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Assay of the Reverse Osmosis Purified Water in the Life Science Building at Bowling Green State University, Ohio

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Assay of The Reverse Osmosis Purified Water in The Life Science

Building at Bowling Green State University, Ohio

Fall 2017

Nick Mendenhall

With Dr. Scott Rogers and Dr. Neocles Leontis

Introduction

It is essential to maintain clean and sterile water for research uses in a lab. Ultrapure water (18.2 Megohm resistance, < 1 ppb total organic carbon) is required for most molecular biological and microbiological projects. Contaminated water can cause many problems for a variety of laboratory research, from causing inaccurate results to complete failure of experiments. During the past two years, the Life Science building has been reporting issues with its water use in labs, mainly those that involve the growth of bacteria or fungi, as well as failures of reliability for many procedures. It is hypothesized that organisms could be growing inside the pipes, as this is a very common place for biofilms to appear¹. The company that is in charge of servicing and maintaining the deionized water system has flushed out the pipes twice during the past six months. However, assays indicated that the decontamination procedure was ineffective the first time. The proposed research will continue these assays to determine the purity of the water.

The goals of this project are to test the water in the building from various classrooms and research laboratories on each floor of the Life Sciences building for the presence of fungi and bacteria. If any are found, each will be sequenced to determine genus and species (and possibly strain or isolate). From there we can determine if they are detrimental to research or possibly to the people in the building.

Up until about five years ago the biology department took care of the water treatment themselves on a regular basis. This changed when the university decided to outsource the maintenance to another company. A private company was contracted to

service and maintain the system. However, they normally deal with food establishments, which require a lower standard of purity than required for the research projects that are being performed in the building. They claim that they keep up with the system checks but from our results so far, it appears that they are failing to meet the high standards needed for biological research projects. Among the noticeable changes include an increase in the pH of the water, as well as an increase in the microbial load, or how many microbes are present in the water.

Another possible source for the change in the quality of the water could be that in February Bowling Green and Waterville started sharing water through a pipe that runs under the Maumee River. It is possible that construction on the pipe could have caused contaminants to leak into the water for the past year or so of construction². However, the problems with the deionized water in the Life Sciences building have been ongoing for at least the past 18-24 months.

Materials and Methods

Water samples were collected in sterilized 1.5 ml microfuge tubes from the deionized water taps throughout the Life Sciences building at Bowling Green State University, Bowling Green Ohio from rooms 129, 426, 445, 515, the herpetology lab, and the autoclave room on the fifth floor after allowing the water to run for at least 15 seconds prior to sampling. A 200-microliter aliquot of each sample was spread with a sterilized glass rod onto LB (10 g tryptone, 5 g yeast extract, 10 g NaCl, 15 g agar all in

one liter of water) and MEA (20 g malt extract, 20 g dextrose, 6 g peptone, 15 g agar all in one liter of water) agar plates in a sterile biosafety laminar flow hood. The sterile room was bathed in UV light for 30 minutes prior to plating and all surfaces were wiped down with bleach. The plates were sealed with parafilm and incubated at 22°C for approximately 2 weeks.

The plates were checked regularly for any growth starting about 2 to 3 days after initial plating. Colonies were denoted by any single colony that could be visibly distinguished from surrounding colonies. The texture, color, and size that were observed were recorded as well. In order to figure out the number of colony forming units per milliliter, the total number of colonies was multiplied by five since the initial aliquot volume was 200 microliters.

The next step will involve isolating the DNA from the samples and running it through polymerase chain reaction (PCR). PCR works by taking the DNA from the samples and making more copies of the same DNA so that it can be used for future research. PCR requires polymerase, DNA primers, deoxyribonucleotides (dNTPs), and buffer. Once PCR is done, the DNA will be sent off to another institution where it will be sequenced to be further identification.

Results

A table of the results is provided in an attached Excel document.

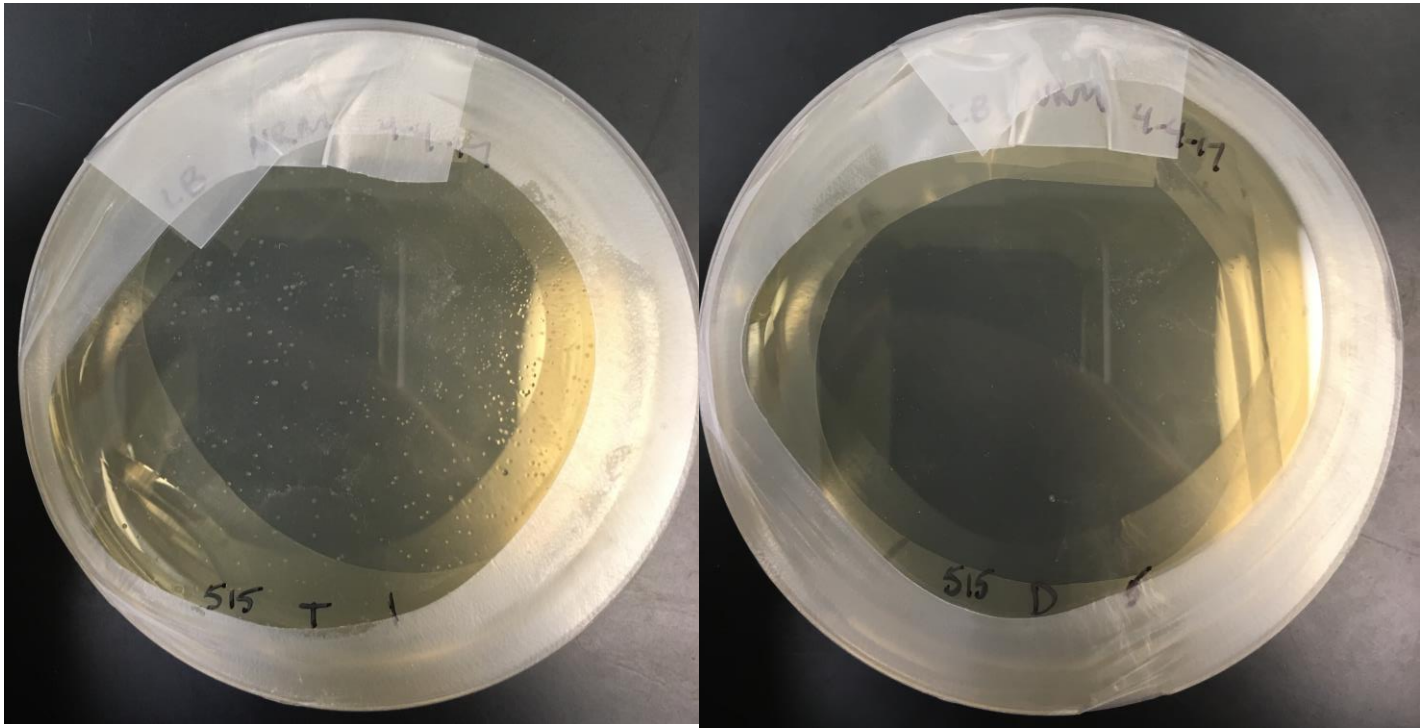


Figure 1 and 2: Plates of 515 tap water and 515 distilled water on LB.

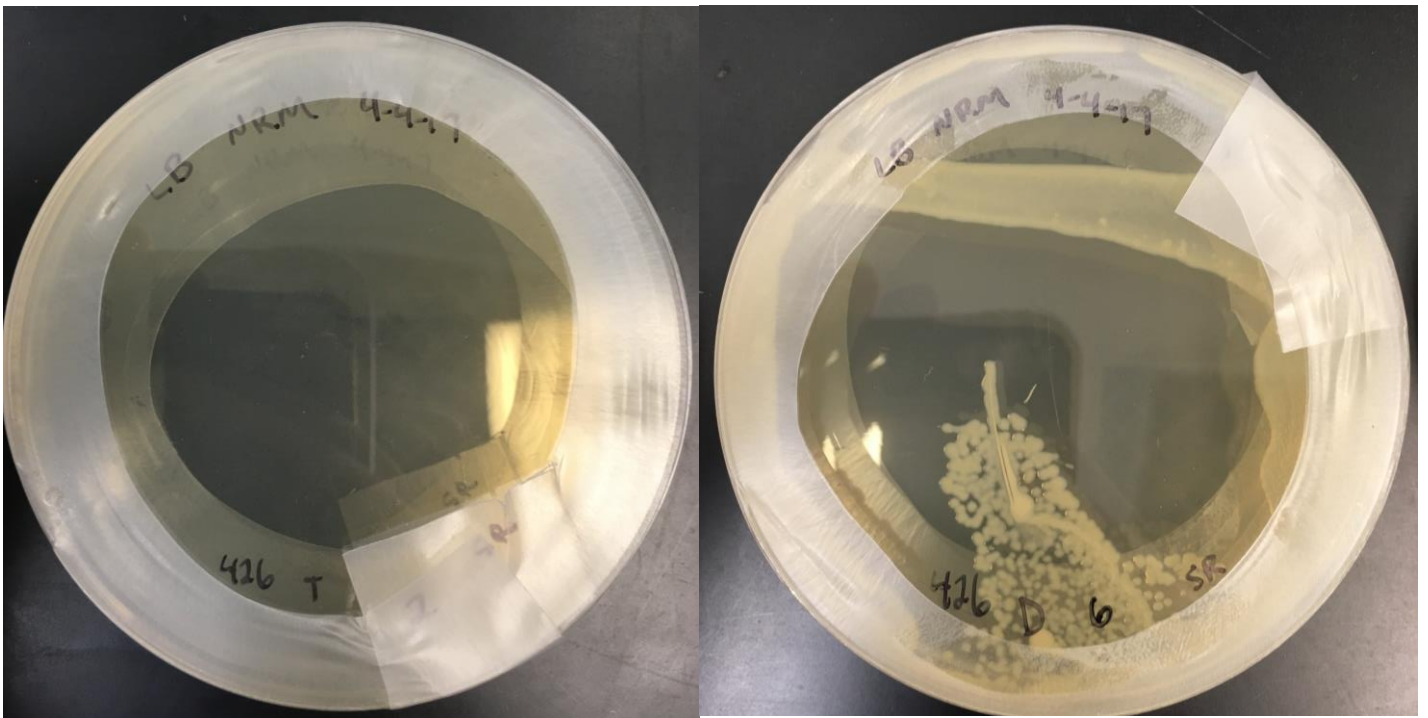


Figure 3 and 4: Plates of 426 side room tap water and 426 side room distilled water on LB.

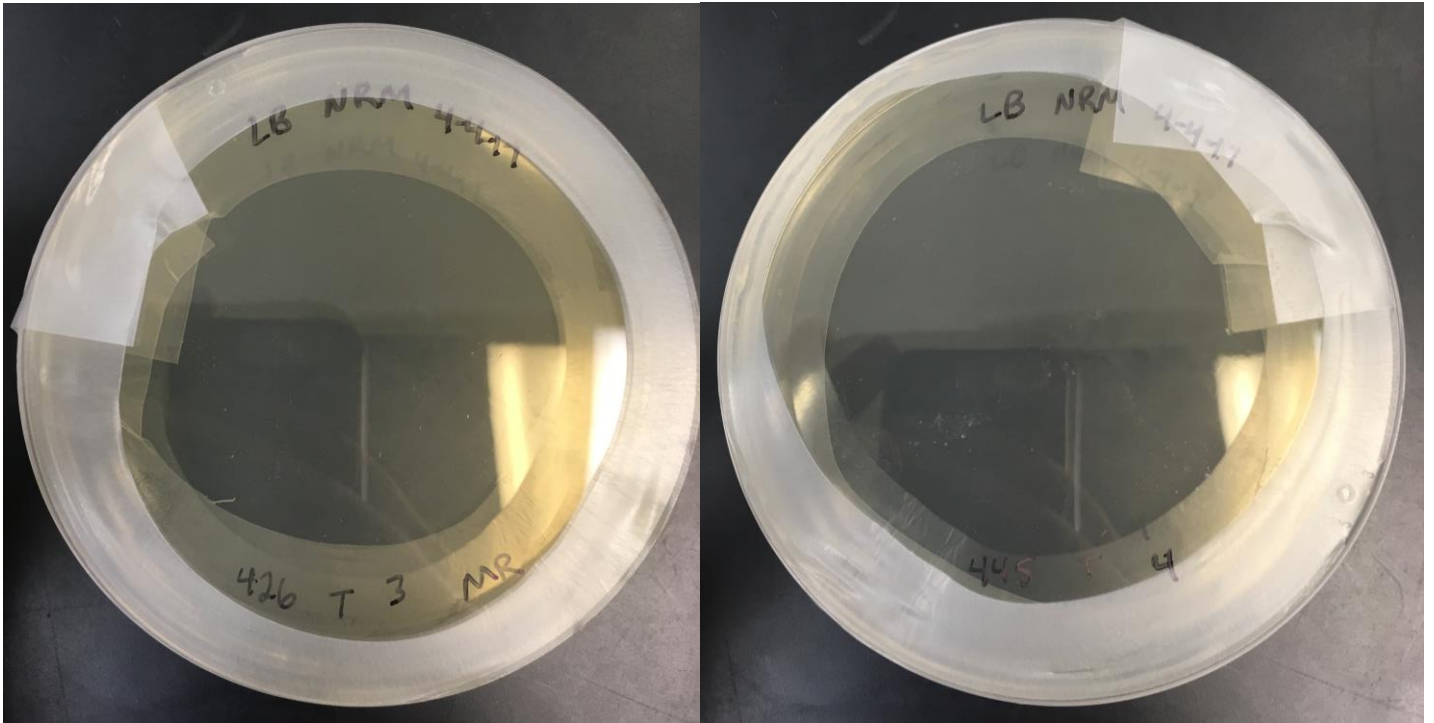


Figure 5 and 6: Plates of 426 main room tap water and 445 tap water on LB.

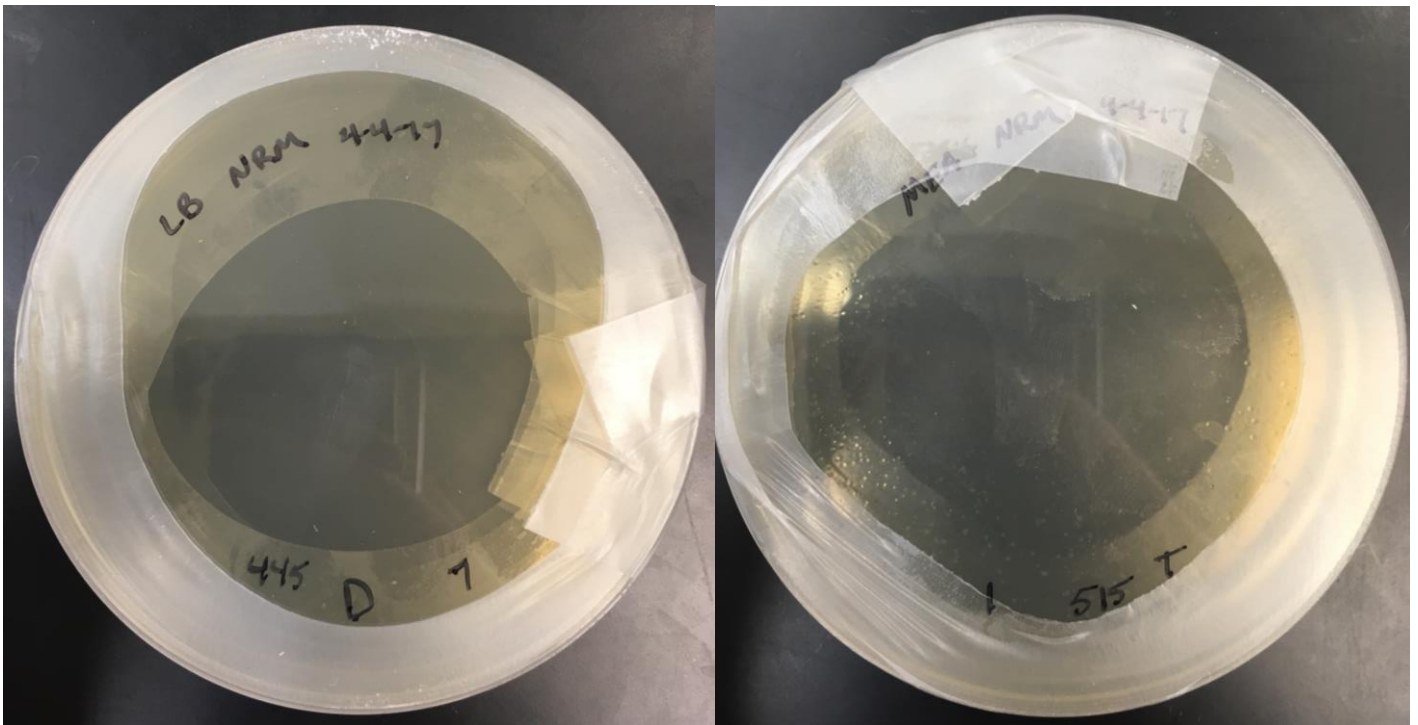


Figure 7 and 8: Plates of 445 distilled water on LB and 515 tap water on MEA.

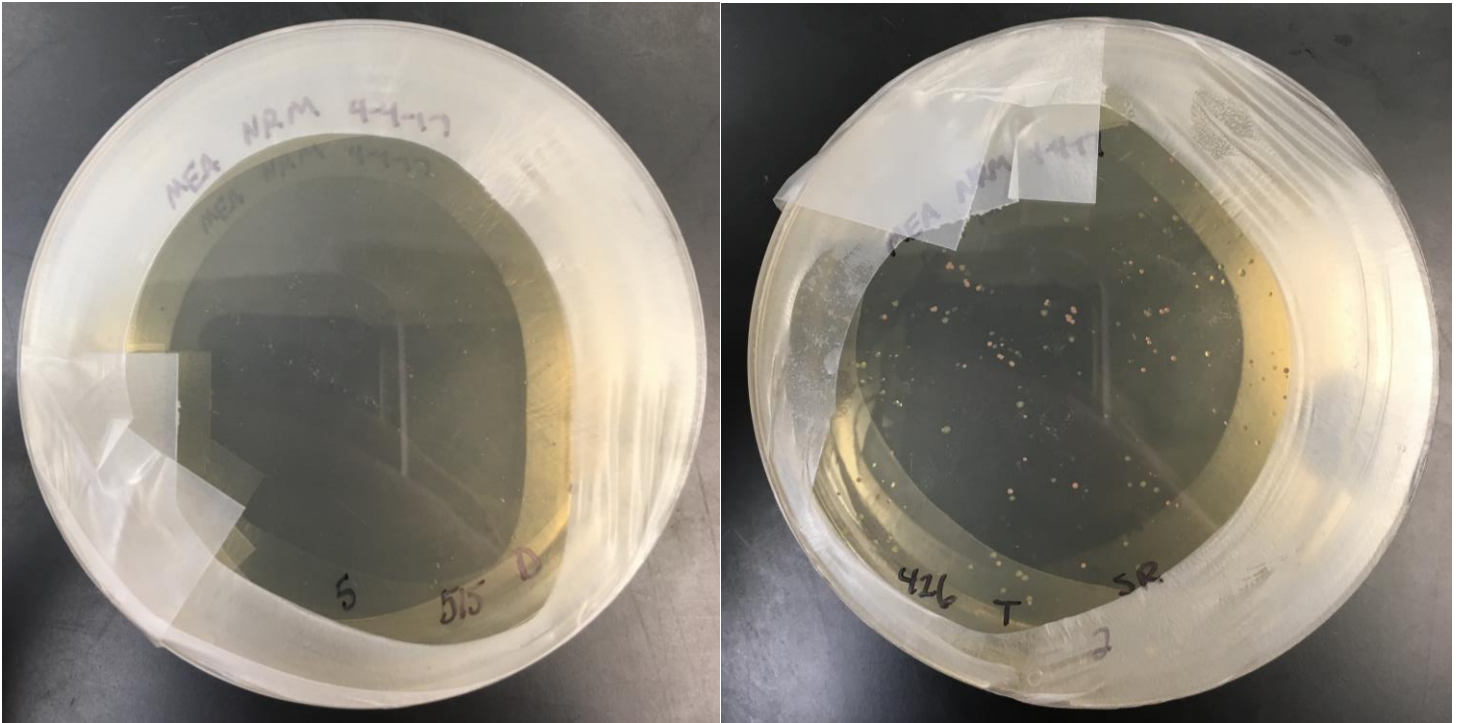


Figure 9 and 10: Plates of 515 distilled water and 426 side room tap water on MEA.

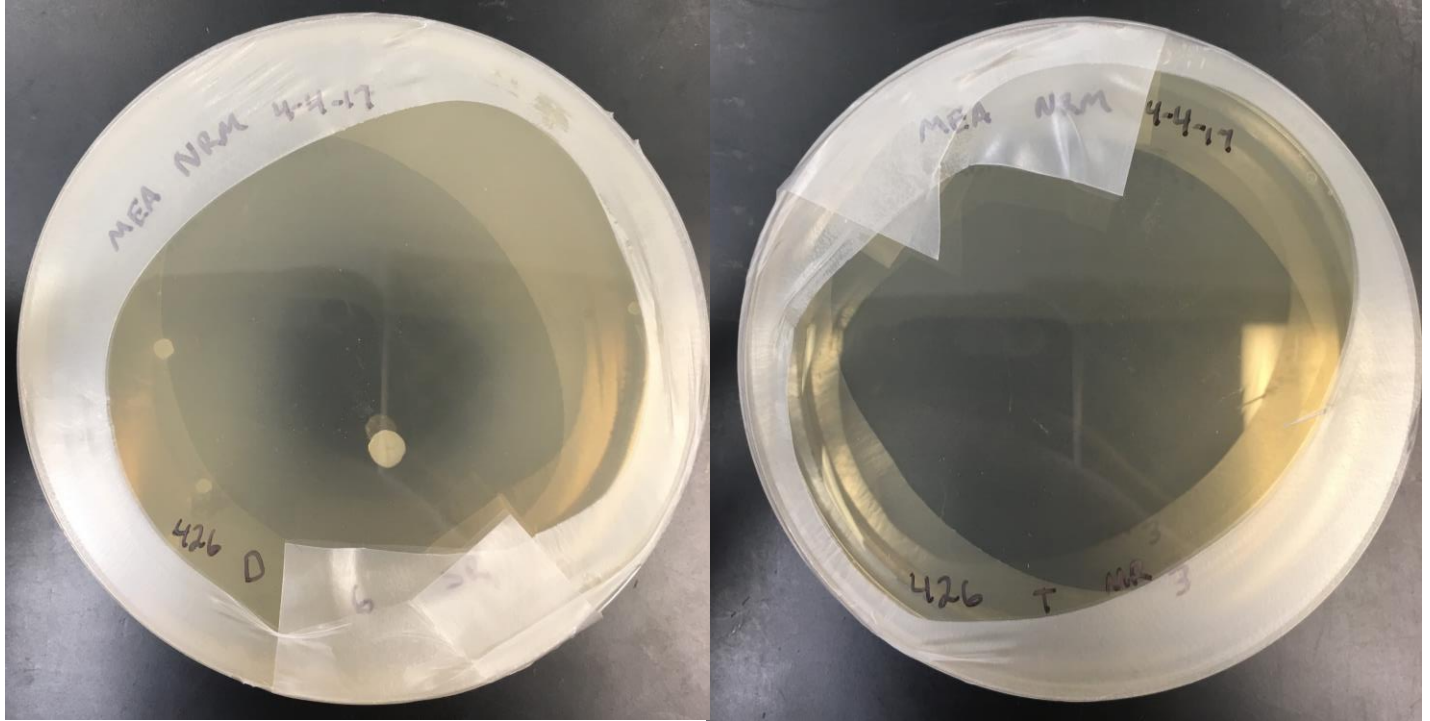


Figure 11 and 12: Plates of 426 side room distilled water and 426 main room tap water on MEA.

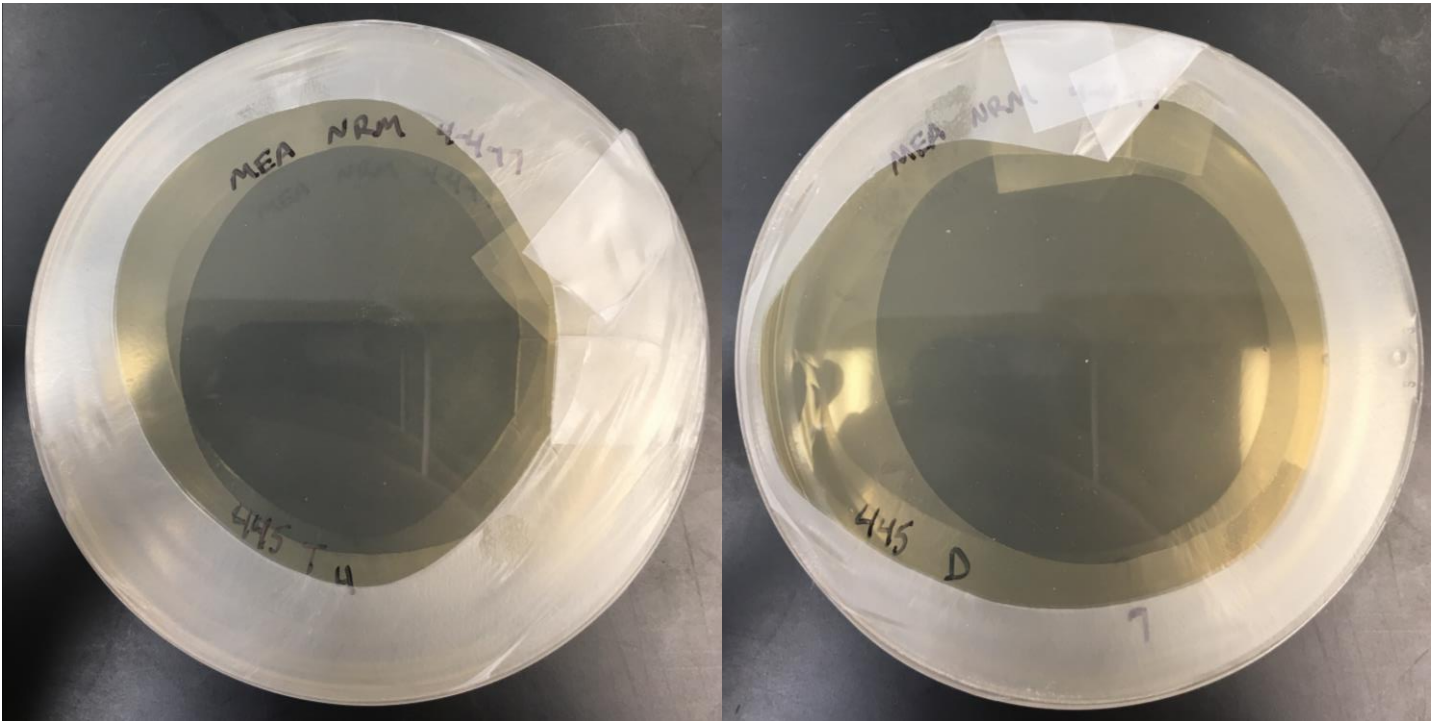


Figure 13 and 14: Plates of 445 tap water and 445 distilled water on MEA.

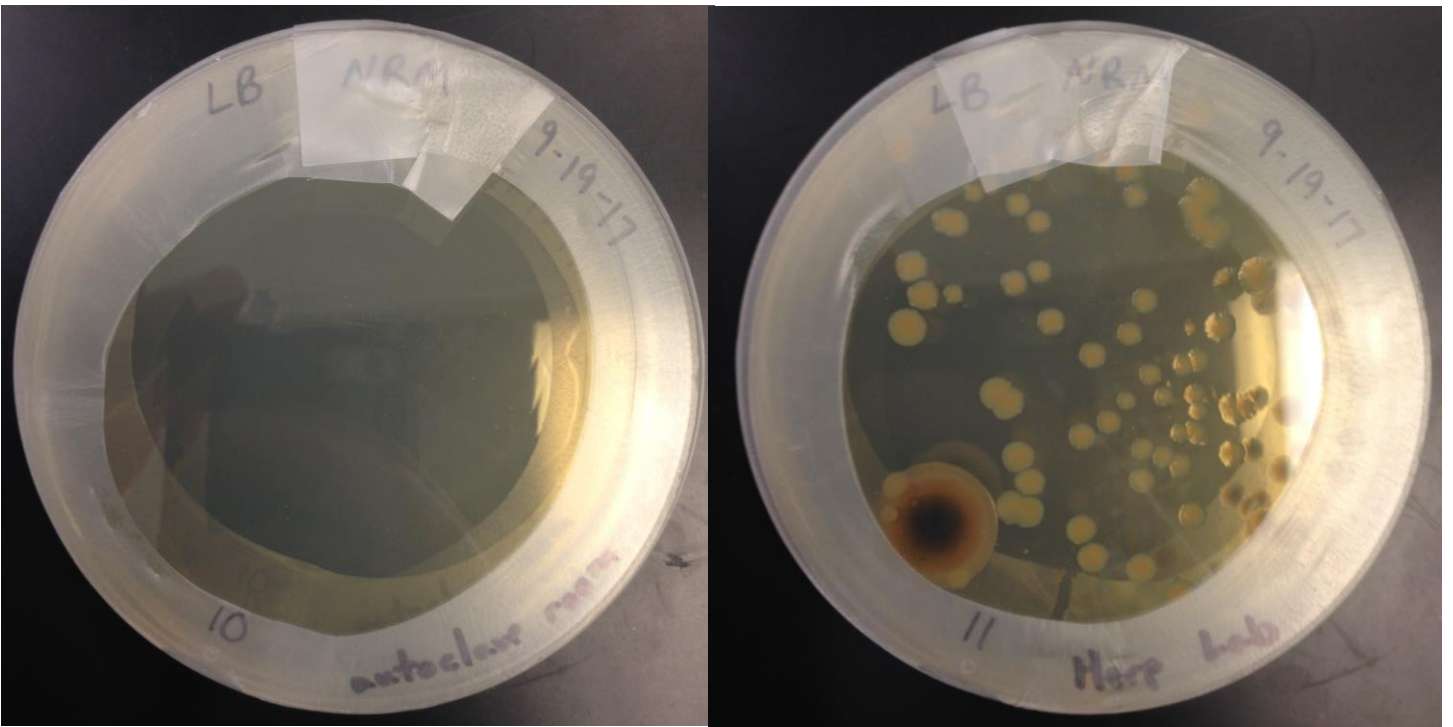


Figure 15 and 16: Plates of autoclave room distilled water and herpetology lab distilled water on LB.

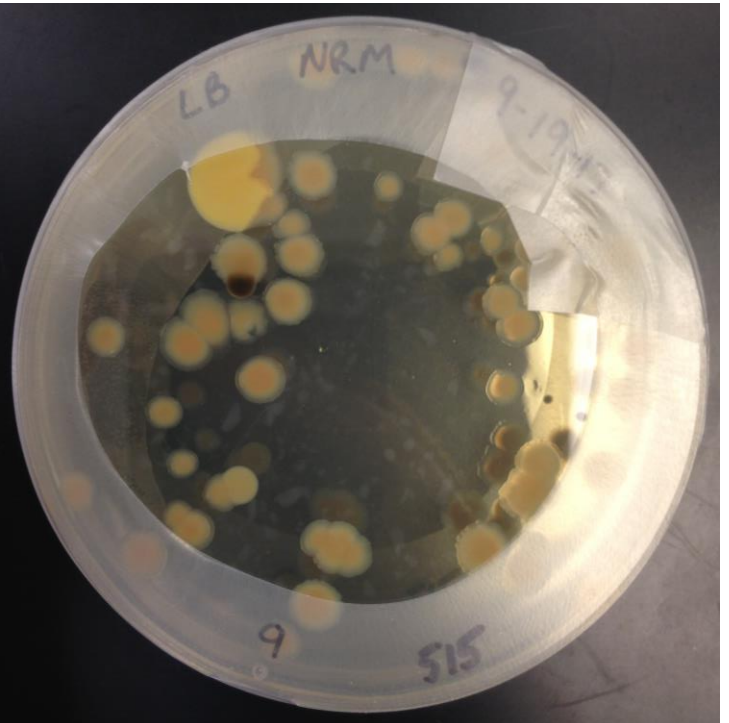
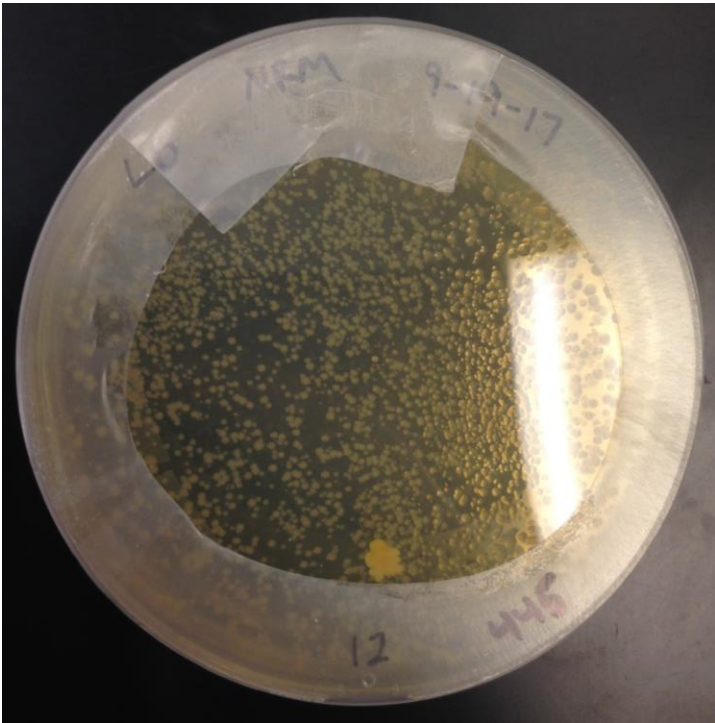


Figure 17 and 18: Plates of 445 distilled water and 515 distilled water on LB.

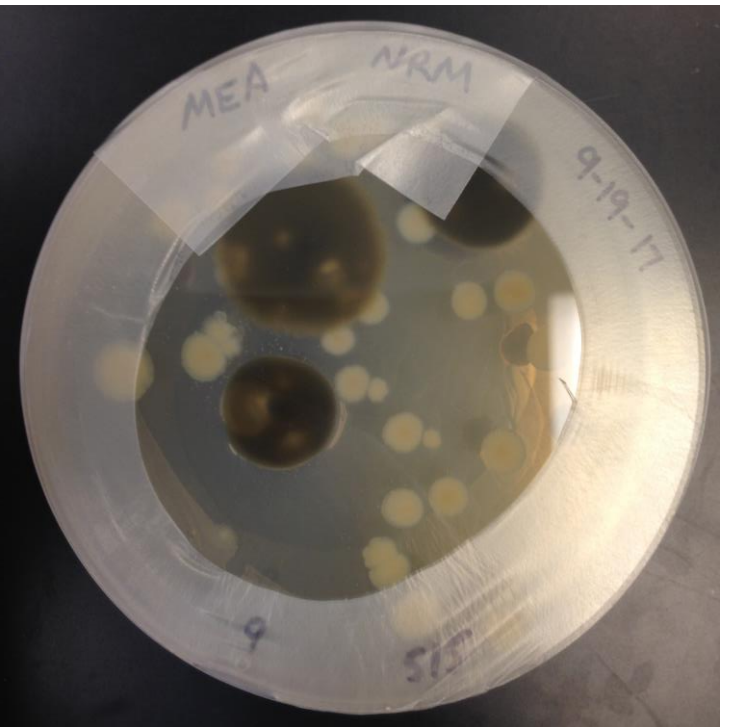
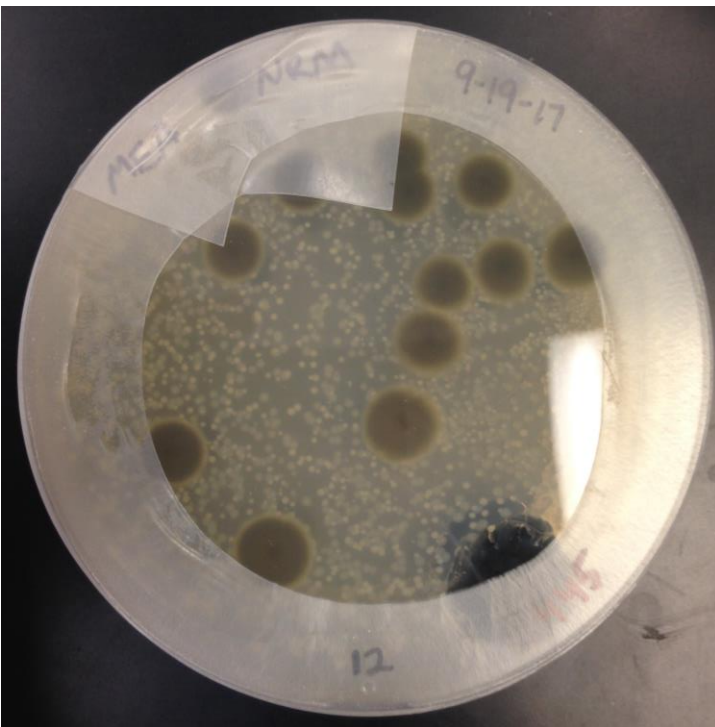


Figure 19 and 20: Plates of 445 distilled water and 515 distilled water on MEA.

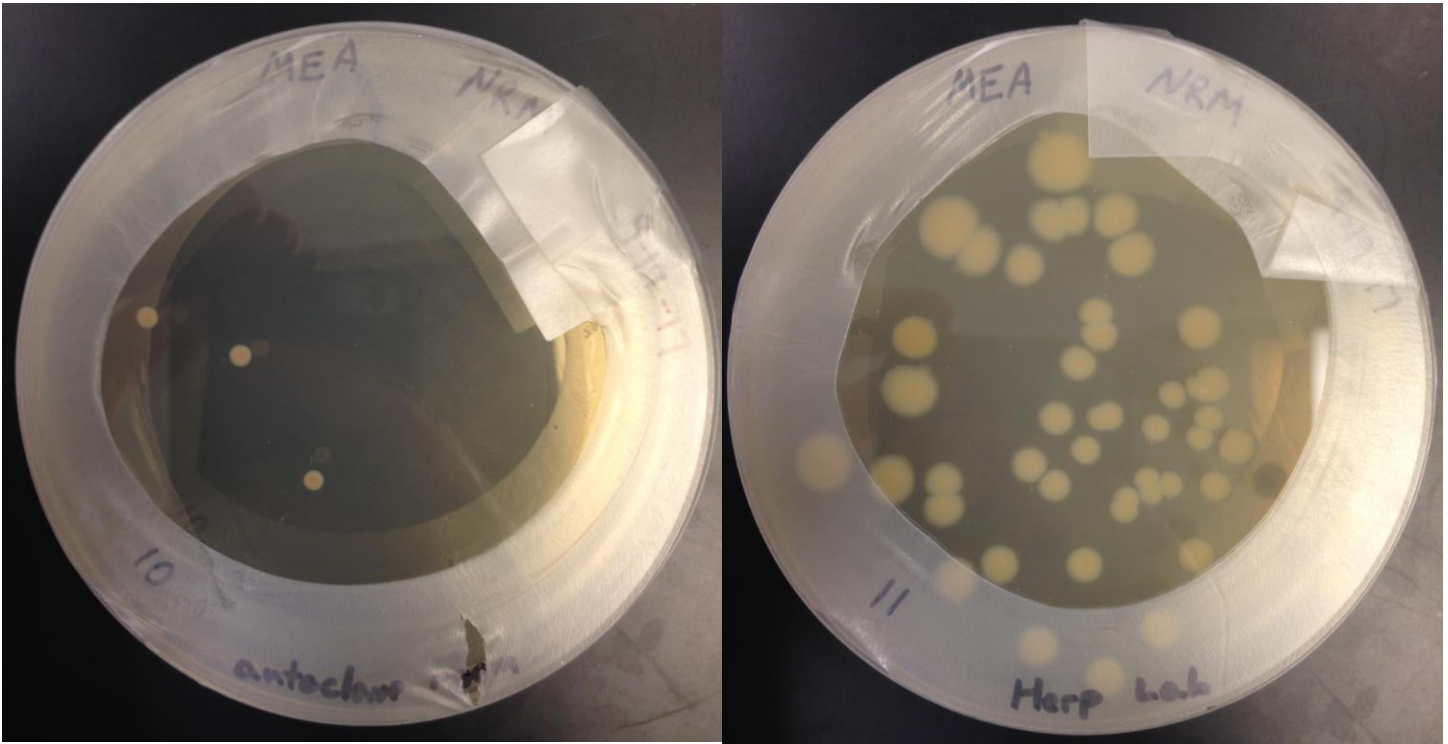


Figure 21 and 22: Plates of autoclave room distilled water and herpetology lab distilled water on MEA.

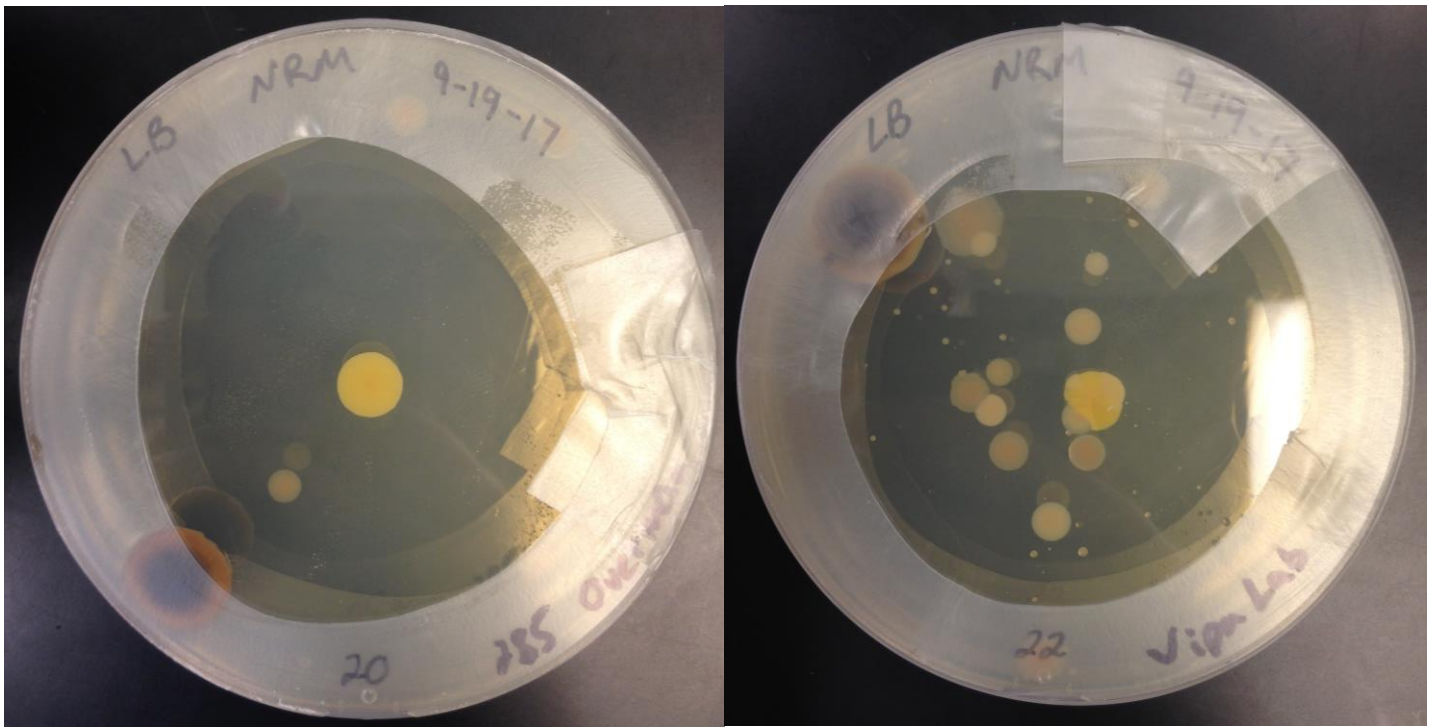


Figure 23 and 24: Plates of 285 Overman distilled water and 129 distilled water on LB.

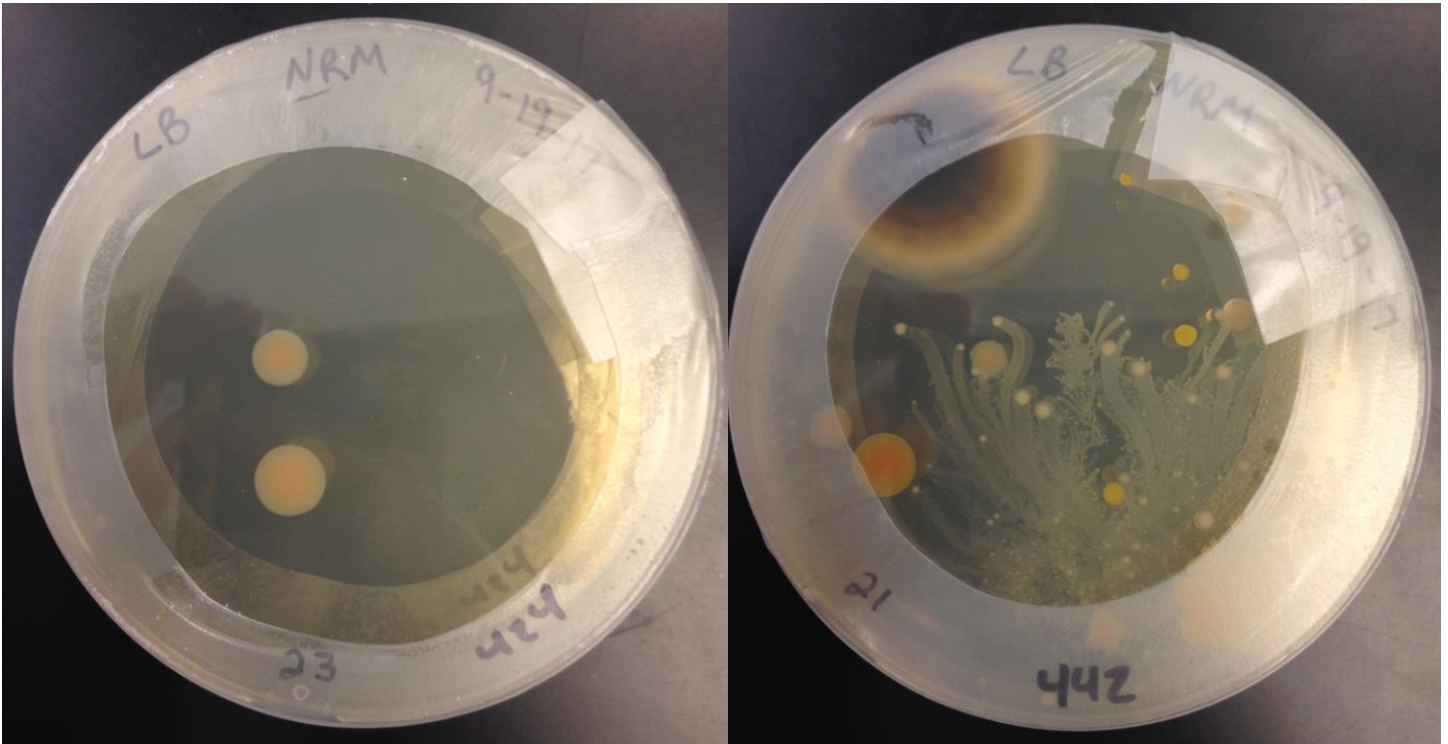


Figure 25 and 26: Plates of 424 distilled water and 442 distilled water on LB.

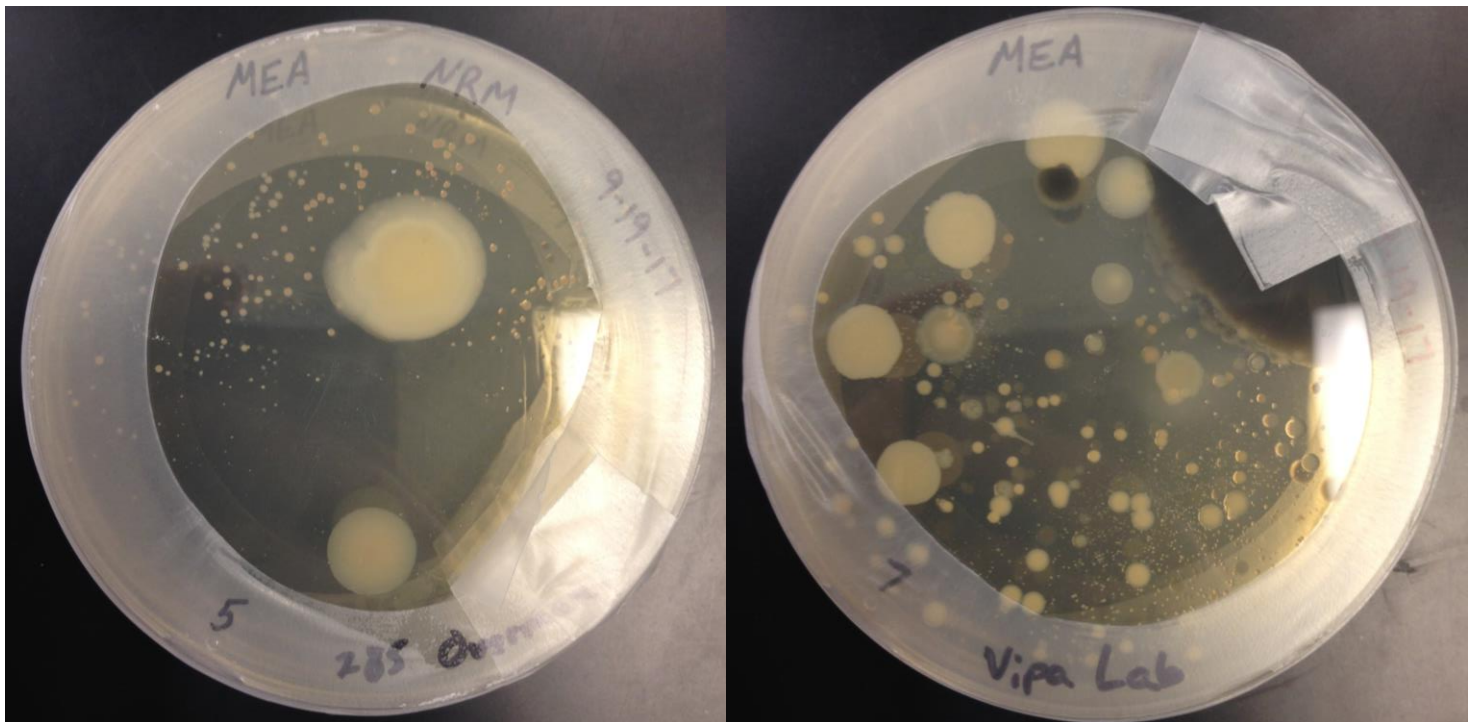


Figure 27 and 28: Plates of 285 Overman distilled water and 129 distilled water on MEA.

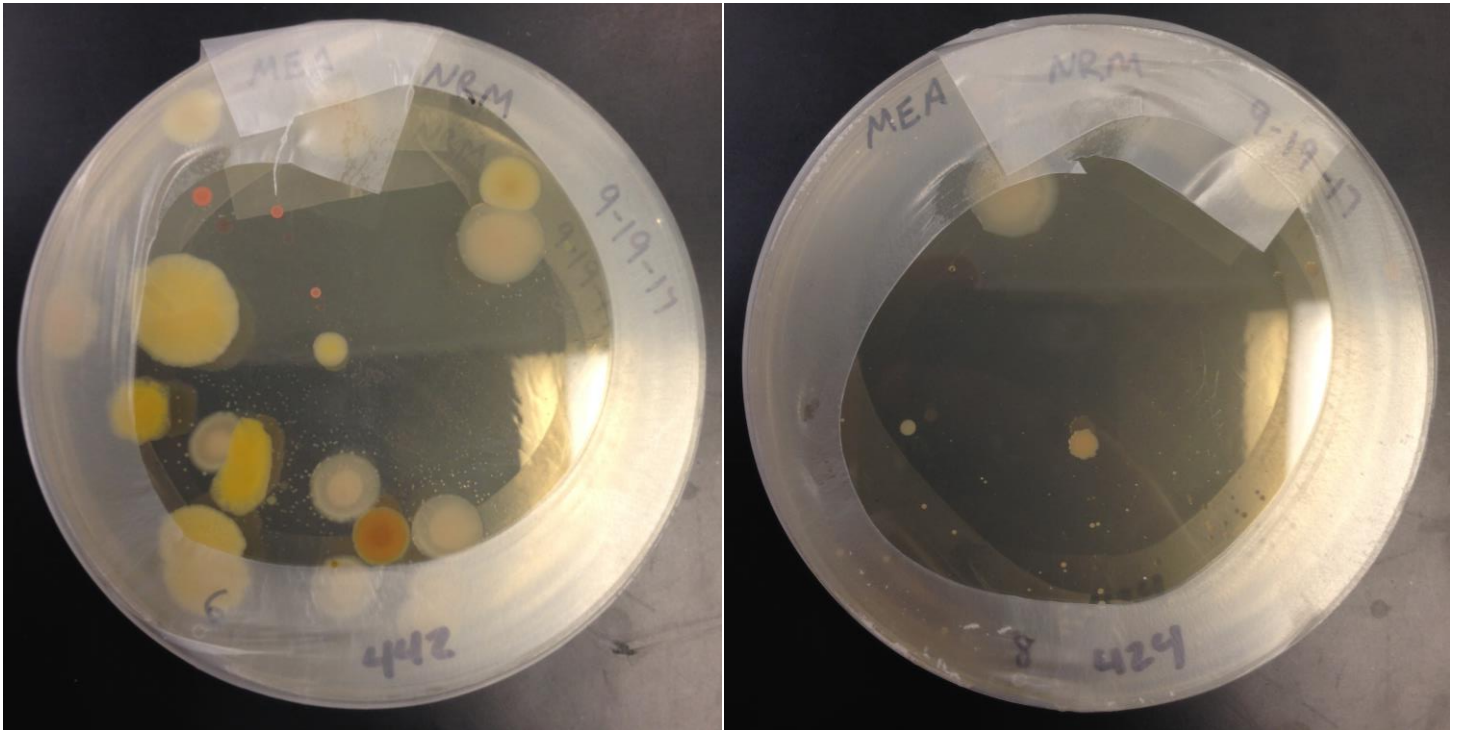


Figure 29 and 30: Plates of 442 distilled water and 424 distilled water on MEA.

Discussion

Almost all samples that were plated had some sort of growth on them. This shows that there is definitely growth inside of the water system in the Life Science Building. The plates also show only approximately 1/3 of what is actually growing, so the problem is much worse than shown. Even after the company claimed they cleaned out the system there were still higher counts from samples.

It's probable that the water is stagnant in the pipes, allowing for any microbes to grow undisturbed. A solution to this would be to keep the water flowing so that microbes cannot grow.

The water system also isn't checked as often as it used to be, so no one would know if there was an issue for a longer period of time. A simple solution to this would be to check on the system more often and change the filters more.

After many discussions with the company running the water system and faculty, a possible solution has been agreed upon. Once results from these experiments were shown to others higher up in the university and they learned of how much the contamination was costing the department, they demanded a change. A direct water line has been dropped into Dr. Rogers' lab from the water system on the roof and has so far produced acceptable quality. If this solution works then other labs will have the same done.

It is still recommended that even after these changes are made that the water be tested regularly to be sure that quality is maintained so the same problems are not repeated.

References

1. Siqueira V.M., Oliveira H.M., Santos C., Paterson R.R.M., Gusmão B.N., Lima N. Filamentous fungi in drinking water, particularly in relation to biofilm formation. *Int. J. Environ. Res. Public Health*. 2011;8:456–469. doi: 10.3390/ijerph8020456.
2. Lemon, Z. (2017, February 21). City of Waterville switches water sources today. Retrieved September 18, 2017, from <http://www.toledoblade.com/local/2017/02/21/City-of-Waterville-switched-water-sources-starting-to-draw-water-from-Bowling-Green-after-shutting-off-connection-to-city-of-Toledo.html>