Microbe Growth in Water Samples

Samuel Humphrey

Covenant Christian School

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Abstract:

This report investigated microbial growth in a variety of water samples. The experiment aims to investigate and identify microbes and their growth from a variety of sources of water. It was hypothesised that the further upstream a source of water, the less polluted it will be. In the experiment water was taken from Manly Dam, Manly Creek and Manly Lagoon. Microbe growth was used to determine the pollution in the water sample. Results indicated an increase in microbe growth from Manly Dam to Manly Creek, however a reduction in microbe growth at Manly Lagoon. Inferences were drawn and possible improvements for further experimentation were suggested. It was concluded that the results were partially supportive of the hypothesis, although further testing is required to draw reliable conclusions.

Table of Contents:

Introduction:

Microbes are single celled microorganisms which can survive in almost every region of the planet. Their effects on human health vary. Microbes can be transmitted to humans in a variety of ways including through water.
Three water samples were taken from areas along the Manly creek water system. These three sample areas were connected to each other through water system. This is illustrated from Figures 1-3.

Source 1: Manly Dam

Surrounding land use in the Manly Dam area is primarily outdoor recreational activities such as hiking and kayaking. There are however some residential homes in the surrounding area. In addition to this the dam has seen previous military use during World War Two.

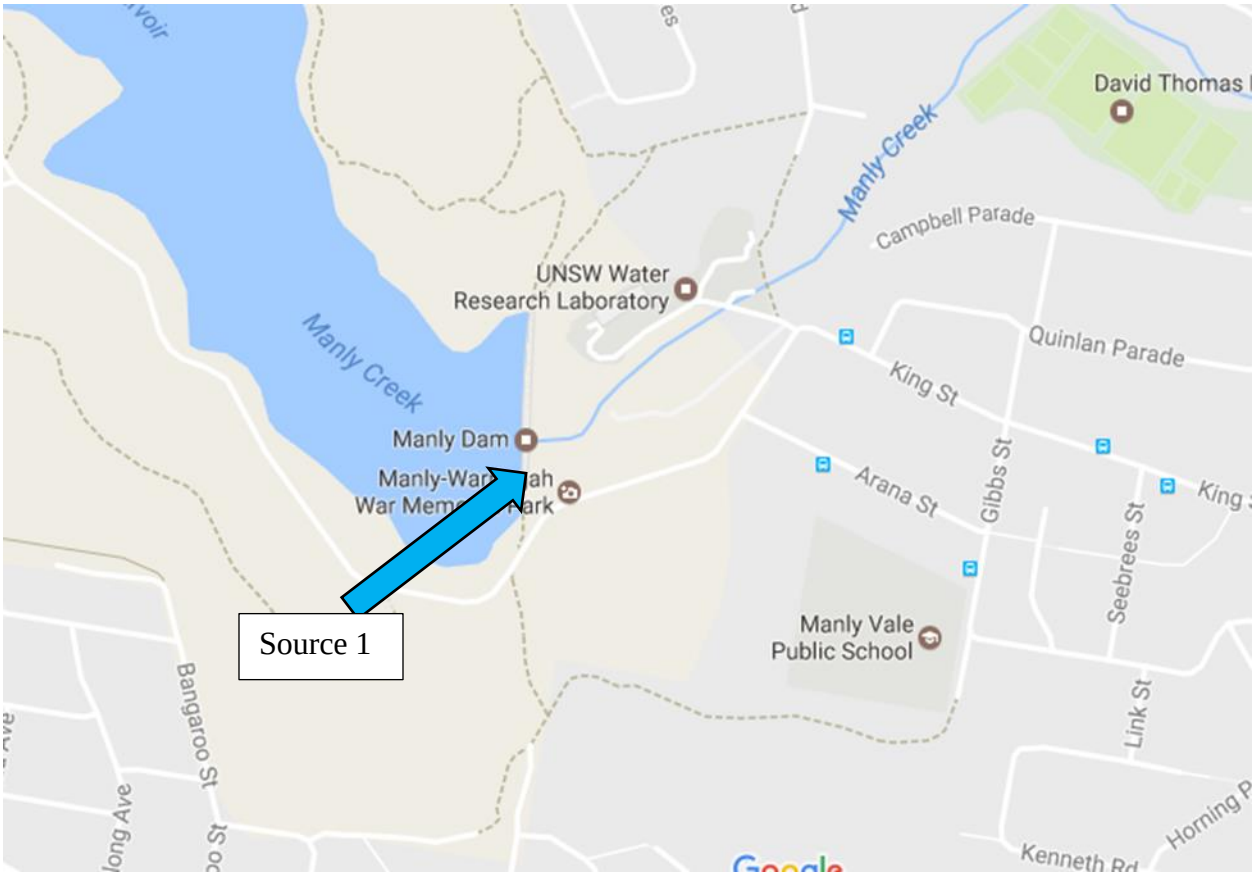


Figure 1. Google Maps. (2017). *Manly Dam catchment area* [Map]
https://www.google.com.au/maps/@-33.7813821,151.2564659,16z

Source 2: Manly Creek

Surrounding land around Manly Creek consists of residential homes and recreational facilities such as sporting grounds.

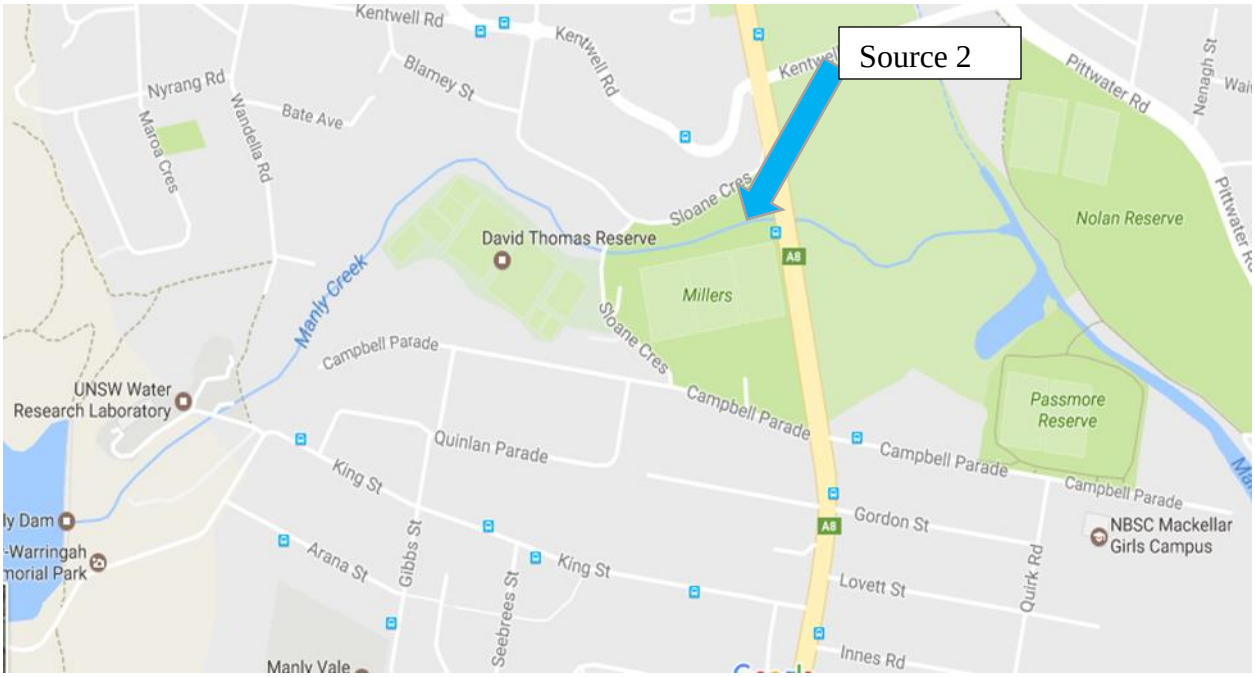


Figure 2. Google Maps. (2017). *Manly creek area* [Map] https://www.google.com.au/maps/@-33.7800979,151.2674093,16z

Source 3: Manly Lagoon

The surrounding land use of the Manly Lagoon consists primarily of residential homes. In addition to this there are recreational facilities and some small businesses.

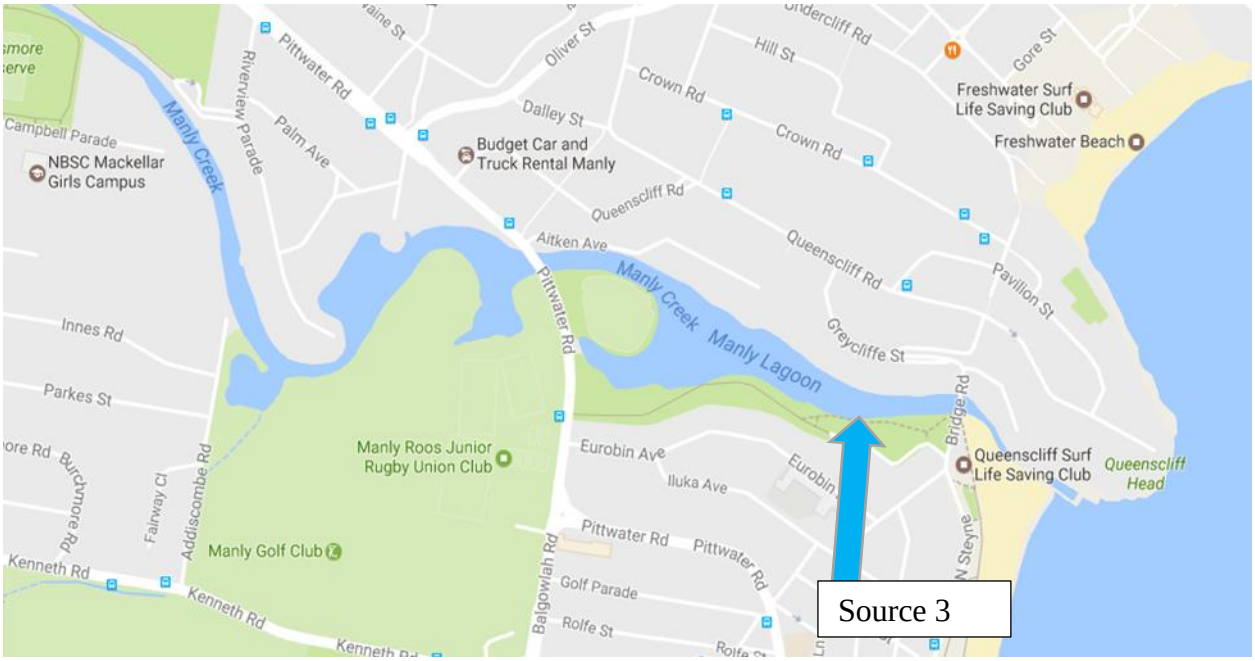


Figure 3. Google Maps. (2017). *Manly Lagoon area* [Map] https://www.google.com.au/maps/@-33.7836471,151.2849188,16z

Aim:

The purpose of this experiment is to investigate and identify microbes from a variety of water sources.

Hypothesis:

It was hypothesised that the further upstream a water source the less polluted the sample will be, while the further downstream water sources will be more polluted. This is because further upstream there is less potential for pollutant microbes to contaminate the water, and, as water flows downstream the more microbes can be absorbed into the water, therefore providing higher pollution rates downstream.

Method:

Equipment List:

- Three Sterilised sample jars
- Fine tip permanent marker
- Safety glasses
- Lab coat
- Gloves
- Ethanol
- Ethanol spray bottle
- Paper towel
- Bunsen burner (with gas)
- Matches
- Heated agar nutrient solution (contained in jar)
- Seven Petri Dishes
- Role of tape
- Scissors
- Inoculating loop
- Incubator

Procedure:

Collecting samples

1. Three sample jars were collected and sterilised to improve the reliability of the experiment.
2. The collector travelled to the first water source and identified a safe collection site where they had firm footing on the edge.
3. Using one of the sample jars water was scooped from the water source. The jar was held above the water upside down (lid toward body of water) to prevent unnecessary contamination. The jar war then scooped in a 'U' shaped movement approximately 10cm into the water. See figure 4.

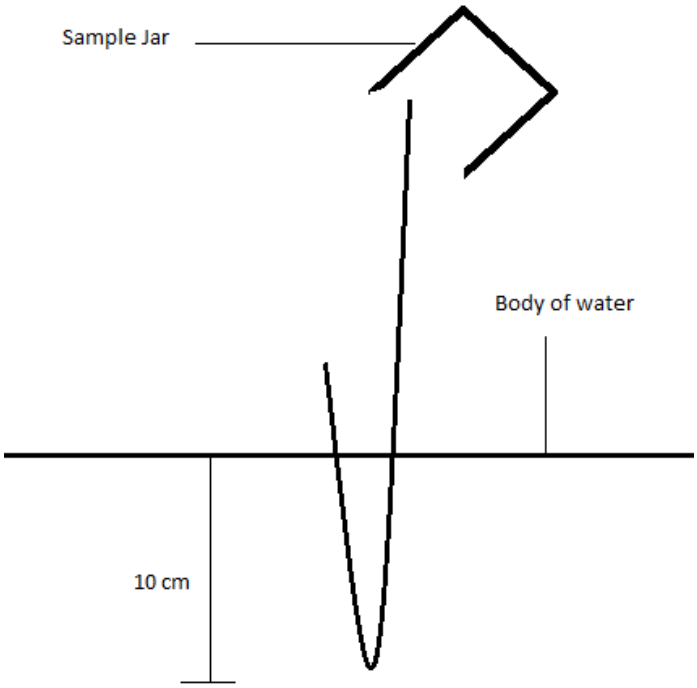


Figure 4. *Water collection technique* [Diagram]

4. The jar was immediately sealed tightly with the lid to prevent contamination.
5. Using the marker, the jar was labelled with the source and the time, temperature, previous rainfall, current speed and tide were recorded in Table 1.
6. The procedure was repeated for water sources two and three.
7. The sample jars were stored out of direct sunlight and at room temperature

Experiment

1. The operator washed hands and wore safety glasses, gloves and lab coat.
2. The workbench and gloves were sprayed with ethanol and swabbed with paper towel.
3. The Bunsen burner was swabbed with ethanol and placed onto the bench.
4. The Bunsen was ignited with matches and left to burn with the safety flame on.
5. The jar containing nutrient agar solution had the lid removed and the rim was sterilised by switching the Bunsen to the blue flame and allowing the slowly rotating the rim through the flame. It should be noted that the burner was switched back to safety flame afterwards.
6. The first petri dish had the lid carefully lifted no more than 45° and the agar solution was poured in up to 2-3mm. See figure 5. The operator took care not to breathe into the solution.

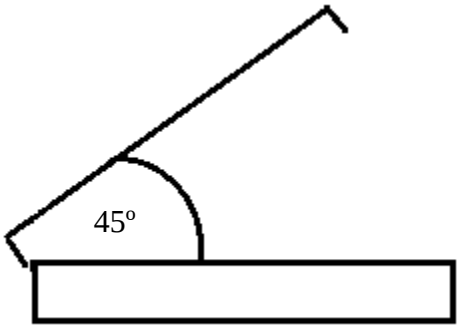


Figure 5. *Opening of petri dish* [Diagram]

7. The agar was allowed to set.
8. Steps 1-7 were repeated for the remaining 6 petri dishes
9. One jar was selected and sealed immediately with tape and flipped upside down to prevent condensation. This was the control and was labelled accordingly.
10. The Bunsen was switched back the blue flame.
11. The centre of the inoculating loop was then heated to red hot and slowly moved toward the loop ensuring the wire remains red hot. After heating it was switched back to the safety flame. This process was to sterilise the loop.
12. The loop was allowed to cool and slowly dispensed into water sample one, this was to ensure the heat didn't kill any microorganisms. The loop was then stirred through the sample jar to collect microorganisms.
13. The inoculation loop was then placed onto a petri dish (lid open no more than 45°) and the loop swirled according to Figure 6. The operator ensured the loop did not dig into the agar.

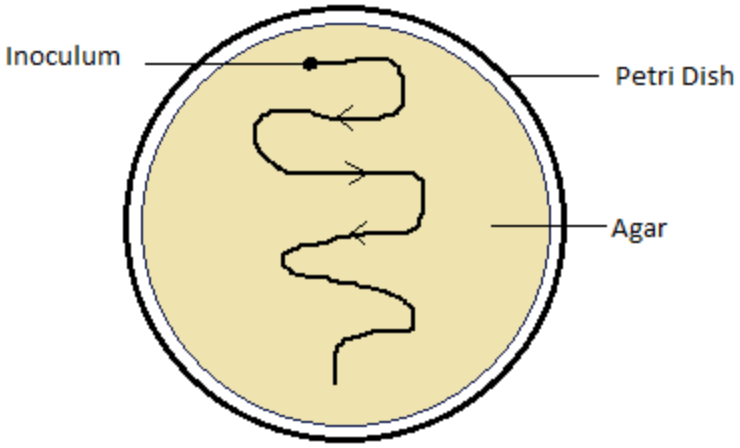


Figure 6. *Inoculation loop* [Diagram]

14. Steps 12 and 13 were repeated with another dish.
15. The two petri dishes were flipped upside down, sealed with tape and labelled accordingly.
16. Steps 10-15 were repeated for samples two and three.
17. The petri dishes were placed in an incubator and the microbes were allowed to grow for three days at a temperature of 27°C.
18. After three days, the microbe colonies that had developed and their forms, elevation characteristics and growth coverage were recorded and formatted into Table 2.

Variables:

Independent Variable:
-Location of the water samples.

Dependant Variable:
-Microbe growth on petri dish

Controlled Variables:
-Incubation time
-Incubator temperature
-Method for collecting water
-Method for inoculating
-Amount of nutrient agar used
-Sterilisation methods
-Opening angle of petri dish

-Prevention of breathing into petri dish
-Sample jars and petri dishes unopened and sterilised

Control:
-A control is used in the experiment by creating an agar filled petri dish with no water sample used. This determines if there is a flaw in the sterilisation processes of the experiment.

Safety Assessment:

Collecting Water:

-The operator is to ensure they constantly maintain firm footing and do not lean too far when collecting water. This is to prevent potential injury.
-The operator is to thoroughly wash hands after collection. This is to remove and kill potential pathogens contained within the water that may be ingested by the operator.

Performing Experiment:

-Long hair must be tied up to prevent injury caused by open flame.
-Protective eyewear should be worn to prevent injury caused by open flame.
-Gloves should be worn to prevent ingestion of potential pathogens in water samples. In addition to this they reduce the risk of injury caused by heated equipment.
-Lab coat should be worn to prevent contact with potentially harmful microorganisms in the water samples. In addition to this it serves as a protective barrier between the open flame and the operator.
-The Bunsen burners yellow safety flame is switched on during periods where it is not in use. This serves to make the open flame more distinguishable and thus preventing harm to the operator
-Petri dishes are to remain sealed to prevent pathogens from escaping.
-In the case of spillage fluids are to be cleaned up immediately and the surrounding area disinfected
-Hands are to be washed thoroughly before and after experiment, this kills and removes sources of pathogens before and after experiment thus reducing chances of ingestion.

Results:

Environmental factors at the time of water collection were recorded and formatted into Table 1.

Table 1. Environmental Conditions at Collection of Water Samples

	Source 1	Source 2	Source 3
Time and date	15:40hrs 21-05-17	15:45hrs 21-05-17	15:50hrs 21-05-17
Temperature (°C)	21	21	20
Current Weather Description	Sunny with some light cloud cover		
Previous Rainfall in 24 hours (mm)	14.6		
Current	Stationary	Slow moving, almost stationary	Slow moving, almost stationary
Tide	Not existant	Not existant	Peak of high tide

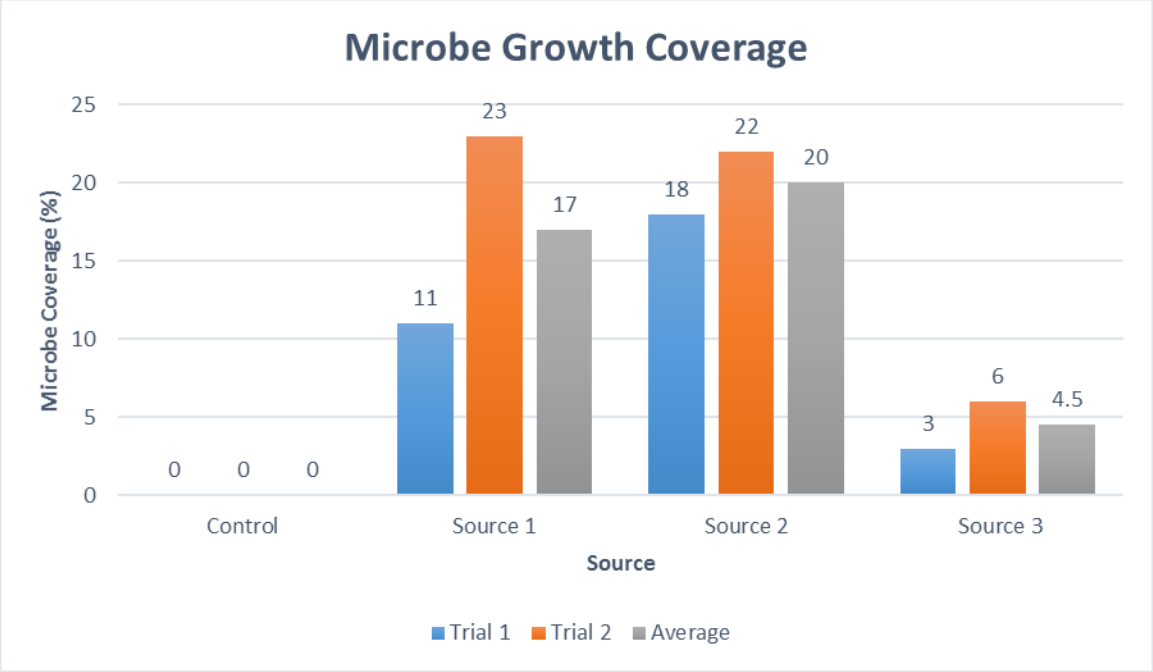
The data from the microbe growth after the experiment was recorded and formatted into Table 2. Photographs of the petri dishes are illustrated in the appendix.

Table 2. Microbe growth data

	Microbe coverage estimate (%)			Description of microbe growth			
	Trial 1	**Trial 2**	**Average**	**Number of colonies**	**Types**	**colonial forms**	**elevation characteristics**
Control	0	N/A	0	0	0	N/A	N/A
Source 1	11	23	17	**Trial 1:** 27 distinquishable colonies **Trial 2:** 30 distinquishable colonies and 7 indistinquishable growths	**Trial 1**: 4 types, -1x milky white -7x cream yellow -1x orange -18x grey **Trial 2:** 4 types -3x milky white -9x cream yellow -6x orange -19x grey	**Trial 1:** 2x irregular growth and 26x circular growth **Trial 2:** 5x circular growth and 32xirregular growth	All growth is convex
Source 2	18	22	20	**Trial 1:** 15 distinquishable colonies and 2 indistinquishable growths **Trial 2:** 10 distinquishable colonies and one large indistinquishable growth	**Trial 1:** 4 types, -3xmilky white -6x cream yellow -6x orange -1x grey (large indistinquishable growth) **Trial 2:** 4 types -1x milky white -5x cream yellow -4x orange -1x grey (large indistinquishable growth)	**Trial 1:** 4x circular growth and 13x irregular growth **Trial 2:** 4x circular growth and 7x irregular growth	All growth is convex
Source 3	3	6	4.5	**Trial 1:** 5 distinquishable colonies and one large indistinquishable growth **Trial 2:** 1 large indistinquishable growth	**Trial 1:** 3 types -1x cream yellow -1x orange 4x grey (includes large indistinquishable growth) **Trial 2:** 1 type -1x large indistinquishable grey growth	**Trial 1:** 4x circular growth and 2x irregular growth **Trial 2:** 1 x irregular	All growth is convex

Microbe growth data regarding percentage of agar plate covered from table 2 was formatted into Graph 1.

Graph 1.



Discussion:

Evaluation of Data and Inferences:

The control developed no microbe growth. This indicated that the sterilisation techniques used in the experiment are effective and contamination within the petri dishes or agar was likely not a cause for error within the experiment.

Microbe growth coverage from Manly Dam varied significantly between trials one and two with a 12% difference in growth. There was a smaller difference between trials at Manly Creek and Manly Lagoon at 4% and 3% respectively as indicated from Graph 1. This indicates there may have potentially been an error in the inoculation of trial two from Manly Dam.

A comparison between the different water samples in Graph 1 indicates that average microbe growth coverage has increased between sources at Manly Dam and Manly Creek. This corresponds with the hypothesis. A possible reason for this increase in pollution is the runoff of pollutants from the surrounding areas such as topsoil, chemicals, rubbish and various forms of organic matter (DPI. n.d.) (AIMS. n.d.). This runoff, and pollutants, are carried by the current further downstream and therefore result in an increase in microbe growth.

Graph 1 does however indicate a dramatic reduction in microbe growth coverage between sources 2 and 3. This is contradictory to the hypothesis and could have been caused by a variety of factors. One prominent factor is the salinity of the water at Manly Lagoon. Although salinity was not tested upon collection of the water sample, records indicate that it was high tide (BOM. 2017). This would have brought salt into Manly Lagoon and thus increased salinity. Salinities effect on microorganisms is highlighted by Dr Elisabeth Fay's experiments regarding salinity and its influence on microbe growth which indicated that *"Low humidity and salinity are the most stressful factors of microbial flora"* in addition to this she established *"high salt concentration has a high bio-energetic taxation since the microorganisms need to maintain osmotic equilibrium".* In Manly Lagoon, the high tide potentially inhibited microorganism growth by altering the osmotic balance and therefore resulting in decreased microbe growth coverage. (Fay. 2012).

The local government's cleaning efforts focused in Manly Lagoon could have also reduced the microbe growth (Northern Beaches Council. n.d.).

Another factor that could also have contributed to the reduction of microbes is the increasing build-up of herbicides and pesticides in the water used in recreational grounds. This could have led to killing significant proportions of microorganisms in the water (Northern Beaches Council. n.d.).

In addition to this it should be noted that the current travelled along Manly Creek at a slow to almost stationary rate. Considering 14.6mm of rain had fallen in the past 24 hours it is possible

that runoff had built up in the water and had begun to flow downstream but had not reached the Manly Lagoon area therefore resulting in lower microbe growth coverage (Weatherzone. 2017).

It should also be noted that the same types of bacteria are present from all sources as indicated from Table 2. Manly Lagoon however displays significant reduction in the number of white, creamy yellow and orange microbes. This was likely caused by a change in environmental conditions at Manly Lagoon.

Evaluation of Experiment:

The experiment has accurately prevented contamination due to thorough sterilisation techniques, as supported by the control, which suggests that no contamination occurred during the inoculation process.

The experiment could however be improved if the inoculation for each water sample was replicated multiple times. This replication would serve to improve the accuracy of the data obtained.

The use of computer based programs could also be implemented which would allow for more accurate estimations on the microbe growth coverage and the number of colonies present.

In addition to this further testing of environmental factors such as chemical residues, salinity and turbidity at the water samples could allow for more accurate inferences to be drawn from experimental data.

Conclusion:

The experiment successfully investigated and identified a variety of microbes from a variety of different water sources. The results only partially confirmed the hypothesis. There was an increase in microbe growth between samples 1 and 2, as predicted, yet a decrease was seen in sample 3, contradictory to the hypothesis. Due to the small sample size tested during the experiment further testing with more replication is required to draw reliable conclusions. This testing should also include testing for environmental factors.

Appendix:

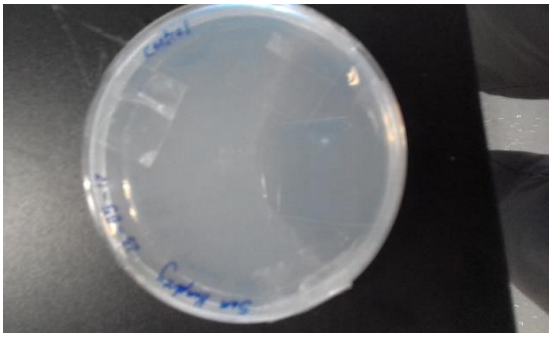


Figure 7. *Control* [Photograph]

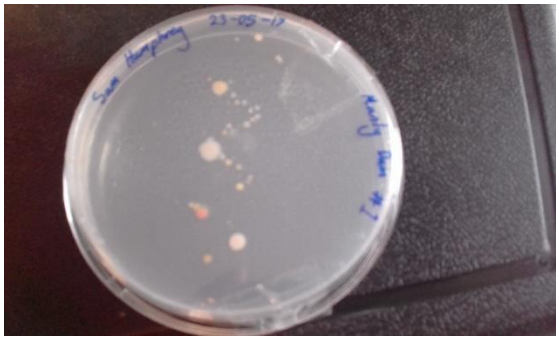


Figure 8. *Manly Dam Trial 1* [Photograph]

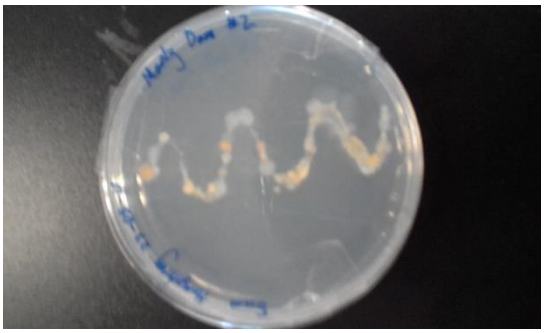


Figure 9. *Manly Dam Trial 2* [Photograph]

Figure 10. *Manly Creek Trial 1* [Photograph]

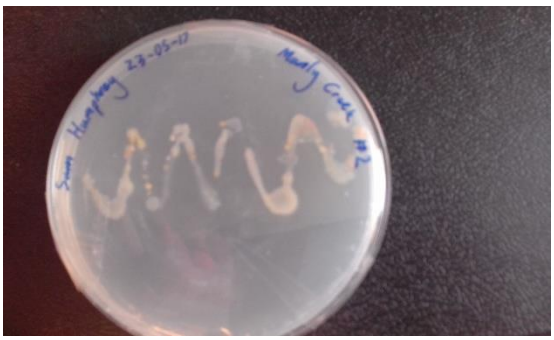


Figure 11. *Manly Creek Trial 2* [Photograph]

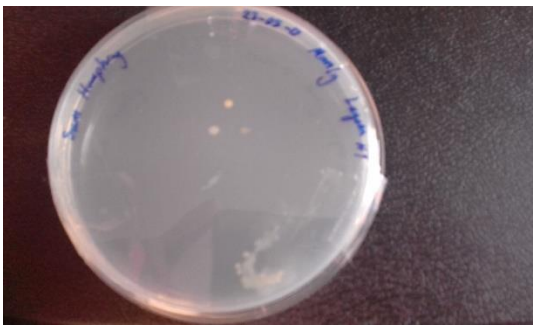


Figure 12. *Manly Lagoon Trial 1*[Photograph]

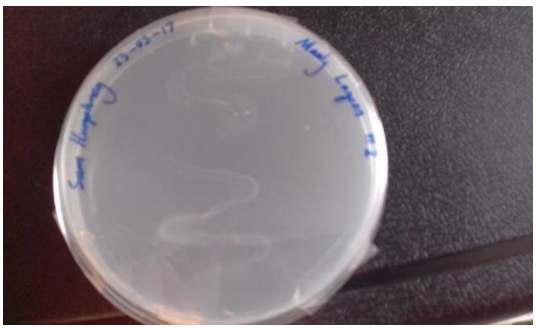


Figure 13. *Manly Lagoon Trial 2* [Photograph]

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