala-Challaghatta Valley in the south of Bangalore. It was originally built as a drinking water storage tank and for irrigation purposes. It is a shallow, wind influenced
hypereutrophic lake (i.e., extremely nutrient-rich) characterized by recurrent algal blooms. Its original area was 216 hectares, however, due to erratic rainfall, siltation and encroachment, the lake now spans an area of 165.7 hectares. Varthur lake is located downstream from a chain of thee lakes, one of them being Bellandur lake, the largest and most polluted lake in the city (Ramachandra et al., 2015). Varthur lake’s downstream location from Bellandur lake’s catchment results in Varthur receiving the overflow from Bellandur, which will then join the Dakshina Pinkani river. Therefore, Varthur lake receives all of the surface runoff, wastewater and sewage from the southern district of Bangalore (Ramachandra et al., 2015). This amount of pollution has resulted in a thick macrophyte layer, depleted DO, the overgrowth of algae and bacteria lowering the nitrate level, and extremely high phosphate levels, threatening the lake’s aquatic life (Jumbe & Nandini, 2009). The depleted DO levels in the lake have resulted in 3 episodes of fish kills in the past decade.

The sustained inflow of sewage into Bellandur and Varthur lake contain several natural and synthetic dissolved organic compounds (DOCs) (Environmental Management & Policy Research Institute, 2018). Biosurfactants (natural) include carboxylic fatty acids from lipids in the water hyacinth and synthetic surfactants include soaps, detergents, personal care products such as shampoos and toothpaste, fats, oils, and grease. Detergents, in particular, contain branch-chained alkyl benzene sulfonate surfactants, these are not biodegradable and produce extremely persistent foam, especially during the monsoons due to consistent rainfall. This foam always exists on the surface of Varthur lake; however, during the monsoon season it overflows into the surrounding lands and streets. Detergents with high levels of phosphates and the lake’s naturally occurring phosphates due to eutrophication accelerate the formation of foam (Ramachandra et al., 2015). Foam houses
hydrophobic compounds, i.e., those repelled by water. Therefore, the foam is primarily composed of nutrients (nitrogen, phosphorus, carbon), cations (sodium, potassium, calcium, magnesium), heavy metals (cadmium, copper, iron, lead, zinc) and chlorinated hydrocarbon (Environmental Management & Policy Research Institute, 2018). These harmful substances enter the food chain for organisms residing in the surface layer, contaminate groundwater, and are dangerous to all those using Varthur’s water for domestic and irrigational purposes (Murthy, Kadali & Chandran, 2017).

There have been several incidents of the foam catching fire, setting ablaze the entire surface of the lake. This has been the result of combustion due to contact with cigarette butts, and the release of methane, a combustible gas released by pollutants within the lake. When Varthur lake burned for the first time, it reduced its own acid neutralizing capacity, making it susceptible to catching fire again in the future, which it constantly did (Murthy, Kadali & Chandran, 2017). The fire burns surrounding vegetation and lands around the lake releasing ash, foam, froth, and other harmful gases into the air. These gases get trapped in the atmosphere and persist, causing respiratory problems such as asthma (Environmental Management & Policy Research Institute, 2018). Alkyl benzene sulfonate, the primary surfactant found in this lake, is an anion and easily forms bonds with cations such as heavy metals thus allowing metals such as zinc and copper to be present in the foam. Consequently, when this foam is ignited, it poses the risk of metal exposures through inhalation from the smoke.

Kasavanahalli and Kaikondrahalli lake. Both Kasavanahalli and Kaikondrahalli lake are part of the Varthur lake series as part of the Koramanagala-Challaghatta valley. Kaikondrahalli lake spans an area of 27.09 hectares and
Kasavanahalli lake spans an area of 8.91 hectares (Ramachandra, Aithal, Shivamurthy, & Setturu, 2015). Both of these lakes were restored in 2009. They support 37 species of fauna and is surrounded by thick vegetation which are nesting grounds for various species of storks. Two drainage channels enter Kasavanahalli lake and one drainage channel enters the Kaikondrahalli lakebed, bringing fresh sewage into both lakes. Earlier, these lakes were used as sources of drinking water, ritual place, agriculture, and washing cattle for the Kasavanahalli and Kaikondrahalli villages. Additionally, both of these lakes have witnessed a major disappearance of native fish species and have become a breeding ground for mosquitos and other disease vectors.

**Doddanekundi lake.** Doddanekundi lake is the third largest lake in eastern Bangalore spanning an area of 60.7 hectares. It is located 13 km north of both Kasavanahalli and Kaikondrahalli lake and connected to them via numerous smaller channels.
Chapter 3: Materials and Methods

Water Sample Collection

Two liters of water were collected from seven lakes, i.e., Agara, Varthur, Bellandur, Kaikondrahalli, Kasavanahalli, Haralur, and Doddanekundi lake corresponding to sample 1,2,3,4,5,6, and 7 respectively. The samples were all collected from 10am to 12pm on 15\textsuperscript{th} December 2019 as convenience sampling. One liter each was collected from two points from every lake and transported to Genotypic Technology Labs in a cooler for analysis immediately.

DNA Extraction and Quality Control

DNA from water samples was isolated by the method described by Satyabrata Bag et al., 2016. Samples were lysed with glass beads followed by enzymatic lysis with Lysozyme, Mutanolysin and Lysostaphin. They were then incubated further with lysis buffer and DNA precipitated by salt and ethanol. DNA was re-suspended in 50 μl of 10mM Tris.Cl, pH 8.0(Cat#T3038-1L). The quantification and quality of the
genomic DNA was assessed using Nanodrop2000 (Thermo Scientific, USA), Qubit (Thermo Scientific, USA) and agarose gel electrophoresis.

**PCR Amplification**

The samples’ DNA were subjected to 16s rRNA gene amplification using region specific primers and LongAmp Taq 2X master mix (NEB). 1.5kb amplicons were obtained for both the samples for which Agarose gel QC was performed. The PCR products were purified by using 1.6X Ampure XP beads (Beckmann Coulter, USA).

**Library Preparation**

A total of 50ng amplicon DNA from each sample was end-repaired (NEBnext ultra II end repair kit, New England Biolabs, MA, USA), cleaned up with 1X AmPure beads (Beckmann Coulter, USA). Barcode adapter ligation (BCA) was performed with NEB blunt/ TA ligase (New England Biolabs, MA, USA) and cleaned with 1X AmPure beads. Qubit quantified adapter ligated DNA samples were barcoded using PCR reaction (LongAmp Ta 2x New England Biolabs, MA, USA) and cleaned up with 1.6x AmPure beads (Beckmann- Coulter, USA). Barcode sequences are detailed in the Table 1.

Table 1

<table>
<thead>
<tr>
<th>Sample</th>
<th>Barcode name</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>BC61</td>
<td>AGAGGGTACTATGTGCCTCAGCAC</td>
</tr>
<tr>
<td>2</td>
<td>BC62</td>
<td>CACCCACACTTACTTCAGGACGT</td>
</tr>
<tr>
<td>3</td>
<td>BC63</td>
<td>TTCTGAAGTTCCTGGGTCTTGAAC</td>
</tr>
<tr>
<td>4</td>
<td>BC64</td>
<td>GACAGACAACCGTTCATCGACTTTC</td>
</tr>
<tr>
<td>5</td>
<td>BC65</td>
<td>TTCTCAGTCTTCCTCCAGAAAAG</td>
</tr>
<tr>
<td>6</td>
<td>BC66</td>
<td>CCGATCCTTGTCCTCCTAATTC</td>
</tr>
<tr>
<td>7</td>
<td>BC67</td>
<td>GTTTTGCTACTCGTGCTCACC</td>
</tr>
</tbody>
</table>
Qubit quantified barcode ligated DNA samples were pooled at equimolar concentration and end-repair was performed using NEBnext ultra II end repair kit (New England Biolabs, MA, USA). End-repaired DNA was cleaned up with 1x AmPure beads. Adapter ligation (AMX) was performed for 15 minutes using NEB blunt/ TA ligase (New England Biolabs, MA, USA). Library mix was cleaned up using Ampure beads and finally eluted in 15 μl of elution buffer. (Figure 5: Overview of Library Preparation).

Figure 5. Overview of library preparation

Nanopore Sequencing

Sequencing was performed on GridION X5 (Oxford Nanopore Technologies, Oxford, UK) using SpotON flow cell R9.4 (FLO-MIN106) in 72 hrs sequencing protocol on MinKNOW 2.1 v19.02.1. Nanopore raw reads (‘fast5’ format) were basecalled (‘fastq5’ format) and demultiplexed using Guppy basecaller v2.3.5.

Data Analysis

The Nanopore raw reads which had 16S regions and high-quality bases were selected. Pre-processing of the reads were performed using Porechop. Processed reads
were then aligned against GREENGENES database using the Centrifuge. The Vegan package in R was used for diversity calculation. The matrix was generated of (species present) vs (species count in sample) and this matrix was used for alpha diversity calculation. Alpha diversity is the microbial diversity assessed within a sample which reflects the diversity of taxonomy within the sample. The diversity was calculated using Shannon diversity index (H) which characterize species diversity.

\[
SH = -\sum p_i \log p_i
\]

Where \(i=1\) and H corresponds to shannon index. The beta diversity was calculated using z indices based on Arrhenius species-area model.

\[
Z = \frac{[\log(2) - \log (2(a + b + c)) + \log (a + b + c)]}{\log (2)}
\]

z is the steepness of the species area curve and is a measure of beta diversity.
Chapter 4: Results

DNA Quality Control

All seven samples passed quality assessment with optimal yield and concentration suitable for Nanopore library preparation. The estimated DNA Concentration is listed in table 2.

Table 2

Estimated DNA Concentration

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Nanodrop QC</th>
<th>Volume (µl)</th>
<th>Yield (ng)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ng/ul</td>
<td>260/280</td>
<td>260/230</td>
</tr>
<tr>
<td>1</td>
<td>36.1</td>
<td>1.35</td>
<td>0.06</td>
</tr>
<tr>
<td>2</td>
<td>293.9</td>
<td>1.26</td>
<td>0.56</td>
</tr>
<tr>
<td>3</td>
<td>60.3</td>
<td>1.52</td>
<td>0.11</td>
</tr>
<tr>
<td>4</td>
<td>61.3</td>
<td>2.03</td>
<td>0.09</td>
</tr>
<tr>
<td>5</td>
<td>639.5</td>
<td>1.65</td>
<td>0.3</td>
</tr>
<tr>
<td>6</td>
<td>641.6</td>
<td>1.32</td>
<td>0.27</td>
</tr>
<tr>
<td>7</td>
<td>285.8</td>
<td>1.54</td>
<td>0.4</td>
</tr>
</tbody>
</table>

PCR Amplification

PCR amplification was observed at expected size (1.5kb) from all seven samples as shown in Figure 2.

Figure 6. Agarose gel showing PCR amplification for 16s rRNA gene

Overall Taxonomic Structure and Community Diversity Analysis
All the samples were successfully sequenced by 16S Metagenome analysis on nanopore reads with a total of 4,20,872 high quality reads and average read lengths of 1450.81 bp for each sample. The diversity was calculated using Shannon diversity index (H) which characterize species diversity. Alpha diversity calculation was performed on all the 7 samples as shown in table 3.

Table 3

*Alpha diversity calculated using Shannon index.*

<table>
<thead>
<tr>
<th>Samples</th>
<th>Alpha Diversity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3.28253284180168</td>
</tr>
<tr>
<td>2</td>
<td>4.21692071962228</td>
</tr>
<tr>
<td>3</td>
<td>1.92327872620666</td>
</tr>
<tr>
<td>4</td>
<td>0.742324592504349</td>
</tr>
<tr>
<td>5</td>
<td>1.74526200822864</td>
</tr>
<tr>
<td>6</td>
<td>4.0763472595449</td>
</tr>
<tr>
<td>7</td>
<td>1.95871025512094</td>
</tr>
</tbody>
</table>

Data from sample 1, 2, and 6 are highly diverse in nature with an alpha diversity of 3.38, 4.22, and 4.08 respectively, while sample 4 is the least diverse with an alpha diversity of 0.7423. The average alpha diversity is 2.56. The classification summary and taxonomic abundance for all the seven samples is shown in table 4.

Table 4

*Classification summary of reads*

<table>
<thead>
<tr>
<th>Samples</th>
<th>Raw reads</th>
<th>Classified reads</th>
<th>Unclassified reads</th>
<th>Microbial reads</th>
<th>Bacterial reads</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>59176</td>
<td>58089</td>
<td>1087</td>
<td>58087</td>
<td>58087</td>
</tr>
<tr>
<td>2</td>
<td>58748</td>
<td>58225</td>
<td>523</td>
<td>58220</td>
<td>58217</td>
</tr>
<tr>
<td>3</td>
<td>72224</td>
<td>71429</td>
<td>795</td>
<td>71426</td>
<td>71425</td>
</tr>
<tr>
<td>4</td>
<td>71850</td>
<td>71060</td>
<td>790</td>
<td>71055</td>
<td>71042</td>
</tr>
</tbody>
</table>
**Overall Biodiversity and Microbial Analysis**

The major phyla in the lake samples were predominantly comprised of Proteobacteria, Bacteroidetes, Firmicutes, and Cyanobacteria. Proteobacteria was the most dominant phylum and accounted for 47.68% of all the phyla for the seven lakes followed by Bacteroidetes (28.88%), Firmicutes (9.82%), and Cyanobacteria (7.58%) (Figure 3). Gammaproteobacteria was most prevalent accounting for 29.20% of all the classes for the seven samples, followed by Flavobacteria (26.54%), Betaproteobacteria (13.59%), and Bacilli (7.18%). The most dominant order was *Flavobacteriales* (27.8%) followed by *Pseudomonadles* (25.14%), *Burkholderiales* (9.58%), and *Bacillales* (5.98%). *Pseudomonas, Flavobacterium, Synechococcus,* and *Exiguobacterium* were the primary genera found in the samples. *Pseudomonas* had the highest prevalence and accounted for 30.8% of all the genera for the seven lakes followed by *Flavobacterium* (30.67%), *Synechococcus* (6.06%), and *Exiguobacterium* (5.78%).
Figure 7 (a). Proportion of the 20 most abundant phyla; 1: Agara, 5: Bellandur, 6: Doddanekundi, 7: Varthur, 2: Kaikondrahalli, 3: Kasavanahalli, 4: Haralur lake.

Figure 7 (b). Proportion of the 20 most abundant classes; 1: Agara, 5: Bellandur, 6: Doddanekundi, 7: Varthur, 2: Kaikondrahalli, 3: Kasavanahalli, 4: Haralur lake.
**Figure 7(c):** Proportion of the 20 most abundant orders; 1: Agara, 5: Bellandur, 6: Doddanekundi, 7: Varthur, 2: Kaikondrahalli, 3: Kasavanahalli, 4: Haralur lake.

**Figure 7 (d):** Proportion of the 20 most abundant genera; 1: Agara, 5: Bellandur, 6: Doddanekundi, 7: Varthur, 2: Kaikondrahalli, 3: Kasavanahalli, 4: Haralur lake.

Sample-wise analysis
**Agara Lake**

A total of 207 types of bacteria were found in Agara lake. The most dominant phyla were Proteobacteria (71%), Bacteroidetes (23%), Firmicutes (4%), and Actinobacteria (2%). The most dominant class was Betaproteobacteria, which comprised 41% of bacteria, followed by Gammaproteobacteria (19%), Alphaproteobacteria (10%), and Firmicutes (4%). The most dominant orders were Burkholderiales (38%), Pseudomonadales (18%), Rhodobacteraceae (6%), and Bacilli (3%). Comamonadaceae (28%) was the most abundant family, followed by Flavobacteriaceae (22%), Pseudomonadaceae (17%), and Oxalobacteraceae (8%). *Flavobacterium* was the most abundant genus found, comprising 21% of the bacterial makeup, followed by *Pseudomonas* (17%), *Variovorax* (13%), and *Acidovorax* (10%).

Of the 207 species found, 38 were pathogenic in nature. There were 24 opportunistic pathogens, seven commensal bacteria, and seven true pathogens. The most abundant of these were *Capnocytophaga ochracea*, *Campylobacter ureolyticus*, *Staphylococcus aureus*, *Stenotrophomonas geniculata*, and *Staphylococcus epidermidis*. Apart from these, *Salmonella enterica*, *Legionella pneumophila*, *Haemophilus parainfluenzae*, *Mycobacterium* spp., and *Clostridium* spp. were detected. In addition to pathogenic species, one extremophile, *Deinococcus geothermalis*, was detected.

Table 5
Summary of bacterial community results for Agara lake.

<table>
<thead>
<tr>
<th>Phylum</th>
<th>Class</th>
<th>Order</th>
<th>Family</th>
<th>Genus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proteobacteria</td>
<td>Betaproteobacteria</td>
<td>Burkholderiales</td>
<td>Comamonaceae</td>
<td>Flavobacterium</td>
</tr>
<tr>
<td>(71%)</td>
<td>(41%)</td>
<td>(38%)</td>
<td>(28%)</td>
<td>(21%)</td>
</tr>
<tr>
<td>Bacteriodetes</td>
<td>Gammaproteobacteria</td>
<td>Pseudomonadales</td>
<td>Flavobacteriaceae</td>
<td>Pseudomonas</td>
</tr>
<tr>
<td>(23%)</td>
<td>(19%)</td>
<td>(18%)</td>
<td>(22%)</td>
<td>(17%)</td>
</tr>
<tr>
<td>Firmicutes</td>
<td>Alphaproteobacteria</td>
<td>Rhodobacteriales</td>
<td>Pseudomonadaceae</td>
<td>Variovorax</td>
</tr>
<tr>
<td>(4%)</td>
<td>(10%)</td>
<td>(6%)</td>
<td>(17%)</td>
<td>(13%)</td>
</tr>
<tr>
<td>Actinobacteria</td>
<td>Firmicutes (4%)</td>
<td>Bacilli (3%)</td>
<td>Oxalobacteriaceae</td>
<td>Acidovorax</td>
</tr>
<tr>
<td>(2%)</td>
<td></td>
<td>(8%)</td>
<td>(10%)</td>
<td></td>
</tr>
</tbody>
</table>

**Kaikondrahalli Lake**

A total of 215 types of bacteria were found in Kaikondrahalli lake. The dominant phyla were Proteobacteria (73%), Bacteriodetes (14%), Firmicutes (6%), and Actinobacteria (5%). The dominant classes include Gammaproteobacteria (30%), Betaproteobacteria (30%), Flavobacteriia (13%), and Alphaproteobacteria (9%). Pseudomonadales was the most dominant order with a prevalence of 25%, followed by Burkholderiales (23%), Flavobacteriales (13%), and Methylphilaceae (5%). The most prevalent family was Pseudomonadaceae (20%) followed by Comamonadaceae (13%), Flavobacteriaceae (12%), and Oxalobacteriaceae (30%). The dominant genera were *Pseudomonas* (20%), *Flavobacterium* (9%), *Janthinobacterium* (7%), and *Variovorax* (6%).

Of the 215 species detected, 42 were pathogenic in nature. There were 21 opportunistic pathogens, six commensal bacteria, four zoonotic bacteria, and 11 true pathogens. The most abundant of these were *Capnocytophaga ochracea*, *Campylobacter ureolyticus*, *Acinetobacter lwoffii*, *Myroides odoratimimus*, and *Paracoccus aminovorans*. Other pathogenic species included *Elizabethkingia meningoseptica*, *Acinetobacter guillouiae*, *Helicobacter pylori*, *Mycobacterium* spp., and *Vibrio* spp. Zoonotic bacteria included *Arcobacter cryaerophilus*, *Leptospira*
biflexa, Bartonella washoensis, and Kurthia gibsonii. Additionally, eight species of extremophiles were found, including Marinomonas primoryensis, Octadecabacter antarcticus, and Spirochaeta thermophila.

Table 6

Summary of bacterial community results for Kaikondrahalli lake.

<table>
<thead>
<tr>
<th>Phylum</th>
<th>Class</th>
<th>Order</th>
<th>Family</th>
<th>Genus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proteobacteria</td>
<td>Gammaproteobacteria</td>
<td>Pseudomonadales</td>
<td>Pseudomonadaceae</td>
<td>Pseudomonas</td>
</tr>
<tr>
<td>(73%)</td>
<td>(30%)</td>
<td>(25%)</td>
<td>(20%)</td>
<td>(20%)</td>
</tr>
<tr>
<td>Bacterioidetes</td>
<td>Betaproteobacteria</td>
<td>Burkholderiales</td>
<td>Comamonadaceae</td>
<td>Flavobacterium</td>
</tr>
<tr>
<td>(14%)</td>
<td>(30%)</td>
<td>(23%)</td>
<td>(13%)</td>
<td>(9%)</td>
</tr>
<tr>
<td>Firmicutes</td>
<td>Flavobacteria</td>
<td>Flavobacteriales</td>
<td>Flavobacteriaceae</td>
<td>Janthinobacterium</td>
</tr>
<tr>
<td>(6%)</td>
<td>(13%)</td>
<td>(13%)</td>
<td>(12%),</td>
<td>(7%)</td>
</tr>
<tr>
<td>Actinobacteria</td>
<td>Alphaproteobacteria</td>
<td>Methylphilaceae</td>
<td>Oxalobacteraceae</td>
<td>Variovorax</td>
</tr>
<tr>
<td>(5%)</td>
<td>(9%)</td>
<td>(5%)</td>
<td>(30%),</td>
<td>(6%)</td>
</tr>
</tbody>
</table>

**Kasavanahalli Lake**

A total of 224 species of bacteria were identified from Kasavanahalli Lake. Dominant phyla included Bacterioidetes (78%), Proteobacteria (11%), Firmicutes (5%), and Actinobiota (4%). Flavobacteriia (77%) was the most prevalent class followed by Alphaproteobacteria (4%), Actinobacteria (4%), Gammaproteobacteria (3%), and Bacilli (3%). The most prevalent orders were Flavobacteriales (77%), Pseudomondales (2%), Burkholderiales (2%), and Clostridiales (2%). The family with the highest prevalence was Flavobacteriaceae (77%) followed by Actinomycetales (4%), Rhodobacteraceae (1%), Pseudomonadaceae (1%). The genera with the highest prevalence included Flavobacterium (73%), Capnocytophaga (2%), Pseudomonas (1%), and Campylobacter (9%).

Of the 224 types of bacteria detected in Kasvanahalli lake, 50 pathogenic species were detected. There were 26 opportunistic pathogens, nine commensal
bacteria, one zoonotic bacterium, and 14 true pathogenic species, with the most abundant being *Capnocytophaga ochracea, Campylobacter ureolyticus, Myroides odoratinimus, Methylobacterium organophilum, and Haemophilus parainfluenzae.*

Other pathogenic species included *Staphylococcus epidermidis, Mycobacterium spp., Rothia mucilaginosa, Salmonella enterica,* and *Streptococcus infantis.* The one zoonotic bacterium was *Leptospira biflexa.* Seven extremophiles were also detected, such as *Deinococcus geothermalis, Geodermatophilus obscurus,* and *Streptomyces radiopugnans* which is also gamma radiation resistant.

Table 7

**Summary of bacterial community results for Kasavanahalli lake**

<table>
<thead>
<tr>
<th>Phylum</th>
<th>Class</th>
<th>Order</th>
<th>Family</th>
<th>Genus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacteroidetes</td>
<td>Flavobacteriia</td>
<td>Flavobacteriales</td>
<td>Flavobacteriaceae</td>
<td>Flavobacterium</td>
</tr>
<tr>
<td>(95%)</td>
<td>(94%)</td>
<td>(94%)</td>
<td>(94%)</td>
<td>(91%)</td>
</tr>
<tr>
<td>Proteobacteria</td>
<td>Alphaproteobacteria</td>
<td>Campylobacteriales</td>
<td>Campylobacteriaceae</td>
<td>Campylobacter</td>
</tr>
<tr>
<td>(3%)</td>
<td>(0.8%)</td>
<td>(0.6%)</td>
<td>(0.6%)</td>
<td>(0.6%)</td>
</tr>
<tr>
<td>Firmicutes (1%)</td>
<td>Gammaproteobacteria</td>
<td>Pseudomonadales</td>
<td>Pseudomonadaceae</td>
<td>Pseudomonas</td>
</tr>
<tr>
<td>(0.8%)</td>
<td>(0.6%)</td>
<td>(0.5%)</td>
<td>(0.5%)</td>
<td>(0.5%)</td>
</tr>
<tr>
<td>Actinobacteria</td>
<td>Bacilli</td>
<td>Bacillales</td>
<td>Rhodobacteriaceae</td>
<td>Rhodobacteraceae</td>
</tr>
<tr>
<td>(0.6%)</td>
<td>(0.8%)</td>
<td>(0.5%)</td>
<td>(0.4%)</td>
<td>(0.4%)</td>
</tr>
</tbody>
</table>

**Haralur lake**

A total of 153 types of bacteria were found in Haralur lake. The phylum with the highest prevalence was Bacteroidetes (95%) followed by Proteobacteria (3%), Firmicutes (1%), and Actinobacteria (0.6%). Dominant classes included Flavobacteriia (94%), Alphaproteobacteria (0.8%), Gammaproteobacteria (0.8%), and Bacilli (0.8%). The highest prevalent order was Flavobacteriales (94%) followed by Campylobacteriales (0.6%), Pseudomonadales (0.6%), and Bacillales (0.5%). The most dominant family was Flavobacteriaceae (94%) followed by Campylobacteriaceae (0.6%), Pseudomonadaceae (0.5%), and Rhodobacteriaceae (0.4%). The genus with
the highest prevalence was *Flavobacterium* (91%), followed by *Campylobacter* (0.6%), *Pseudomonas* (0.5%), and *Rhodobacteraceae* (0.4%).

Of the 153 types of bacteria detected, 31 were pathogenic species with 15 opportunistic pathogens, seven commensal bacteria, one zoonotic bacterium and eight true pathogens. The most abundant of these were *Capnocytophaga ochracea*, *Campylobacter ureolyticus*, *Myroides odoratimimus*, *Bacteroides fragilis*, and *Bacteroides acidifaciens*. Other pathogenic species included *Staphylococcus aureus*, *Pseudomonas alcaligenes*, *Streptococcus anginosus*, and *Micrococcus luteus*. The one zoonotic bacterium detected was *Pasteurella multocida*. Additionally, eight extremophiles were also detected such as *Polaribacter irgensi*, *Flavobacterium frigidarium*, *Spirochaeta thermophila*, and *Psychroflexus torquis*.

**Table 8**

*Summary of bacterial community results for Bellandur lake*

<table>
<thead>
<tr>
<th>Phylum</th>
<th>Class</th>
<th>Order</th>
<th>Family</th>
<th>Genus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proteobacteria</td>
<td>Gammaproteobacteria</td>
<td>Pseudomonadales</td>
<td>Bacillaceae (22%)</td>
<td>Bacillus (13%)</td>
</tr>
<tr>
<td>(89%)</td>
<td>(87%)</td>
<td>(87%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Actinobacteria</td>
<td>Coriobacteria</td>
<td>Flavobacteriales</td>
<td>Moraxellaceae (21%)</td>
<td>Flavobacterium</td>
</tr>
<tr>
<td>(5%)</td>
<td>(5%)</td>
<td>(12%)</td>
<td></td>
<td>(11%)</td>
</tr>
<tr>
<td>Firmicutes (5%)</td>
<td>Bacilli (4%)</td>
<td>Clostridiales (5%)</td>
<td>Flavobacteriacae (12%)</td>
<td>Acinetobacter (12%)</td>
</tr>
<tr>
<td>Bacteroidetes (0.3%)</td>
<td>Clostridia (1%)</td>
<td>Rhodobacteriales (5%)</td>
<td>Planococcaceae (5%)</td>
<td>Psychrobacter (9%)</td>
</tr>
</tbody>
</table>

**Bellandur Lake**

There was a total of 187 types of species of bacteria in Bellandur lake. The dominant phyla were Proteobacteria (89%), Actinobacteria (5%), Firmicutes (5%), and Bacteroidetes (0.3%). The class with the highest prevalence was Gammaproteobacteria (87%), Coriobacteria (5%), Bacilli (4%), and Clostridia (1%). The most dominant order was Pseudomonadales (87%) followed by Coriobacteriales
(5%), Lactobacillales (4%), Clostridiales (1%), and Bacteroidales (0.2%). The most prevalent family was Pseudomonadaceae (85%), followed by Coriobacteriaceae (5%), Lactobacillaceae (3%), Streptococcaceae, and Ruminococcaceae (0.4%). The most prevalent genus was *Pseudomonas* (85%) followed by *Collinsella* (5%), *Lactobacillus* (3%), *Streptococcus* (1%) and *Faecalibacterium* (0.4%).

Of the 187 types of bacteria detected in Bellandur lake, 36 species were pathogenic in nature, including 14 opportunistic pathogens, eight commensal bacteria, one zoonotic bacterium, and 13 true pathogens. The pathogenic species with the highest prevalence were *Pseudomonas alcaligenes*, *Acinetobacter lwoffii*, *Acinetobacter johnsonii*, *Campylobacter ureolyticus*, and *Pseudomonas balearica*. Other pathogenic species detected were *Propionibacterium acnes*, *Serratia marcescens*, *Clostridium perfringens*, *Plesiomonas shigelloides*, and *Clostridium neonatale*. The one zoonotic bacterium detected was *Arcobacter cryaerophilus*. There were also 4 extremophiles detected; *Collinsella aerofaciens*, *Shewanella benthica*, *Geobacillus thermodenitrificans*, and *Marinomonas primoryensis*.

Table 9

*Summary of bacterial community results for Bellandur lake*

<table>
<thead>
<tr>
<th>Phylum</th>
<th>Class</th>
<th>Order</th>
<th>Family</th>
<th>Genus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Firmicutes</td>
<td>Bacilli (36%)</td>
<td>Bacillales (33%)</td>
<td>Bacillaceae (22%)</td>
<td>Bacillus (13%)</td>
</tr>
<tr>
<td>Proteobacteria</td>
<td>Gammaproteobacteria (26%)</td>
<td>Pseudomonadales (23%)</td>
<td>Moraxellaceae (21%)</td>
<td>Flavobacterium (11%)</td>
</tr>
<tr>
<td>Bacteroidetes</td>
<td>Flavobacteriia (12%)</td>
<td>Flavobacteriales (12%)</td>
<td>Flavobacteriaceae (12%)</td>
<td>Acinetobacter (12%)</td>
</tr>
<tr>
<td>Actinobacteria</td>
<td>Alphaproteobacteria (8%)</td>
<td>Clostridiales (5%)</td>
<td>Planococcaceae (5%)</td>
<td>Psychrobacter (9%)</td>
</tr>
</tbody>
</table>

**Doddanekundi Lake**
There was a total of 246 types of species of bacteria found in this lake. The dominant phyla were Firmicutes (41%), Proteobacteria (40%), Bacteroidetes (14%), and Actinobacteria (3%). Bacilli was the class that had the highest prevalence (36%) followed by Gammaproteobacteria (26%), Flavobacteriia (12%), and Alphaproteobacteria (8%). Dominant orders included Bacillales (33%), Pseudomonadales (23%), Flavobacteriales (12%), and Clostridiales (5%). The family with the highest prevalence was Bacillaceae (22%) followed by Moraxellaceae (21%), Flavobacteriaceae (12%), and Planococcaceae (5%). *Bacillus* was the genus with the highest prevalence (13%) followed by *Flavobacterium* (11%), *Acinetobacter* (12%), and *Psychrobacter* (9%).

Of the 246 species detected, 41 were pathogenic in nature including 22 opportunistic pathogens, eight commensal bacteria, two zoonotic bacterium, and nine true pathogens. The most abundant species were *Acinetobacter lwofii*, *Acinetobacter johnsonii*, *Haemophilus parainfluenzae*, *Staphylococcus aureus*, and *Staphylococcus epidermidis*. The two zoonotic bacteria detected were *Kurthia gibsonii* and *Arcobacter cryaerophilus*. Additionally, there were 11 extremophiles detected, with having the highest prevalence, followed by *Jeotgalicoccus psychrophilus*, *Brevibacillus thermoruber*, and *Psychrobacter pacificensis*. Table 10

**Summary of bacterial community results for Doddanekundi lake**

<table>
<thead>
<tr>
<th>Phylum</th>
<th>Class</th>
<th>Order</th>
<th>Family</th>
<th>Genus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proteobacteria (84%)</td>
<td>Gammaproteobacteria (82%)</td>
<td>Pseudomonadales (81%)</td>
<td>Pseudomonadaceae (81%)</td>
<td>Pseudomonas (81%)</td>
</tr>
<tr>
<td>Actinobacteria (8%)</td>
<td>Coriobacteriia (7%)</td>
<td>Coriobacteriales (7%)</td>
<td>Coriobacteriaceae (7%)</td>
<td>Collinsella (7%)</td>
</tr>
<tr>
<td>Firmicutes (7%)</td>
<td>Bacilli (5%)</td>
<td>Lactobacillales (5%)</td>
<td>Lactobacillaceae (3%)</td>
<td>Lactobacillus (3%)</td>
</tr>
<tr>
<td>Bacteroidetes (0.6%)</td>
<td>Clostridia (2%)</td>
<td>Clostridiales (2%)</td>
<td>Streptococcaceae (1%)</td>
<td>Streptococcus (1%)</td>
</tr>
</tbody>
</table>
Varthur Lake

There was a total of 197 species of bacteria found in Varthur lake. Dominant phyla included Proteobacteria (84%), Actinobacteria (8%), Firmicutes (7%), and Bacteroidetes (0.6%). The most prevalent class was Gammaproteobacteria (82%) followed by Coriobacteriia (7%), Bacilli (5%), and Clostridia (2%). Dominant orders included Pseudomonadales (81%), Coriobacteriales (7%), Lactobacillales (5%), and Clostridiales (2%). The family with the highest prevalence was Pseudomonadaceae (81%) followed by Coriobacteriaceae (7%), Lactobacillaceae (3%), and Streptococcaceae (1%). The most dominant genus was Pseudomonas (81%), followed by Collinsella (7%), Lactobacillus (3%), and Streptococcus (1%).

Of the 197 types of bacteria detected in Varthur lake, 31 were pathogenic in nature including 17 opportunistic pathogens, five commensal bacteria, one zoonotic bacterium, and eight true pathogens. The most abundant pathogenic species were Pseudomonas alcaligenes, Campylobacter ureolyticus, Acinetobacter johnsonii, Campylobacter ureolyticus, and Veillonella dispar. Other pathogenic species included Acinetobacter Iwoffi, Eggerthella lenta, Streptococcus anginosus, Haemophilus influenzae, and Enterococci spp. The one zoonotic bacterium was Arcobacter cryaerophilus. Additionally, six species of extremophiles were detected, such as Thermoanaerobacterium saccharolyticum, Psychrobacter pacificensis, and Geobacillus thermodenitrificans.

Table 11

<table>
<thead>
<tr>
<th>Prevalence</th>
<th>Phylum</th>
<th>Class</th>
<th>Order</th>
<th>Family</th>
<th>Genus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Proteobacteria</td>
<td>Gammaproteobacteria</td>
<td>Pseudomonadales</td>
<td>Pseudomonadaceae</td>
<td>Pseudomonas</td>
</tr>
<tr>
<td>(84%)</td>
<td>(84%)</td>
<td>(82%)</td>
<td>(81%)</td>
<td>(81%)</td>
<td>(81%)</td>
</tr>
<tr>
<td>Phylum</td>
<td>Class</td>
<td>Order</td>
<td>Family</td>
<td>Genus</td>
<td></td>
</tr>
<tr>
<td>-------------------</td>
<td>------------------</td>
<td>------------------</td>
<td>------------------</td>
<td>------------</td>
<td></td>
</tr>
<tr>
<td>Actinobacteria</td>
<td>Coriobacteriia</td>
<td>Coriobacteriales</td>
<td>Coriobacteriaceae</td>
<td>Collinsella</td>
<td></td>
</tr>
<tr>
<td>(8%)</td>
<td>(7%)</td>
<td>(7%)</td>
<td>(7%)</td>
<td>(7%)</td>
<td></td>
</tr>
<tr>
<td>Firmicutes</td>
<td>Bacilli</td>
<td>Lactobacillales</td>
<td>Lactobacillaceae</td>
<td>Lactobacillus</td>
<td></td>
</tr>
<tr>
<td>(7%)</td>
<td>(5%)</td>
<td>(5%)</td>
<td>(3%)</td>
<td>(3%)</td>
<td></td>
</tr>
<tr>
<td>Bacteroidetes</td>
<td>Clostridia</td>
<td>Clostridiales</td>
<td>Streptococcaceae</td>
<td>Streptococcus</td>
<td></td>
</tr>
<tr>
<td>(0.6%)</td>
<td>(2%)</td>
<td>(2%)</td>
<td>(1%)</td>
<td>(1%)</td>
<td></td>
</tr>
</tbody>
</table>
Chapter 5: Discussion

Nanopore sequencing of 16S rRNA genes provides unique knowledge of bacterial sequencing as a whole in environmental microbiology, particularly in waterbodies such as lakes (Kai et al., 2019). Overall, Kasavanahalli lake had the highest number of opportunistic pathogens (24), pathogenic species (14), and commensal species (9). Kasavanahalli lake also had seven species of extremophiles, higher than the average of three to four found in other lakes, and three gamma radiation resistant bacteria. Since Bellandur lake receives majority of the pollutants and contaminants amongst the lakes analyzed in this study, it’s surprising that Kasavanahalli lake had higher a prevalence of pathogenic type bacteria. Therefore, this lake poses the most health risks to humans and all flora and fauna surrounding the lake, when compared to the six other lakes.

Through next generation sequencing (NGS), this study has reported vast number of pathogenic bacteria in the Koramangala-Challaghatta lake system, such as Acinetobacter, Staphylococcus, Pseudomonas, and Prevotella. Among the pathogenic bacteria identified, the most prevalent opportunistic pathogens across the lakes sampled were Methylobacterium spp., Campylobacter. ureolyticus, Legionella pneumophila, and Staphylococcus aureus.

Methylobacterium spp., are opportunistic premise plumbing pathogens (OPPPs) with increasing incidence. OPPPs are waterborne microorganisms that emerge in drinking water distribution systems (DWDSs), and cause disease in individuals with predisposing conditions, such as age (> 70 years), cancer or immunodeficiency (Isaac & Sherchan, 2020). Methylobacterium spp. have been found in lakebeds before and have been isolated from a polluted lake in Seattle (Dourado,
Neves, Santos, & Araújo, 2015). Once *Methylobacterium* spp. get into the water table, they can become widespread OPPPs across the region.

*C. ureolyticus* is also an emerging opportunistic pathogen. This species has been implicated in a range of gastrointestinal conditions, including enteritis, Crohn’s disease, colorectal cancer and irritable bowel disease (IBD). Additionally, there is a correlation between *C. ureolyticus* and water quality indicators; the high prevalence of this species in the lake system indicates poor water quality (Percival, 2014, p. 66). This species is found in high numbers in domestic sewage and non-disinfected treated sewage effluents (Percival, 2014, p. 3). Therefore, this bacterium has most likely entered the lake system through the sewage being discharged into Bellandur lake from the STP.

*L. pneumophilia* and other forms of *Legionella* spp. are responsible for Legionnaire’s disease and cause a deadly type of pneumonia resulting in a mortality rate of 40-80% in immunocompromised patients (Wang et al., 2017). Legionellosis is transmitted via the inhalation of this pathogen in aerosol form. A study conducted in Toronto identified several meteorological conditions that influence the incidence of legionellosis. During hot months in Toronto, typically ranging from 24°C to 27°C, the city experiences a higher incidence of cases. This is due to the pathogen’s increase chance of being aerosolized, due to higher evaporation rates allowing *legionella* spp. to be introduced into the lung via microaspiration when in close contact with the source (Ng et al., 2009). Bangalore maintains a temperature over 24°C for eight months in the course of a year, making this mechanism even more likely. Due to the absence of active level Legionnaire’s disease surveillance systems in India, disease prevalence and seasonality are unknown, however, in 2017, Valavane et al. identified *L. pneumophila* in 21.5% non-potable water samples and 6.1% in potable
water samples across northern India (Chaudhary, Srinath, Agarwal, & Valavane, 2017; Valavane et al., 2017).

*S. aureus* is a dangerous pathogen for both community- acquired as well as hospital-acquired infections such as sepsis and endocarditis (INSAR, 2013). This species is ubiquitous in freshwater and marine environments, a study conducted in Ohio found *S. aureus* in 22.8% of their samples from freshwater lakes across the state (Thapaliya et al., 2017). Sugumar and Anandharaj (2016) also detected the presence of *S. aureus* is a lake system in Tamil Nadu indicating significant levels of microbial pollution.

One of the most abundant zoonotic bacteria present in the lakes was *Leptospira biflexa*. *L. biflexa* is a neglected infectious agent, which has been associated with many poor clinical outcomes ranging from subclinical infections to fatal pulmonary hemorrhaging and causes leptospirosis in those infected with it (Fraga, Carvalho, Isaac, & Barbosa, 2015). What makes *L. biflexa* infections even more challenging is that the protean manifestations of the disease make it hard to diagnose. Access to health care and clinical resources in Bangalore, for people without private insurance is difficult (Bhojani et al., 2012). With limited access to high quality clinical care, actual mortality rates associated with Leptospirosis could be higher than estimated mortality rates due to misdiagnoses. Additionally, the disease can only be treated by antibiotics, and the consumption of contaminated waters from these lakes could result in the ingestion of antibiotic resistant genes (ARGs), making the disease lethal.

The presence of opportunistic pathogens can be grouped into two lake series. The bacterial communities in Agara, Kaikondrahalli, Kasavanahalli, and Haralur were quite similar and those in Varthur, Bellandur, and Doddanekundi resembled each
other. This can be attributed to the proximity and number of channels between Bellandur lake, and Varthur and Doddanekundi lake as compared to Bellandur lake’s proximity and number of channels with Agara lake. Agara lake is connected to Bellandur lake via two large drainage channels, and then this lake drains into Kaikondrahalli, Kasavanahalli, and Haralur lake. Additionally, Bellandur lake directly drains into Varthur lake and is much closer to it in terms of distance. It also has smaller channels feeding Doddanekundi, therefore, Varthur lake and Doddanekundi lakes’ bacterial communities will show much more resemblance to Bellandur than Agara and its connecting lakes will.

There were several human commensals found in each lake such as *Burkholderia glathei*, *Bacteroides fragilis*, *Propionibacterium granulosum*, *Bacteroides acidifaciens*, and *Staphylococcus sciuri*. *B. fragilis* is an anaerobic organism commonly isolated from normal stool flora (Garza & Cohen, 2011). *P. granulosum* are commensal bacteria on the skin and are non-pathogenic, and *B. acidifaciens*, *S. sciuri*, and *B. fragilis* are common gut commensals (Yang et al., 2013; Nystrom, Wyatt, & Noiseux, 2013). In addition to these, most of the commensals found were specific to the human gut indicating human fecal contamination and must be entering Bellandur lake through the STP. Other fecal contaminators found in all the lakes studies were *E coli.*, *Shigella*, *Legionella* and *Campylobacter* species.

Interestingly, a range of extremophilic bacteria were detected in all seven lakes. Extremophiles are bacteria that thrive in extreme environments of pH, temperature, salt concentration, pressure, etc. and develop the ability to adapt to these harsh environmental conditions due to specialized cellular mechanisms (Kulkarni, Dhakar, & Joshi, 2019). Twenty species of extremophiles were detected across the seven lakes, including psychrophiles, thermophiles, and alkaliophiles.
*Shevanella benthica* is a psychrophile that was detected in six of the seven lakes sampled from. It thrives in deep sea environments with low temperature and high hydrostatic pressure (Suka et al., 2019). This species has previously been identified in the world’s deepest sediments such as the Mariana trench and the Tonga-Kermadec trench (Lauro et al., 2013). The prevalence of this species in freshwater lakes globally is very low, they are usually present in marine environments, and when *S. benthica* is detected in lakes, they are most often extremely polluted and heavily eutrophic, both of which are true of the Koramangala-Challaghatta lake system making this bacteria’s presence here unsurprising.

However, five other species of psychrophiles that are native to polar water and soils were detected in five lakes, these were *Marinomonas primoryensis*, *Octadecabacter antarcticus*, *Flavobacterium gelidilacus*, *Polaribacter irgensii*, and *Psychrobacter pacificensis*. Psychrophiles are extremophiles that are capable of growth and reproduction in cold climates, ranging from -20°C to 10°C (Maayer, Anderson, Cary, & Cowan, 2014). *P. irgensii* especially, has an extremely high freeze tolerance and is a novel psychrophile. It is interesting that these bacteria that are typically only found in subfreezing environments on Earth have been found in this lake system. Psychrophiles have evolved mechanisms to successfully counteract stressful conditions in addition to the cold, such as radiation, extreme pH, high pressure and low nutrient availability (Maayer, Anderson, Cary, & Cowan, 2014). Such limiting ecological factors promote the growth of certain psychrophilic bacteria. According to a water quality assessment, Bellandur lake’s turbidity (ranges from 14 NTU to 30.90 NTU) drastically exceeds permissible limits of 4 NTU, electrical conductivity is 1120 μs/cm to 1220 μs/cm above the WHO recommendation of 500 μs/cm, and DO is between 1.9 mg/l to 4.8 mg/l across the lake, where concentrations
below 5 mg/l are detrimental to life, and below 2 mg/l cause fish kills (Ramesh & Krishnaiah, 2014). From this, it is apparent that extreme conditions exist in this lake creating certain ecological niches for psychrophiles to thrive in from which they can migrate to any of the other lakes in the Koramangala-Challaghatta lake system through the 96-cascade system interconnecting channels leaving Bellandur lake, explaining their ubiquity in the system as well.

Another interesting point to note was the detection of three gamma radiation resistant bacteria, i.e., Deinococcus geothermalis, Streptomyces radiopugnans, and Geodermatophilus obscurus. D. geothermalis is thermophilic (grows at optimal temperature of 40°C to 50°C) and known for its resistance to extreme environmental conditions such as the presence of heavy metals, oxidative stress, and ionizing radiation (Bornot et al., 2013). S. radiopugnans is also thermophilic and resistant to 60Co gamma radiation, up to the dose of 15 kGy. (Bhave, Shanbhag, Sonawane, Parab, & Mahajan, 2013). This species has primarily been detected in deep sea sponges, radioactive polluted soils, or waterbodies in the environment (Dhamodaran, D., Naine, J. S., Keziah M. S., and Subathra, D.C., 2019). The third species found was G. obscurus, a gamma radiation resistant bacterium found in hyper arid desert soils (Castro et al., 2018). G. obscurus contains gene clusters that ensure adaptations to extreme environmental conditions. Therefore, similar to the presence of psychrophiles, these gamma radiation resistant bacteria have found ecological niches with favorable conditions for their survival in these lakes. While this theory supports the life of such extremophiles in these lakes, it is important to acknowledge that nanopore NGS identifies all DNA, irrespective of whether the bacteria are live or dead, in the sample and it is a possibility that many of these extremophiles once
existed in these lakes, however. are no longer living. Therefore, nanopore NGS might have detected the DNA from these dead bacteria.
Chapter 6: Conclusion

In conclusion, an average of 204 bacteria were detected by NGS in the seven lakes sampled from in the Koramangala-Challaghatta lake system. Although the bacterial communities were identified in all seven lakes, the results of sequencing only one sample from each lake in the year does not yield fairly representative results, especially through different seasons, as this composition will look quite different during the monsoon season.

Further research should be conducted to understand the prevalence of ARGs in these lakes. The gargantuan amount of sewage entering these lakes ensures the possibility of the existence of an array of ARGs. All the pathogens mentioned above are treated by antibiotics, however, a study examining antibiotic residues in south east Asia revealed that only 20-30% of municipal wastewater is treated in treatment plants and that too is not effective enough to ARGs (Taneja & Sharma, 2019). With the sheer amount of sewage entering Bellandur lake and spreading through the Koramangala-Challaghatta lake system, many of the pathogenic species mentioned above, such as *S. aureus* and *L. biflexa* could become difficult to treat due to the presence of ARGs. This is critical as other opportunistic pathogens could mass spread across the region if people are also consuming ARGs, making medical treatment difficult, in addition to economic hardships already faced in finding such treatment. Additionally, further research should be conducted to analyze the chemicals, such as PFOS, heavy metals, and pharmaceuticals entering these lakes through the adjacent sewage and fiberglass industries.

This study also reports the detection of novel psychrophiles in five of seven lakes sampled from, which is the first time this has been demonstrated. This study also detected the presence of three species of gamma radiation resistant bacteria.
People living in surrounding villages, such as Bellandur, Agara, Kaikondrahalli, and Haralur villages, are extremely dependent on lake water and ground water use. However, the lack of accessible water forces these populations to use these lakes’ polluted water for domestic purposes, irrigation, pisciculture, and washing vegetables. And consumption of such contaminated lake water, which contains various heavy metals, antibiotic resistant bacteria, and fecal indicators poses several adverse health effects. This lake water also goes down to the water table, further polluting ground water. This not only effects neighboring villages, but spreads to neighboring urban settlements as well such as H.S.R. layout, Koramangala, Yemalur, and Marathahalli, affecting hundreds of thousands of people. The interconnectivity of the seven lakes makes this even more dangerous as chemicals, pathogenic species of bacteria, and other harmful contaminants increase the risk of waterborne diseases in the Koramangala-Challaghatta valley.

The results of this study have made it apparent that urgent action is required to remediate these lakes and prevent further growth of pathogenic species. The presence of extremophiles and gamma radiation bacteria illustrates what biologically stressful aquatic environments these lakes are.
References


