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BACTERIOLOGICAL EXAMINATION OF WATER

Abstract

This research project aimed to assess the microbiological quality of water sources through a comprehensive water testing process. Our original hypothesis was the presence of fecal coliform bacteria in water samples would indicate contamination and pose a risk to human health. We used standard methods for water testing, including filtration and chemical treatment techniques, to detect and enumerate fecal coliform bacteria in water samples from various sources. Our results showed that all the water sources, including Brita Filter and Charles River Water, were contaminated with fecal coliform bacteria. We conclude that regular water testing is critical for ensuring safe and healthy water supplies for human consumption. Our findings highlight the need for continued monitoring and management of water quality to prevent contamination and protect public health. The methods used in this study could be useful in future studies evaluating the quality of water sources in the regions.

Introduction

Water is a fundamental resource for all living organisms, and ensuring its quality is essential for public health and environmental sustainability. However, maintaining water quality is a significant challenge, especially in areas with limited assess to clean water. Poor water quality can result in the spread of waterborne diseases, which can be fatal. Therefore, investigating the microbial composition of water sources is an essential step in ensuring that it is safe for consumption and other purposes. For example, many individuals in rural areas rely on well water as their primary source of potable water. However, studies indicate that over 50% of private water supplies in the United States have never been tested, despite the vital role they play in providing clean water for human consumption. Furthermore, over half of the tested water sources do not meet drinking water standards. Even municipal sources, which are typically thought of as reliable, can be poorly maintained or neglected, as evidenced by the water crisis in Flint, Michigan[1].

In this water lab, we aim to compare the effectiveness of different water treatment methods in removing coliforms and other contaminants from a water sample. We will primarily focus on coliforms as potential microbial pathogen contamination, despite the fact that there are several types of contaminants (such as pesticides, heavy metals, toxins, etc.) that may also pose a risk to human health. To be specific, coliforms are a type of bacteria that are non-endospore forming, Gram-negative, facultatively anaerobic bacilli, capable of fermenting lactose to produce acid and gas within 24 hours at 37°C[2]. Since coliforms are commonly found in the intestine of warm-blooded animals, their detection in water samples suggests the presence of fecal matter from animals or humans. Measuring the levels of coliforms in water samples can help determine the extent of fecal contamination and, therefore, the risk of waterborne diseases. As such, coliforms provide a valuable indication of the overall quality of water sources and the potential health risks associated with their consumption or use.

In the Presumptive Test, we tested how effectively two filtration methods (a Brita filter and a backwoods filter made from sand) and one chemical treatment method (Aquamira droplets) remove coliforms from a water sample taken from the Charles River[3]. The Charles River is a feature of the Greater Boston Area and is one of the major sources of recreation for the surrounding Boston communities[4]. The test involved inoculating a number of lactose fermentation broths with the water sample and observing the growth of coliform bacteria, which indicated the presence of fecal contamination. By comparing the results of the test for different water treatment methods, we can identify the most effective method for removing coliforms and other contaminants from water. Since many non-coliform bacteria could ferment lactose, we needed to confirm that any lactose fermenters we obtained were actually coliforms. Our finding indicated that all the treatment methods were effective at removing coliforms, but the backwoods filter showed the most significant reduction in coliform bacteria. The results highlight the importance of properly treating water to remove harmful bacteria and the potential of simple filtration methods to improve water quality. Overall, the study emphasizes the significance of regular water testing and treatment to ensure the safety of water sources for human consumption and other purposes.

Methods

Sample Collection:

To conduct the Presumptive Test, we collected samples of single-strength lactose broth (SSLB), double-strength lactose broth (DSLB), 10-mL pipette, 1-mL pipette, Sawyer Filter, Aquamira droplets, Brita Filter, and unfiltered Charles River water. For the Confirmed Test, we used a Levine EMB Agar plate, an Endo Agar plate, Bunsen Burner, Striker, and Inoculation Loop. Lastly, we prepared a NA plate, a Lactose Broth, and an incubated EMB/Endo plate to perform the Completed Test.

Presumptive Test:

This experiment was conducted to determine the presence of coliforms in water samples. To perform this test, a series of lactose fermentation broths were inoculated with different volumes of our water samples and then incubated at 37°C for 24 hours[5]. During this time, coliforms that were present in the test tubes fermented the lactose in the broth, producing gas. The number of positive fermentation results that produced gas could be compared with a table, which will provide an estimate of the concentration of coliforms in the original water samples.

Confirmed Test:

We selected a lactose broth from the Presumptive Test that displayed positive results for both acid and gas production, indicated by a yellow color change and a bubble in the Durham tube. We then performed an isolation streak of the broth on both an EMB and Endo plate, following established protocols[6]. After incubating the plates for 24 hours at 37°C, we identified Gram-negative, lactose-fermenting bacteria by examining the colonies on the plates. Blue or iridescent colonies on the EMB plate and dark pink iridescent colonies on the Endo plate were indicative of such bacteria.

Completed Test:

In the Confirmed Test, a positive result indicated the presence of Gram-negative lactose fermenters, but this did not confirm that the bacteria were coliforms. Therefore, we must perform additional tests to determine if the positive colonies belonged to the rod-shaped, non-spore forming organism and whether they can produce gas from lactose fermentation[7]. To carry out these tests, we inoculated a lactose broth with a positive Confirmed Test colony to determine gas production from lactose fermentation. Additionally, we prepared an isolation streak plate for Gram and spore staining from the same colony. These tests would help confirm the presence the coliforms and their ability to produce gas from lactose fermentation.

Data Analysis:

In the data analysis, we first combined the number of positive results obtained from each dilution in the Presumptive Test. We then used a table to determine the most likely concentration of coliforms in our original water samples based on the total number of positive results obtained. Additionally, we calculated a 95% confidence interval for the concentration of coliforms in our samples. This interval helps us estimate the range of values within which the true concentration of coliforms in our samples is likely to fall.

Results

In the Presumptive Test, the result for Aquamira Chlorine Drops was positive, indicating a combination with an MPN Index of 33 per 100 mL. For the Sawyer Filter test, our MPN Index was equal to 110 per 100 mL with errors of 10 for low and 100 for high. Besides, the MPN Index for Brita Filter was approximately 920. Its errors were 2600 for high and 220 for low. For our last water source, Charles River Water, the MPN Index was 21 per 100 mL with a high error of 42 and a low error of 6.8. Positive results were determined by the presence of gas in the Durham Tube which contained broths. In the summary for our Presumptive Test, we calculated the positive errors for the MPN Index of the four water sources which were 67, 140, 1680, and 21 respectively. In addition, the negative errors for our water sources were correspondingly 23, 76, 700, and 13.2. The attached bar chart below **(Image 1)** shows the MPN of coliforms (+/- 95% confidence interval bard) for each sample tested.

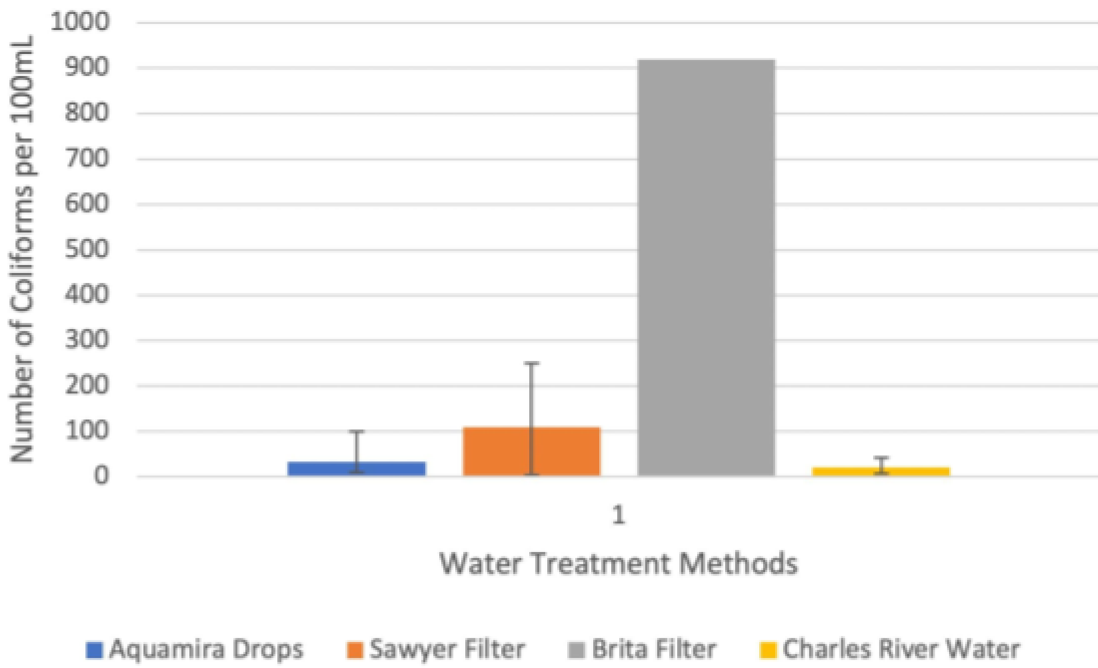

Image 1

In our next experiment with the Confirmed Test, we observed that there was growth in Levine EMB and Endo plates, both plates formed colonies **(Image 2)**. These colonies indicated the presence of Gram-negative, lactose-fermenting bacteria, consistent with coliforms, in the initial water samples.

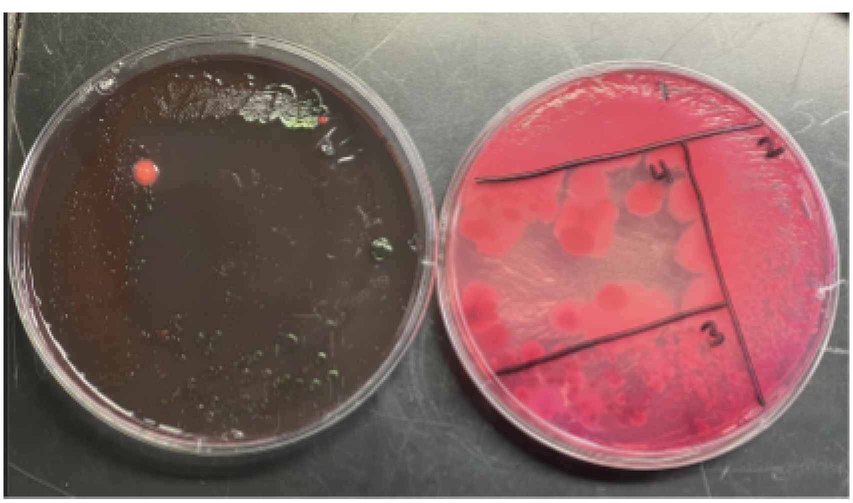

Image 2

Following the Confirmed Test, in the Completed Test, our experiment indicated that for Aquamira Drops and Charles River Water, their gram and spore stains were both positive, indicating the presence of coliform bacteria. However, the lactose result for Aquamira was negative, indicating that the bacteria present did not match the expected characteristics of coliform bacteria. Besides that, Charles River Water matched coliform bacteria because of its positive lactose test. In the experiment of Brita Filter and Sayer Filter, we found negative results for both gram and spore stains, which meant our test did not match coliform. Based on the positive lactose tests for both water sources, we could confirm that our results matched coliform bacteria.

The pH values for four water sources were also recorded during the experiment. The pH for Aquamira Chlorine Drops, Brita Filter, and Charles River Water were all recorded as 6, while for Sawyer Filer, it was recorded as 6.5.

Discussion

The research aimed to assess the microbiological quality of water sources through a comprehensive water testing process, primarily focusing on the detection of coliforms as potential microbial pathogen contamination. The study was also to compare the effectiveness of different water treatment methods in removing coliforms and other contaminants from water samples taken from various sources. The results indicated that the tested water sources, including Brita Filter and Charles River Water, were contaminated with fecal coliform bacteria, while Sawyer Filter and Aquamira Chlorine Drops were not. Brita Filter and Charles River Water contained coliforms because they met all criteria for coliform traits: Gram-negative, facultatively anaerobic, rod-shaped, non-spore forming, and lactose fermented.

In the Presumptive Test, the presence of a gas bubble in the Durham Tube indicated that the microorganisms in the broth were fermenting lactose, which is a key characteristic of coliforms. However, this alone did not confirm that the microorganisms were indeed coliforms; additional tests were necessary. Therefore, we conducted the Confirmed Test, which revealed that the lactose fermenters had high pH values, indicating that they were relatively more acidic. The EMB and Endo Agar plates were differential and selective, and preferred Gram-negative bacteria. They contained lactose and pH-sensitive dye that produced distinct colors for growing colonies that fermented lactose to produce acid. The lactose fermenters appeared blue on the EMB agar plate and pink on the Endo agar plate, indicating that they were more acidic due to the fact that acidic byproducts of their fermentation would cause them to absorb the dye from the media. In the subsequent Completed Test, we determined that all our sources contained coliforms, but these coliforms did not have the same characteristics.

According to the Washington State Department of Heath article on "Coliform Bacteria in Drinking Water," the presence of coliform bacteria in drinking water does not necessarily cause illness[8]. However, their presence serves as an indicator that potentially harmful pathogens could be present in the water system. These pathogens have the potential to cause a range of illnesses, from mild gastrointestinal discomfort to severe illnesses such as typhoid fever and hepatitis A. Therefore, it is essential to monitor the levels of coliform bacteria in drinking water to ensure that it remains safe for consumption.

However, there are several limitations to water testing that should be considered. First, the accuracy of water testing can be affected by the location and time of sample collection. Water quality can vary depending on the source, location, and time of day, so it's important to collect representative samples to obtain accurate results. Second, water testing can only detect the presence of known contaminants and pathogens. There may be other unknown or emerging contaminants that are not yet included in standard water tests. Finally, water testing does not guarantee that water is safe to drink. Even if test results are within acceptable levels, there is still a risk of exposure to harmful contaminants and pathogens, especially in cases of unexpected events such as natural disasters or accidents.

Future research on water testing could focus on improving the speed and accuracy of detecting pathogens in water. One recommendation would be to develop more sensitive and specific tests for a wider range of pathogens, including emerging contaminants and antibiotic-resistant bacteria. Additionally, the research could focus on developing more efficient and cost-effective methods for treating contaminated water sources, such as using advanced oxidation processes or other innovative water treatment technologies. Another area of future research could be to explore the effectiveness of combining various water treatment methods to achieve better water quality. Furthermore, there is a need for continued monitoring of water quality and implementing public education campaigns to encourage individuals and communities to take action to prevent contamination of water sources.

References

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