Which water source contains the most bacteria count and what type of bacteria does it contain?

Abstract

In the investigation, the types and number of bacteria in various water sources were observed using nutrient agar dishes. Water samples were collected using sterile water collection containers and dropped onto agar slides which then was spread and kept in an incubator. The samples were observed under microscope and counted using grid square mechanisms. The online (web) references were used to identify the different types of bacteria and find their characteristics and suitable environment to grow. This information gave further insight on the pollution of some of Melbourne's main water sources and how the increasing agriculture and development of the world is impacting water sources.

Introduction

The experiment is a form of microbiological water analysis. This type of water analysis is a process to estimate the number of bacteria present in a water source. The method used is plate count, which is a count that relies on the bacteria growing on the nutrient medium in a controlled environment. The colonies formed become visible to the naked eye and can be counted. Water analysis can also mean the observation of different species of bacteria such as e coli (the fastest growing waterborne bacteria) and P.Aeruginosa. These bacteria can indicate the presence of sewage or animal waste in a water source.

The procedure involves collecting water from various water sources around Melbourne. The main water sources considered were Yarra River, a local pond, Sanctuary lakes reservoir, Altona beach and Werribee River.

Bacteria is a member of a large group of unicellular organisms which have cell walls but do not possess organelles and an organised nucleus, at times it may cause disease. Bacteria can be found in soil, water, plants, and animals. To investigate how bacteria grows and its characteristics, it can be swabbed and placed on a medium to grow which is called an agar plate.

Four main things bacteria need to grow are oxygen, proper temperature, and adequate nutrition. Bacteria uses nutrients that come through the pores in its membranes to undergo a series of chemical transformations. The increase in biomass is accompanied by an increase in cell size and by replication of bacterial DNA, possibly with some errors (mutations). Eventually, the cell divides into two daughter cells, in a process called binary fission. Although what triggers this is not known, bacteria have an incredible metabolism (growth rate) for example, e coli, (the most common bacteria found in water) can double its population every 20 minutes.

Nutrient Agar is a polysaccharide derived from red seaweed. Agar is a good platform for bacteria to grow because it provides a hard surface for bacterial growth and is an insert and non-nutritive. Bacteria can be grown in a controlled environment called an incubator at a set temperature of 37 degrees.

There can be many different types of bacteria in water such as E. Coli, C. Albicans, P. Aeruginosa, S. Aureus, Vibrio Vulnificus and a protein called Amylase. Their form and characteristics can be used to

help identify them. This can be used to see whether a water source has sewage or not and observe which resource has the most bacteria.

E. coli is a type of bacteria that causes both minor and severe illness. It is shaped in a coliform way. It is the most cause of diarrhea. It is bacteria found in foods and digestive tracts of people. Some strands of e. Coli are harmless, whereas some can cause illness. Some e. Coli can cause diarrhea whilst some can cause urinary tract infection, respiratory illness and pneumonia and other illnesses. This is released to the environment through the deposition of faecal material, and it is used as an indicator of sewage or faecal contamination of water ways. As part of the water analysis performed, the number of e. Coli is used to identify the most organically polluted water source. When the e. Coli count in recreational areas such as ponds, beaches, and lakes, they are shut down as they are a risk of health and safety. Some possible sources of faecal contamination include agricultural runoff, wildlife that use the area as their natural habitat, run off from areas contaminated with pet manure, wastewater plant treatments plants, and on-site septic systems.

S. aureus, is a major pathogen in humans. The main reservoirs of the bacteria is the skin and mucous membrane of humans and animals. S. Aureus infections can cause abscesses(boils) , furuncles and cellulitis. The presence of S. Aureus in drinking water is of concern because of its susceptibility to human infection and anti-microbial resistance. Different types of staph bacteria cause different problems in different parts of your body like skin, breast or chest, bones, lungs, and heart. Bacteria causes skin infection, and this can cause boils, blisters, and redness on the skin.

P. Aeruginosa is a bacterium that can cause infections in blood. The organism often occurs in fresh water and aquatic environments but not marine. IT often inhabits soil, water, and vegetation. It can cause variety of infection in both immunocompetent and immunocompromised horses.

Vibrio Vulnificus affects the environment by affecting marine life as well. It affects vertebrates and fishes often causing them to die. It is found in coastal areas and warm sea water. It is apart of a community of vibrio's that are called halophilic because they require salt.

B.subtilis is a bacterium found in soil and gastrointestinal tracts of ruminants, humans, and marine sponges. Infections attributed to B. subtilis include bacteraemia, endocarditis, pneumonia, and septicaemia.

The water sources were considered due to various factors such as day to day usage of water in it, the impact of salt content in water such as sea and the multiple ecosystem that are present in it. We have considered two of Victoria's main rivers as it should be subject to being used as a source of tap water and for recreation purposes. It plays a large part in day-to-day life of citizens and this experiment observes how the pathogens growing in the water impact these water sources.

Aim

The aim of this investigation is to observe the bacteria count and variety in the different water sources - Yarra River, Werribee River, Wetland, Sanctuary Lakes Reservoir and Altona Beach.

Hypothesis

If the bacteria in Yarra River is observed, there will be more bacterial colonies and variety.

Materials

- 2 sets of Gloves
- 5 disposable Swabs
- 1 Lab coat
- 1 Microscope
- 5 Disposable Droppers
- 1 Mask
- 100ml sterile water container
- Hand sanitiser
- Soap
- 10 nutrient Agar plates
- Incubator
- 50 ml of water from each water source

Method

1. Collect water from each of the water sources into a sterile container.
2. After putting on gloves, set out two agar plates to each water sample.
3. Label each of the agar plates with a black sharpie.
4. Place the agar plates upside down and remove the lid.
5. Use a disposable dropper to drop 0.1ml of water onto the agar surface.
6. Use a disposable swab to evenly spread the liquid on the surface.
7. Repeat on the rest of the agar dishes.
8. Wait till the water dries.
9. Put the lid onto each of the agar plates and flip over
10. Seal with some stretchy tape to ensure it does not leak.
11. Place in incubator for 1-2 days.
12. Dispose of gloves inside out
13. Wear gloves and mask and take out of incubator to observe.

Hazards

Potential hazard	Why is it dangerous	Safety Rules to Follow
Bacteria	• If not kept in a controlled environment, the bacteria could endanger my health	• Grow in an incubator under a

		professional's watch.
Collection of Water	• In areas where the water has great depth such as in the Yara River, we need to be mindful not to fall in. • Contaminating water in areas where microorganisms thrive, we should make sure during the collection of water that we do not contaminate it and destroy their habitat. • Cross contamination we may spread the bacteria throughout the house which could make it an unsafe area	• Only collect from authorized areas where you are allowed to go. • Wash hands and wear clean gloves when collecting water to ensure bacteria is not impacted. • Grow in an incubator and take measures such as sanitizing before and after and wearing masks and gloves

Results

Types of Bacteria Observed in different water sources.

Reservoir

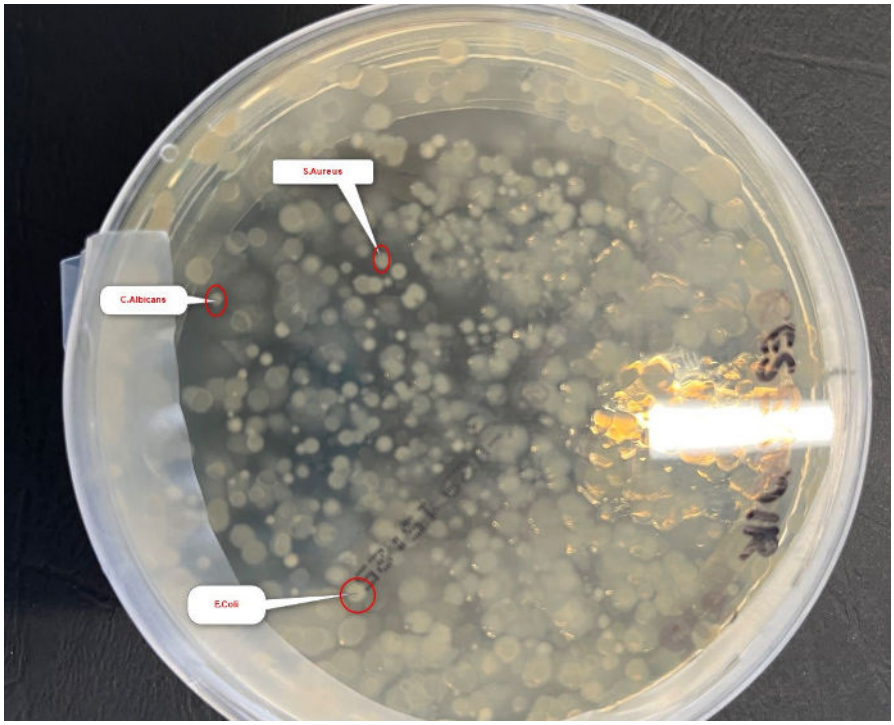


Figure 1 : Reservoir Sample 1

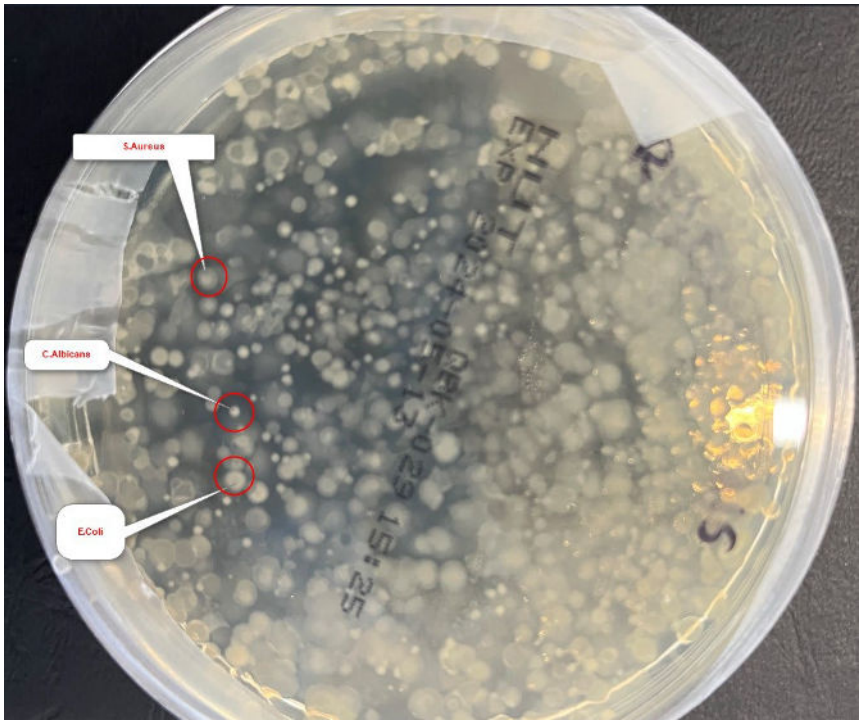


Figure 2: Reservoir Sample2

Sea Water (Altona Beach)

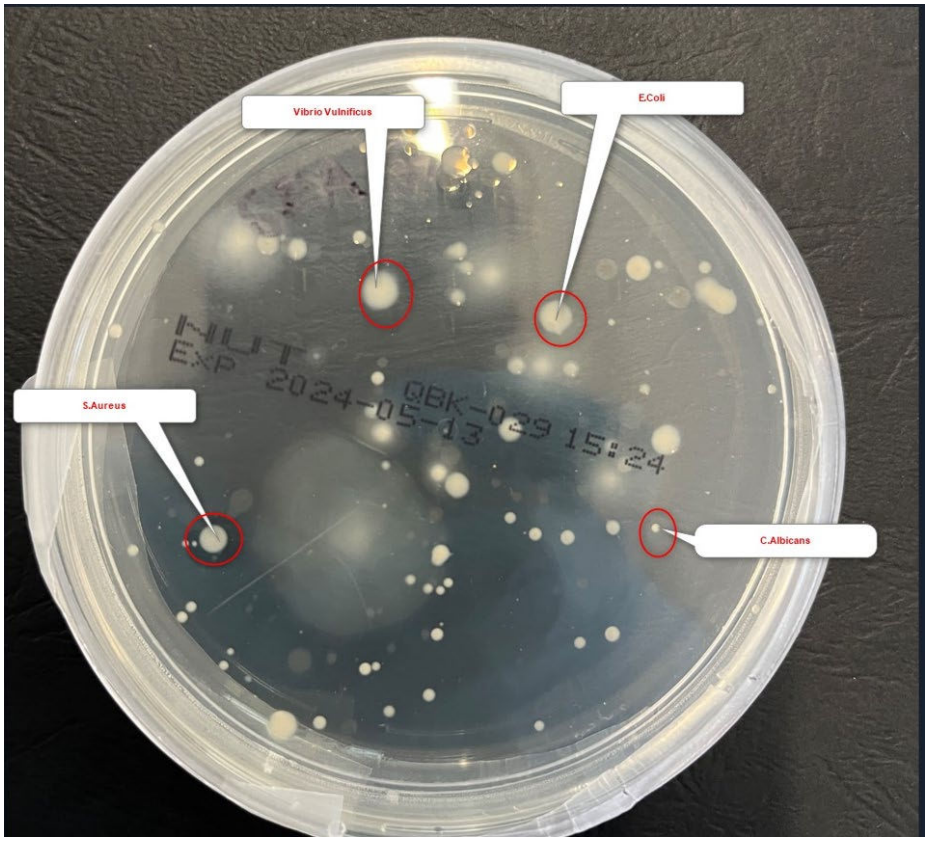


Figure 3: Sea Sample1

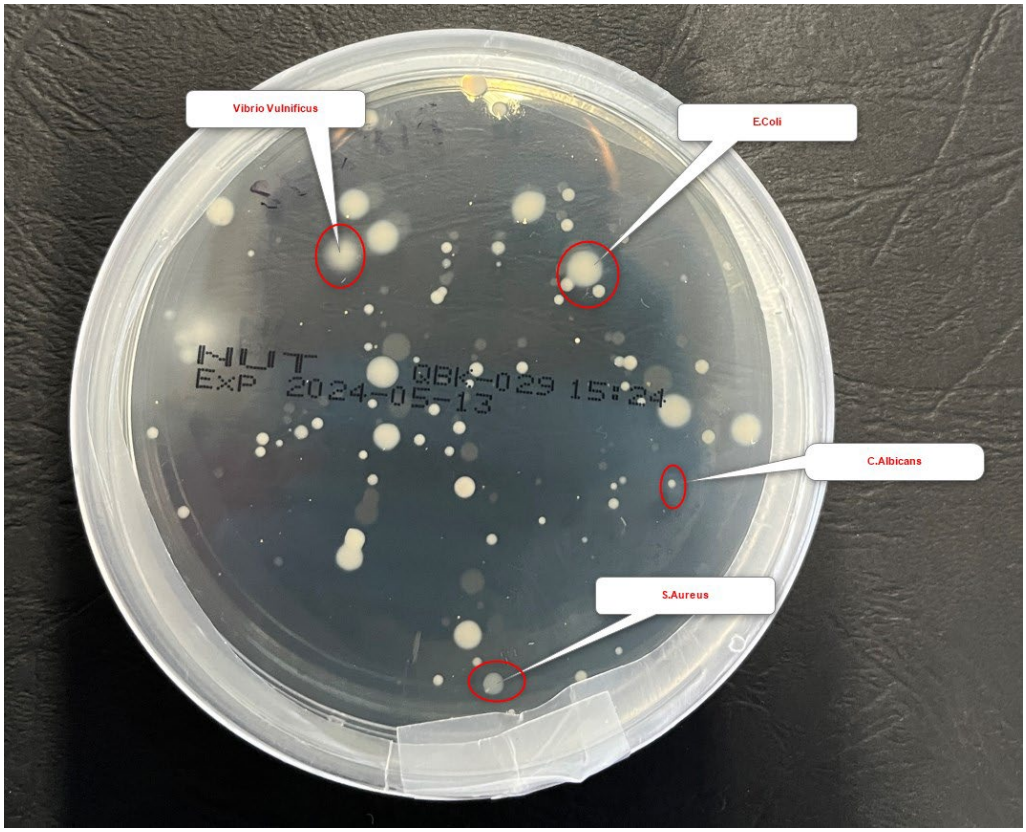


Figure 4 : Sea Sample2

Werribee River

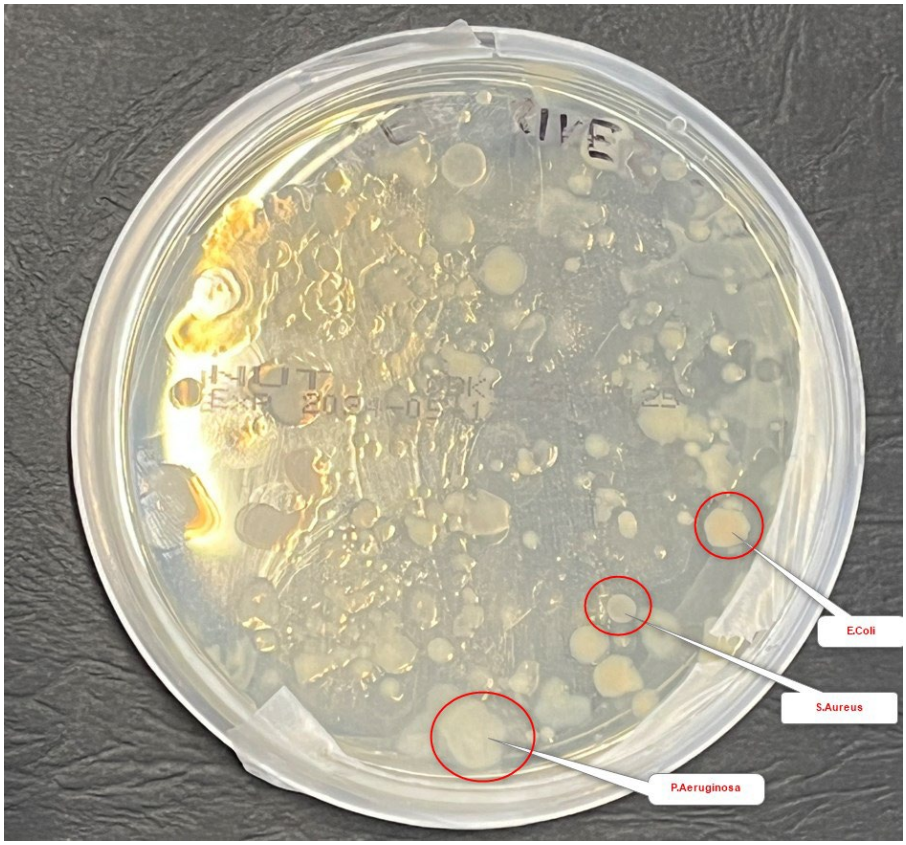


Figure 5: Werribee River Sample1

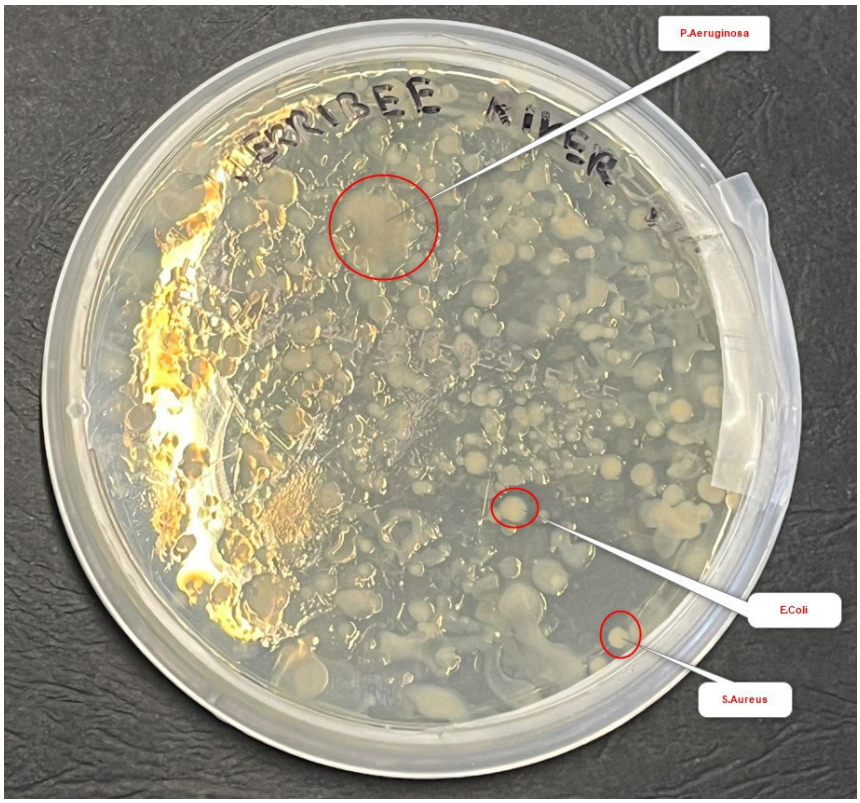


Figure 6: Werribee River Sample 2

Wetland

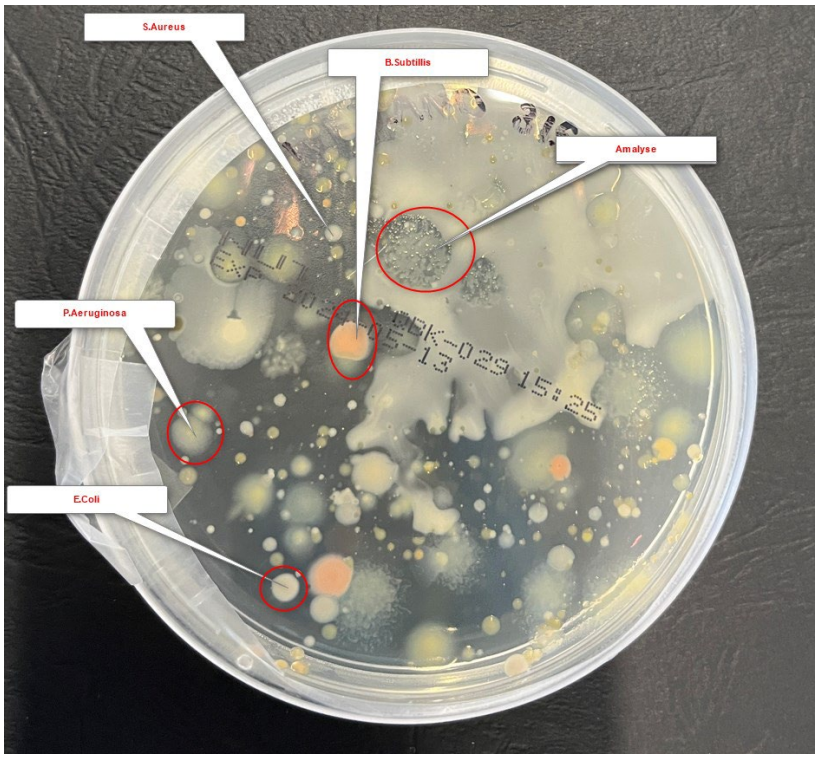


Figure 7: Wetland Sample 1

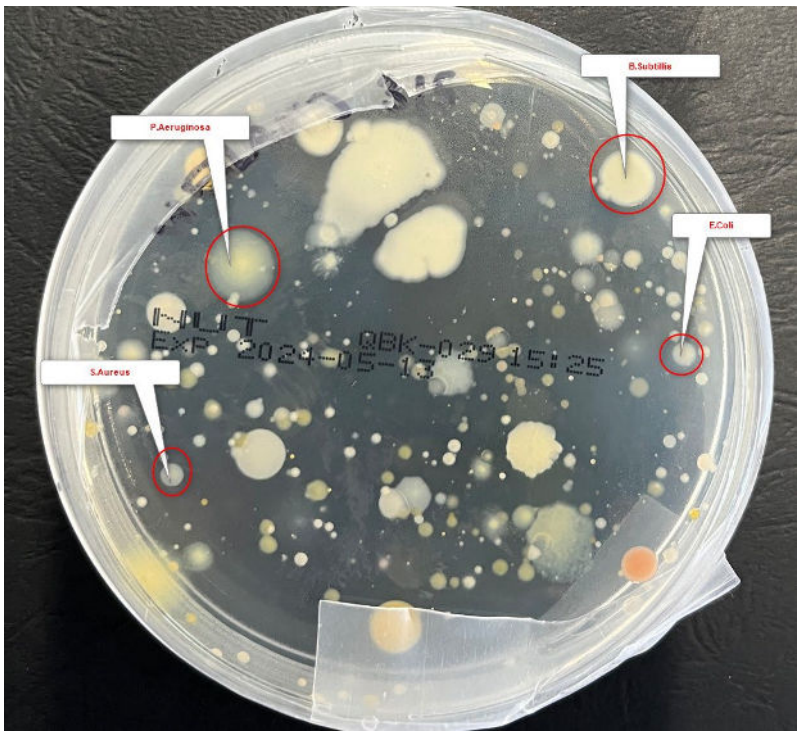


Figure 8: Wetland Sample 2

Yarra River

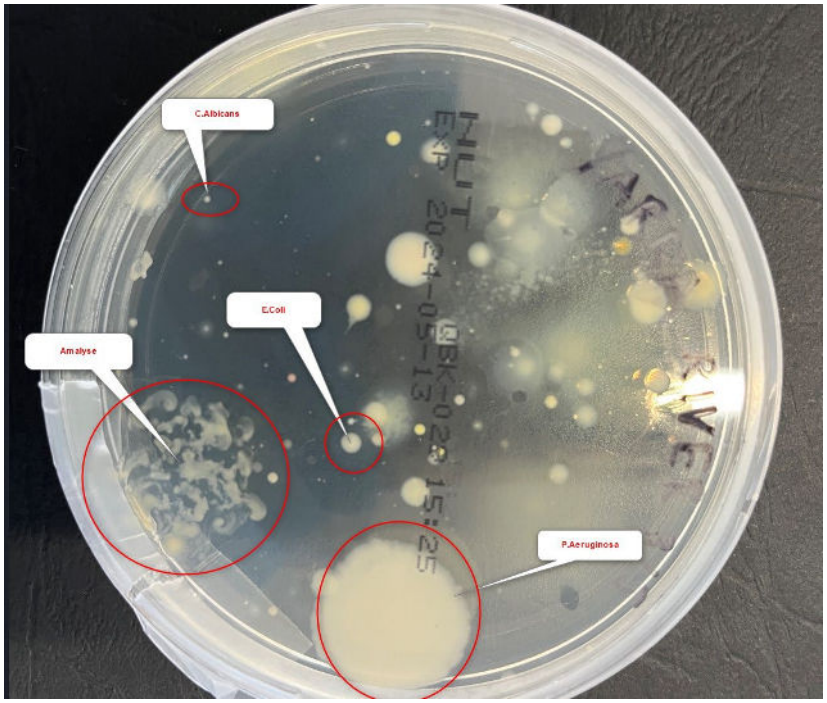


Figure 10: Yarra River Sample 2

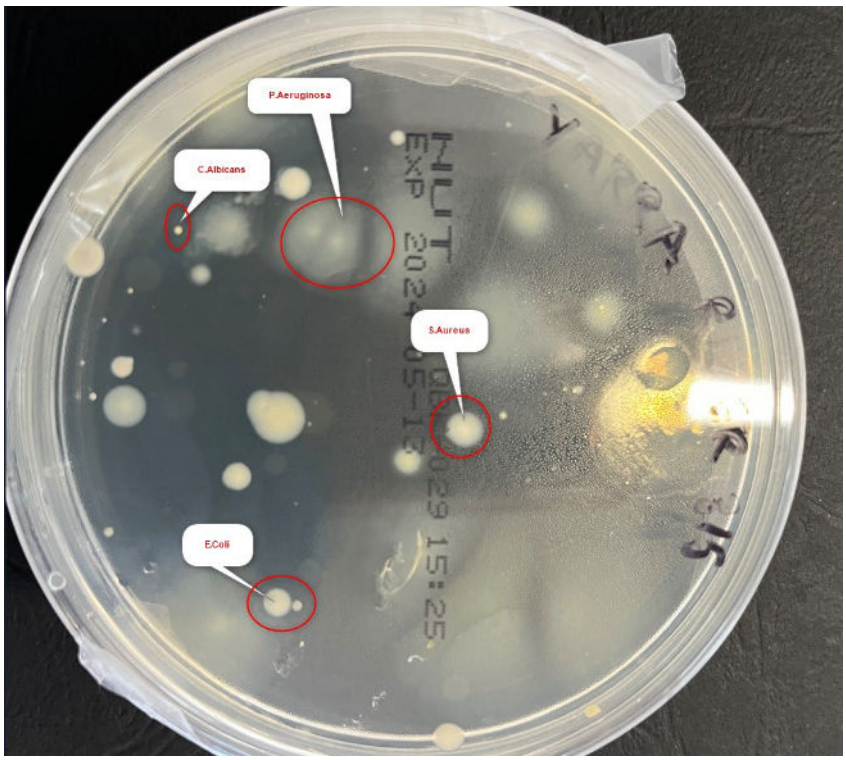
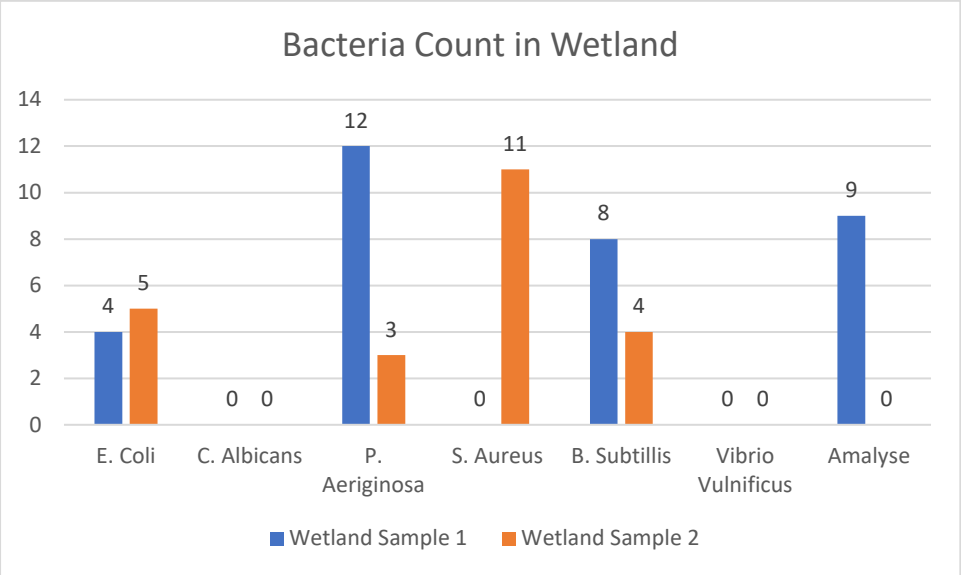
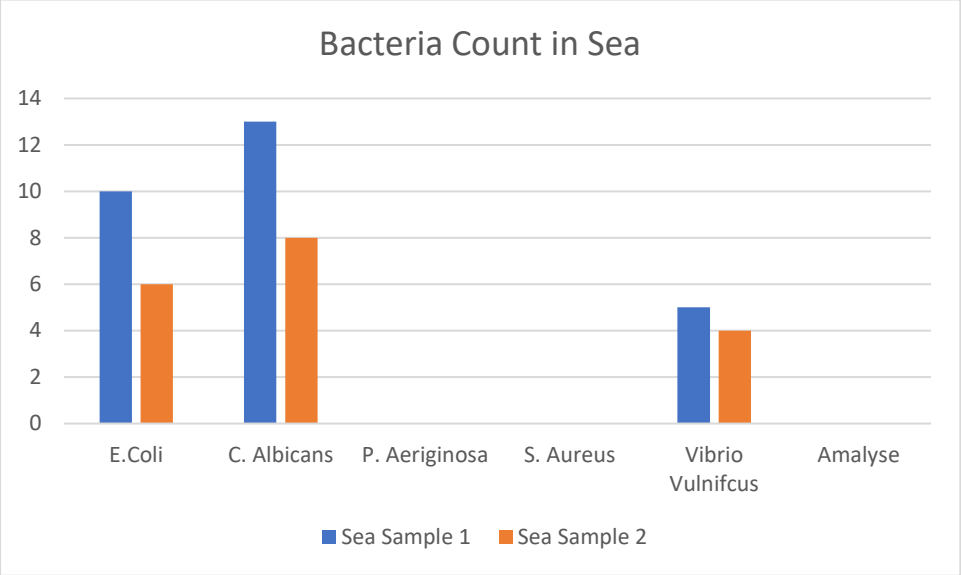
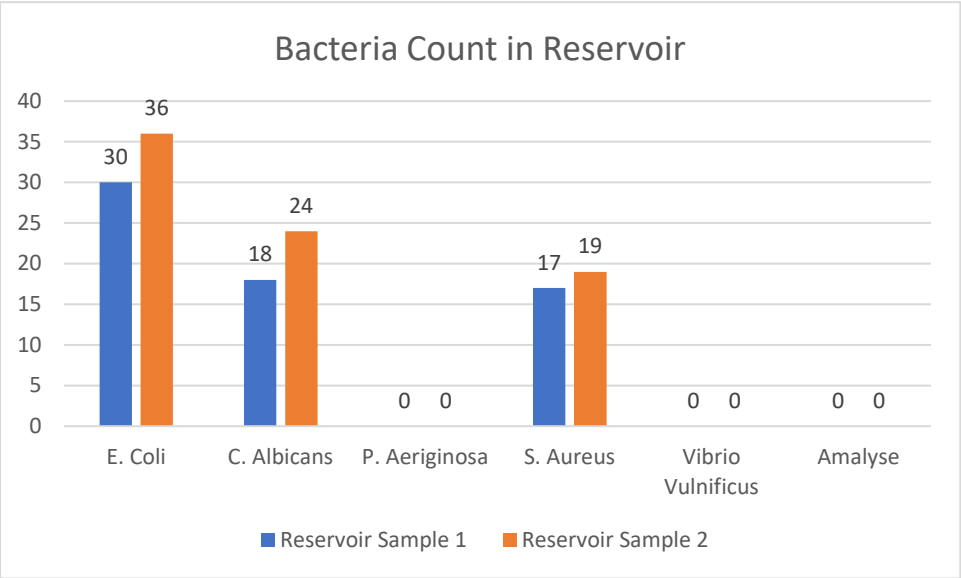


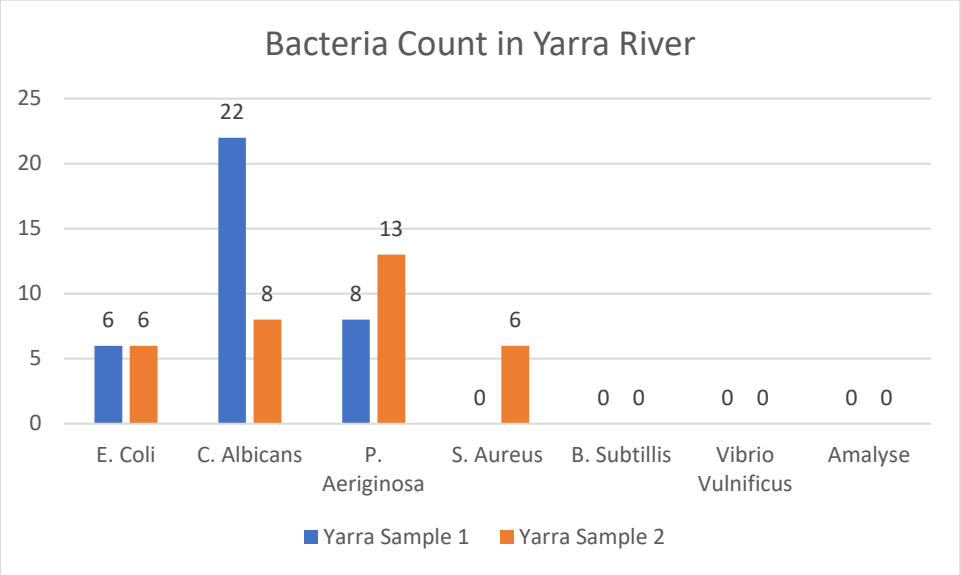
Figure 9: Yarra River Sample 1

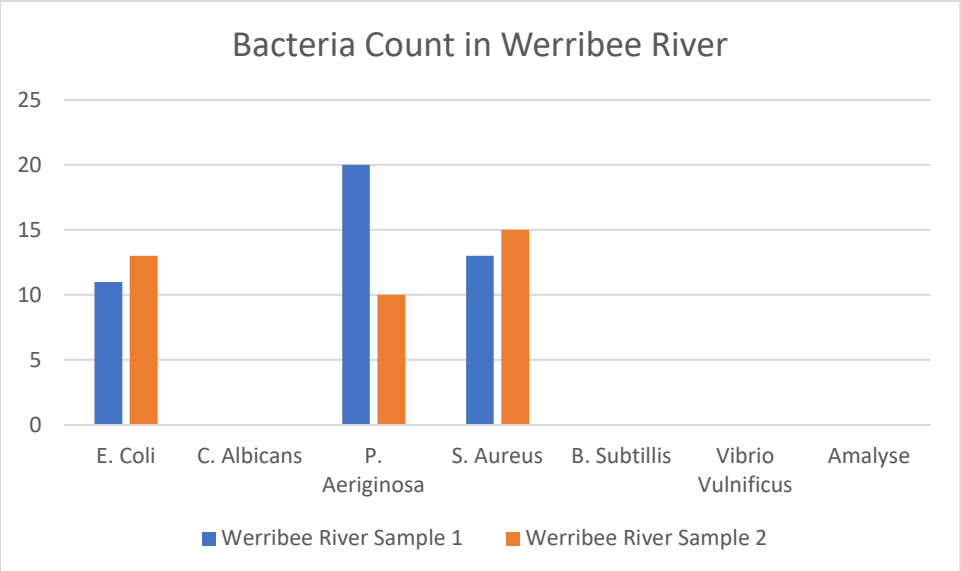
Count of Bacteria observed in each Water sources



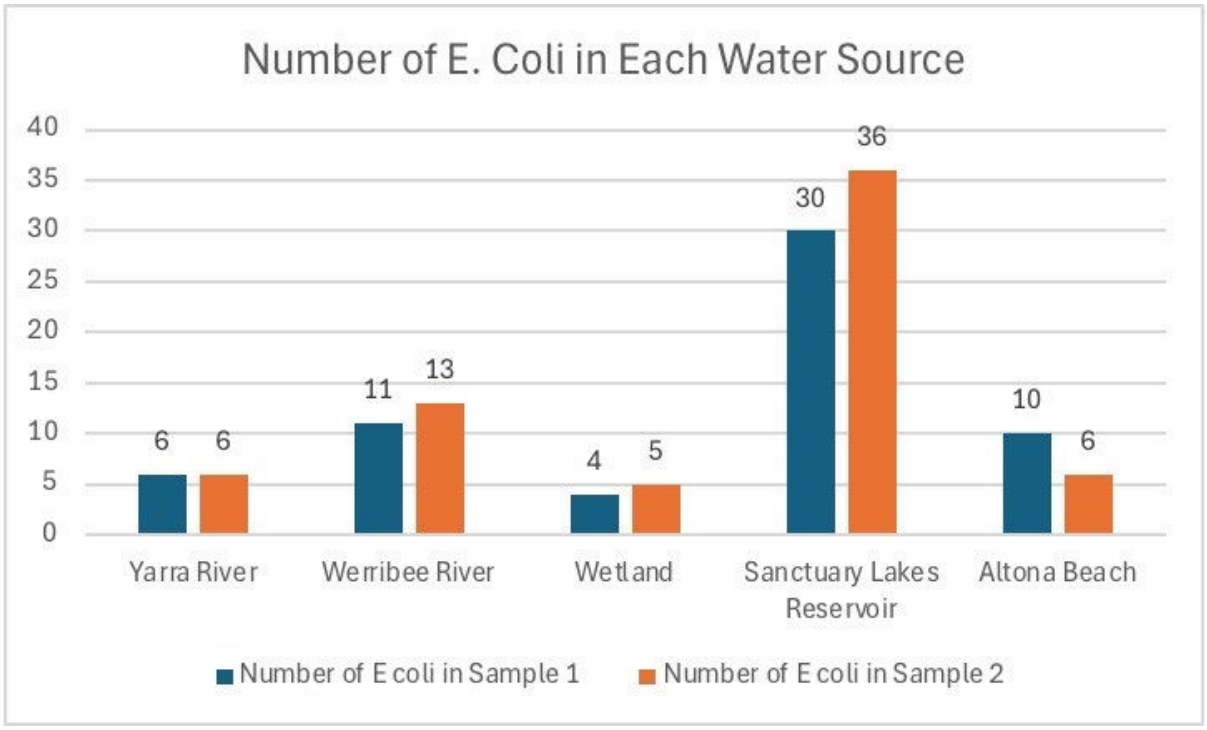



Bacteria Count in Reservoir
40
35
30
25
20
15
10
5
0
30
36
18
24
0 0
17
19
0 0
0 0
E. Coli
C. Albicans
P. Aeriginosa
S. Aureus
Vibrio Vulnificus
Amalyse
Reservoir Sample 1
Reservoir Sample 2


Bacteria Count in Yarra River
25
20
15
10
5
0
6 6
22
8
8
13
0
6
0 0
0 0
0 0
E. Coli
C. Albicans
P. Aeriginosa
S. Aureus
B. Subtillis
Vibrio Vulnificus
Amalyse
Yarra Sample 1
Yarra Sample 2


Bacteria Count in Werribee River
25
20
15
10
5
0
E. Coli
C. Albicans
P. Aeriginosa
S. Aureus
B. Subtillis
Vibrio Vulnificus
Amalyse
Werribee River Sample 1
Werribee River Sample 2

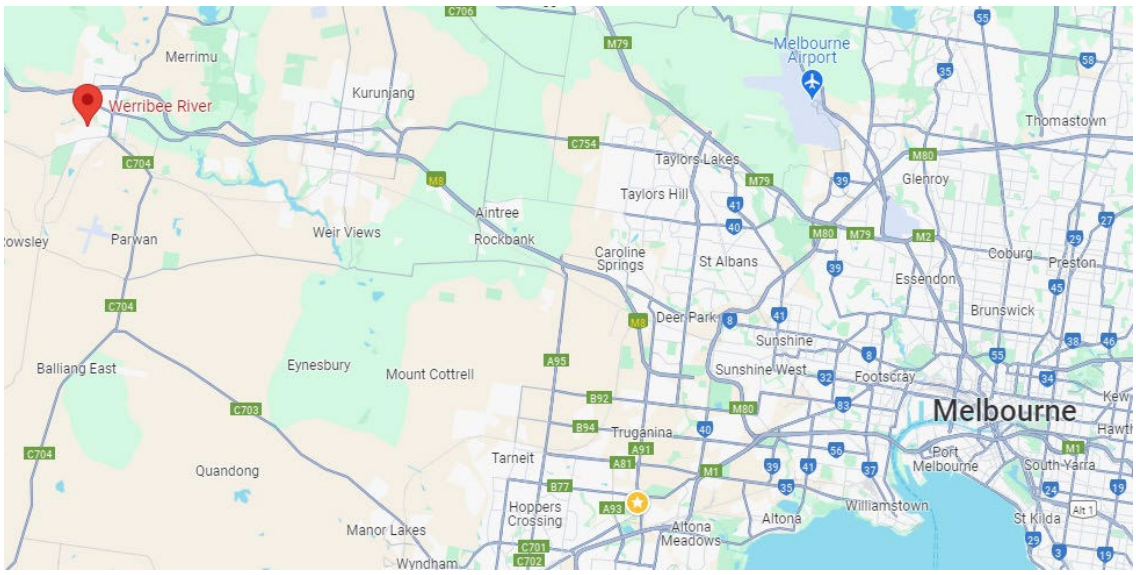
E. Coli Indicator of Different Water Sources



Discussions

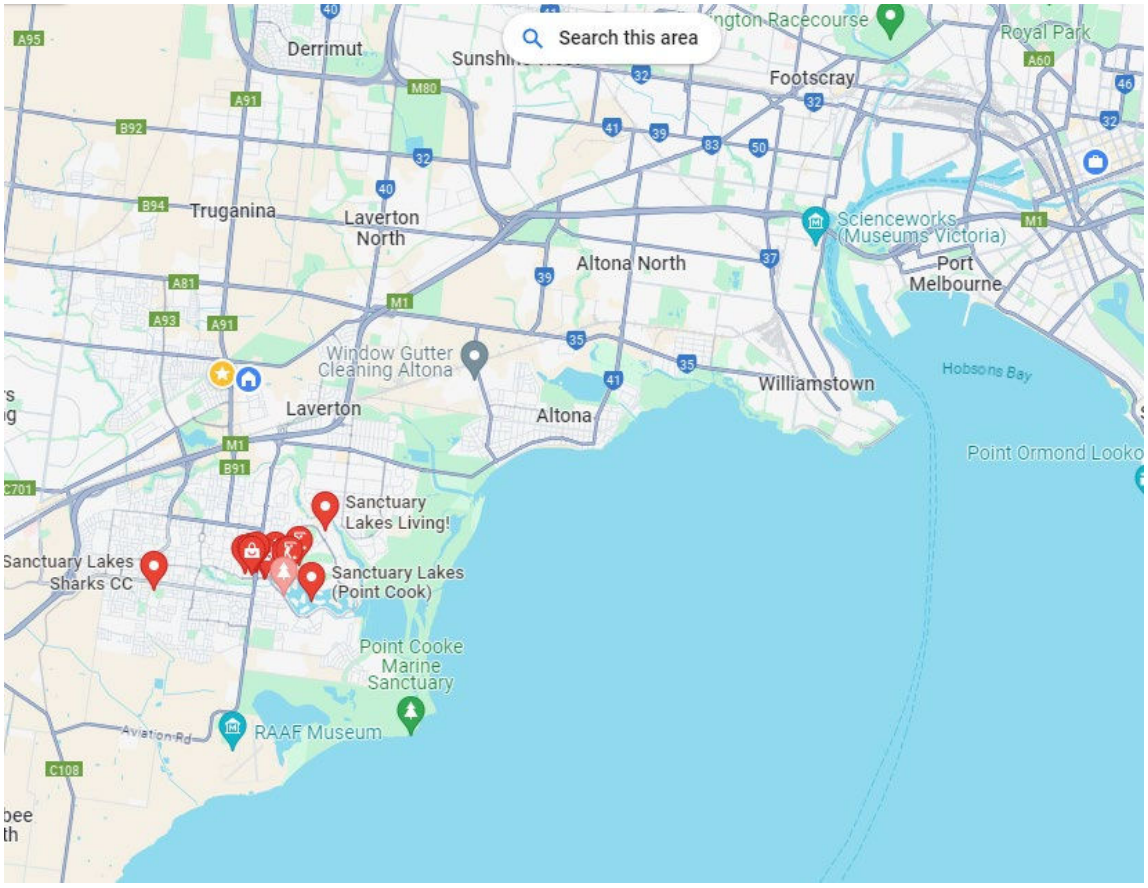
The independent variable was the water source and the dependent variable was the amount of bacteria and variety. Below there is information referring to both the variables. Some results was the minor amount of bacteria in the Yarra River.

Werribee River



It had the second most total bacteria content out of all the water sources. This is due to the increased urbanisation and agricultural development happening around it. Extensive amounts of sediment and nutrients enter the river through tributaries. This could also be due to the area the water was collected from. Areas in higher regions have less development around it results in less pollution accessing the river whereas the lower areas would be more accessible to humans, agriculture, and debris. The extraction of the water from the Werribee River catchment could also contribute to it. Streamflow is a major issue the river faces as the catchment is in the lower areas with comparatively low rainfall and less climate change impact. Fishes, birds, frogs, Mammals, water bugs, and plants live in the river leading to the amylase found in the river. E. Coli is an indicator of sewage and animal waste in the river. The E.coli content could be a result of either the diverse species that call the river home or the mass pollution that goes into the river such as human waste because there is no chlorine to treat it. This then kills the species in the river with all the bacteria and infects the species. The P. Aeruginosa content found in the river indicates that the water is not of drinkable state. P. Aeruginosa is also an indicator of sewage which shows the great impact of pollution in the river. S.Aureus in often found in areas containing wastewater treatment plants. It is also an indicator of human environmental waste, sewage due to septic systems in houses and overflow of sewages during storms and floods. The strong presence of sewage in the river is of concern as it questions if the river is adequate to be used as one of Melbourne's main water sources.

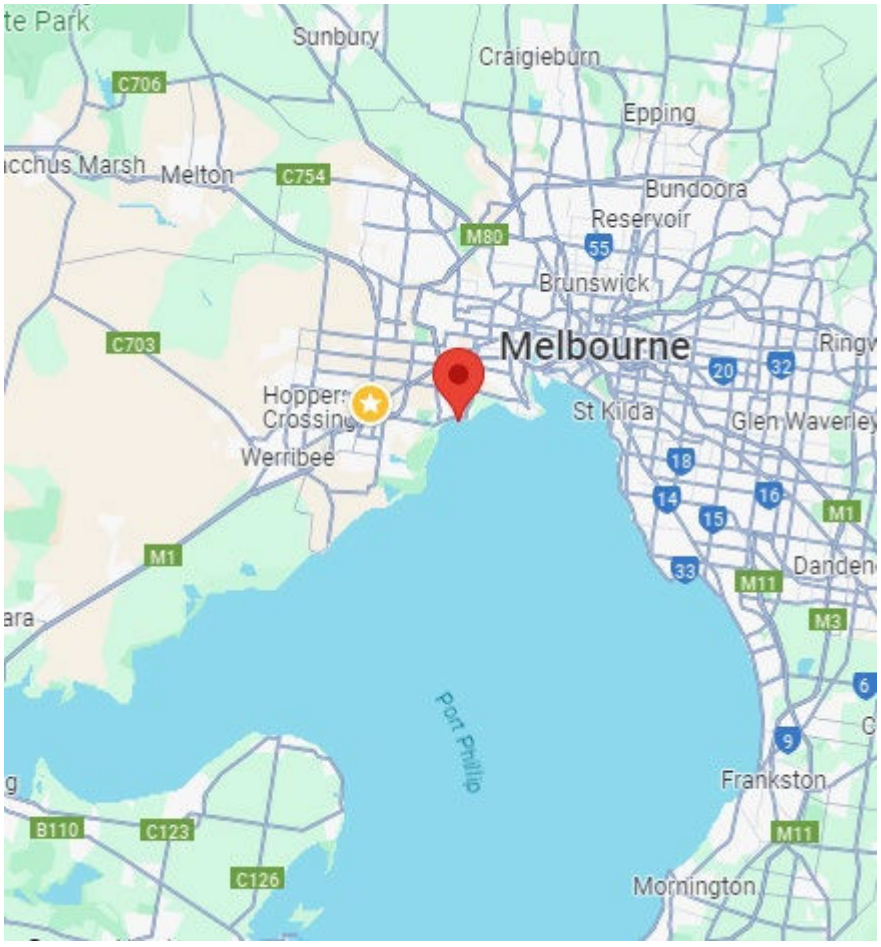
Sanctury Lakes Reservoir



It had the greatest number of bacteria. The extensive number of bacteria in reservoir is because reservoirs host bacteria. They harbour agents of the bacteria but do not grow or develop them, instead it will provide an environment for them to grow, live and reproduce. C. Albicans plays an

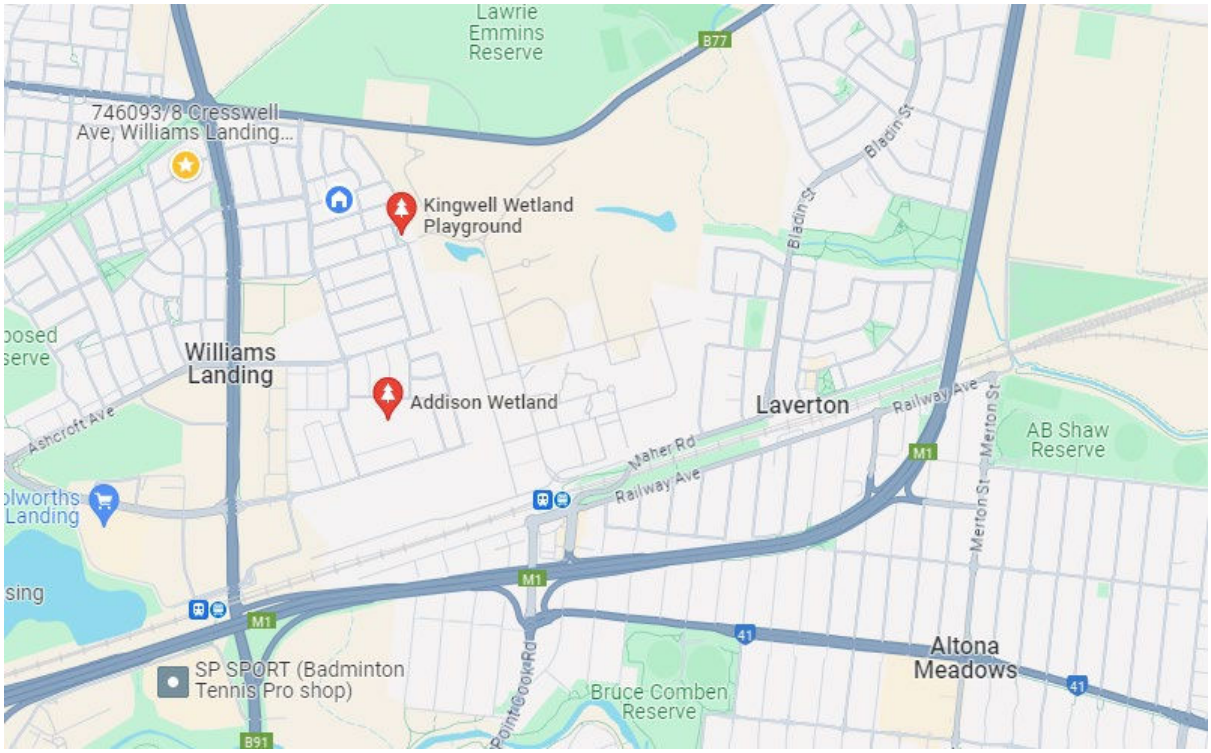
important role in the ecosystem of the reservoir. It breaks down organic matter that other microorganisms cannot cope with, indicating that it plays an important part in maintaining species and is commonly found in polluted water. C. Albicans is also found in the digestive tracts of warm-blooded animals which indicates there is fauna that inhabits the reservoir. S. Aureus and E. Coli are indicators of sewage and animal stool in a water source. They are both waterborne bacteria that grows because of chlorine concentration levels below the recommended levels. The bacteria level could also be impacted by the area the water was collected. The sample was collected from a recreational area that was accompanied by a park and was extremely accessible. There were many visitors and no restrictions to who could access the water including signs that it was a common spot to kayak and fish in. They could have sourced the pollution found in the reservoir and contributed the extensive amount growing there. The surrounding area with sand has also turned into the sludge and the area is also subject to storm water runoff. The presence of humans in the water environment decreases species diversity and causes the ecosystem to break down. The entering of bacteria can cause an influx of pollutants in the water source and disrupt the structure of the microorganisms living there. Unlike bacteria growing in the water, sand is a host to bacteria all year around. They can support the bacteria growth even outside of the times the water is in optimal temperature to grow it. In fact, it is not only a consistent reservoir for growth but at times has more e. Coli content than water.

Sea (Altona Beach)



The sea collectively had the least number of bacteria in whole. The point of collection of the water sample was in a residential area where the release of sewage into the ocean was visible. The main reason for increased bacterial counts in seawater is either sewage entering it or increase in water temperature due to global warming resulting in a more suitable environment for bacteria to grow. There are lots of particles in the ocean which bacteria can attach to and grow on. Vibrio vulnificus grows in coastal areas and seawater because it thrives in the warm water conditions of 18 degrees and above. It is also an hallophic bacteria that requires salt to grow. Vibrio Vulnificus is a threat to marine animals such as fish as it can infect them and kill them. It disrupts the balance of not only the marine ecosystem but also the food change and can be the cause of prolonged negative affects. One of the main reasons animals die is not due to to Vibrio Vulnificus itself but due to it inducing other bacteria into it. Vibrio vulnificus is also an indicator of sewage and can occur due to sewage spills and when infrastructure fails due to rapid and intense storms. The warming environments due to global warming also impact how much vibrio vulnificus grows in water. As the temperature increases, it is becoming a more suitable and easy growing area for the bacteria. S. Aureus is also an indicator of sewage in water and is a bacterium that is not deterred by high salt content but grows well in areas of high salt content as it is a halophile. The beach provides many possible routes for the water to get infected as the bacteria grows well through sand and water. The presence of C.albicans indicates faecal matter and sewage as well as E.Coli. C.albicans in tropical waters may also mean that the water is receiving organic loading. It thrives in the sewage, seawater and animals faeces that are in the saltwater.

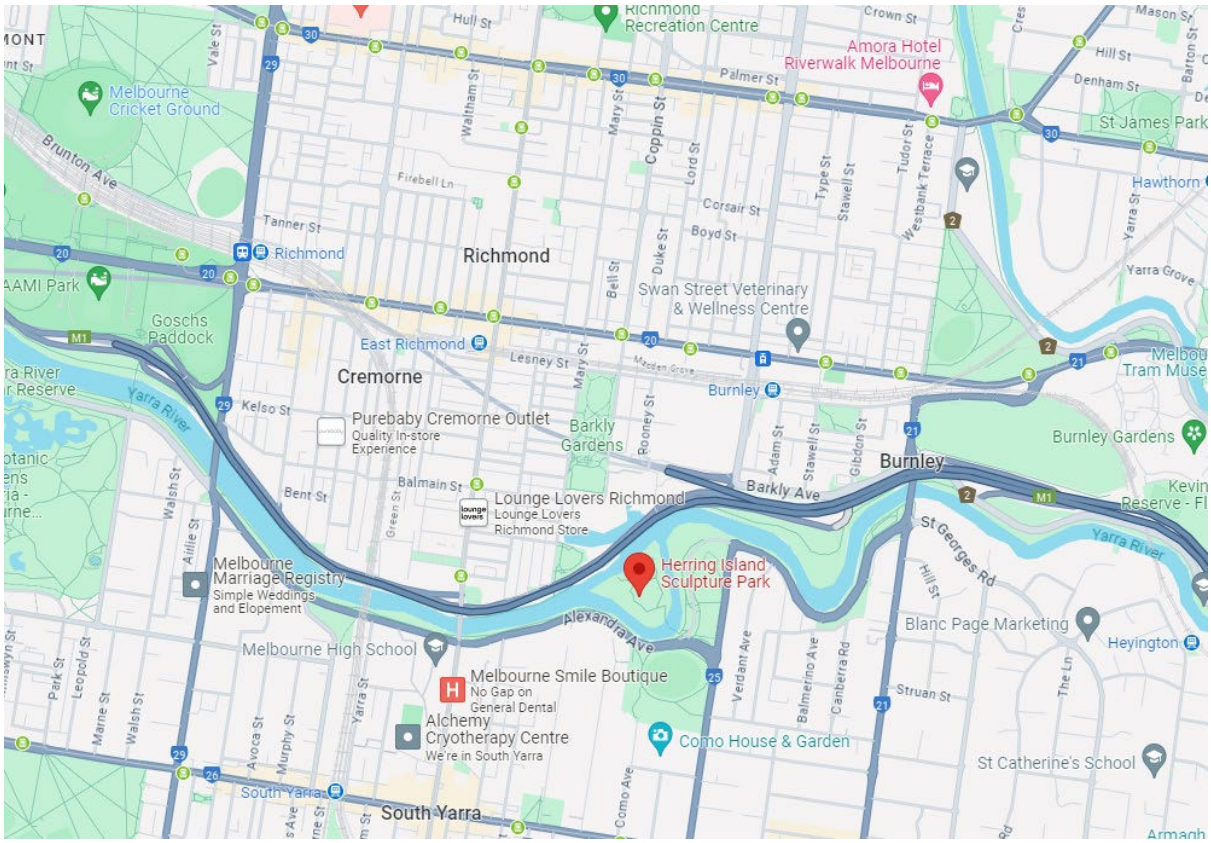
Wetland



The wetland has the third most bacterial growth in total. This is because the soil in wetlands typically provide a perfect habitat for bacteria to thrive in its highly saturated and low oxygen content soils. However, though some of the bacteria negatively impacts the wetland environment, they do play and essential part in the cycle and nutrient recycling processes which create a perfect area to

harbour to diverse microbial communities. Since this a local wetland, it is very easy for the residential sewage to access the area and get absorbed in the soil. E. coli is an indicator of sewage and animal waste. There are many microorganisms that thrive in the area and s. Aureus in the water indicates that there is animal waste in the area. The presence of b.subtitles on surface water indicates food contamination, filtration, and chlorination. P.Aeruginosa indicates sewage in the water and is helpful for organisms to thrive in the wetland soil.

Yarra River



Yarra River had the second least number of bacteria out of all the water sources which was a surprising statistic. The Yarra normally has bacteria in it due to heavy rains and stormwater flushes down it. However, the mainly sunny weather during the sample collection could have contributed to the low bacteria count. Many species around the Yarra use it as drinking water but also an area for their faeces resulting in the presence of E. Coli in the water. C. Albicans and P.Aeruginosa are both indicators of high sewage contamination. The Yarra River area in the city is a common spot for joggers and people to spend their recreational time which could have also been of impact on the type of bacteria in the river.

Although this is what is believed about the different water sources, it may not necessarily be true as what one observes under the naked eye can be different from person to person. The power of school microscopes is not strong enough as a commercial one that can clearly define a close-up shape.

The experiment had a few errors such as not wearing gloves during collection of water. The bacteria were not always stored in the incubator for the 48 hours due to its quick growing pace. The pictures

were not very clear as the light was glaring on the slide subtracting from the quality to see the bacteria.

To improve the validity of the experiment, a sample of tap water could be provided as a control for the experiment to give a base to typical bacterial growth in chlorine tested water. The sample could have been tested several times and averaged to achieve a more reliable result. Gloves should have been used to avoid further bacterial contamination of the water. Agar dishes should have been immediately refrigerated to avoid moulding of the agar.

Conclusion

The investigation did not support my hypothesis as in both samples' reservoir had over 100 bacterial colonies and Yarra River only had 60 and 28 in the first and second sample respectively. There was also more variety in the Wetland water source. The experiment was relevant to the aim as the different types of bacteria and bacteria count in each water source was thoroughly investigated. The different types of bacteria were found such as, vibrio vulnificus, s. aureus, P.Aeruginosa, c. albicans and e. Coli. The bacteria counted ranking each bacterium from least to most bacteria count and were modified into graphs relevant to the type of bacteria.

Bibliography

I acknowledge my parents for driving me and aiding me to collect water samples from the water sources.

I also acknowledge Miss. Mc Coy for sourcing the materials to transfer the bacteria and grow it and the medium to grow it.

Dervaux, J, Magniez, J C & Libchaber, A 2014, On growth and form of Bacillus subtilis biofilms, Interface Focus, viewed 12 May 2024, < https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4213440/ >

Valdes-collazo, L, & Schultz Aj, Hazen, T 1987, Survival of Candida albicans in tropical marine and fresh waters, Applied and Environmental Microbiology, viewed 11 May 2024, < https://pubmed.ncbi.nlm.nih.gov/3310885/ >

Conrad, J W & Harwood, VJ n.d., Sewage Promotes Vibrio vulnificus Growth and Alters Gene Transcription in Vibrio vulnificus CMCP6, Microbiology Spectrum, viewed 10 May 2024, < https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8849060/ >

C=au, & O=the State Of Queensland 2024, Vibrio vulnificus infection, viewed 11 May 2024, < https://www.qld.gov.au/health/condition/infections-and-parasites/bacterial-infections/vibrio-vulnificus-infection >

User, S n.d., Sanctuary Lakes Resort - Sanctuary Lakes Resort, viewed 9 May 2024, < http://www.sanctuarylakesresort.com.au/index.php >

Cudowski, A, Pietryczuk, A& Górniak, A n.d., Effect of Humic Acid on the Growth and Metabolism of Candida albicans Isolated from Surface Waters in North-Eastern Poland, International Journal of Environmental Research and Public Health, viewed 11 May 2024, < https://pubmed.ncbi.nlm.nih.gov/35954766/ >

Pawłowski, Jarosław, Majchrowska, Sylwia, Golan & Tomasz 2022, Generation of microbial colonies dataset with deep learning style transfer - Scientific Reports, Scientific Reports, viewed 14 May 2024, < https://www.nature.com/articles/s41598-022-09264-z >