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Re-purposing an Invasive Species: The Use of the Xylem Tissue of *Pinus taeda* as a Point-of-Use Filter of Waterborne Pathogens

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Abstract 3.4 million people, mostly children under the age of 5, die every year from waterborne diseases. The most common waterborne diseases are caused by bacteria such as *Escherichia coli*, *Salmonella typhi*, and *Vibrio cholerae*. Common technologies to filter out or kill waterborne bacteria are costly in terms of money, resources, and time, which limit their implementation in developing countries. A potential filter of waterborne bacteria exists in the form of plant xylem, the porous material that conducts fluid in plants. The xylem tissue of gymnosperms has evolved to have pores that are an ideal size for filtering out waterborne pathogens. Gymnosperms, namely *Pinus taeda*, are invasive in several developing countries and have resulted in a loss of biodiversity and an overall negative effect on agriculture. This raises the interesting question of whether or not the invasive *P. taeda* can be repurposed to be used as a point-of-use filter of waterborne pathogens. It was predicted before the study began that the difference between the bacterial rejection rate of filters derived from the xylem tissue of *Pinus taeda* and those of costly methods of filtration such as boiling and membrane-based filtration would not be statistically significant. This study found that the *P. taeda* filter rejected 95% and 96% of inactivated *Escherichia coli*. The difference between the performance of the *P. taeda* filter and common methods of filtration was not statistically significant. This study concludes that filters derived from *P. taeda* can solve current global problems.

Keywords: *water sustainability, waterborne diseases, xylem, point-of-use filtration, waterborne pathogens*

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1. Introduction

The scarcity of clean and safe drinking water is a leading cause of mortality in developing countries [1]. 3.4 million people, mostly children under the age of 5, die every year from waterborne diseases [7]. Bacteria such as *Escherichia coli*, *Salmonella typhi*, and *Vibrio cholerae* cause the most common waterborne diseases [1]. Common technologies to filter out or kill waterborne bacteria include chlorination, UV-disinfection, boiling, and membrane based filtration (Madaeni, 1999). Though these treatments are effective, they are costly in terms of money, resources, and time, which limit their implementation in developing countries [2]. Controlling water quality at the point-of-use is often most effective due to the issues of microbial regrowth, byproducts of disinfection, and contamination in the distribution system [5]. Another sustainable way of providing water to future generation in developing countries is harvesting and filtering rainwater due its abundance and cost-effectiveness [3]. A low-cost, point-of-use filtration method that can filter rainwater is needed to combat the prevalence of waterborne diseases in developing countries.

A potential solution exists in the form of plant xylem, the porous material that conducts fluid in plants [2]. Xylem tissue of gymnosperms has evolved to have pores with sizes ranging from 5 to 500 nanometers (nm) to prevent the formation of air bubbles, also known as cavitation [6]. These pores are an ideal size for filtering out waterborne pathogens [2].

The xylem tissue of *Pinus strobus* effectively filtered out *Escherichia coli* from water [2]. *Pinus strobus* is a species of pine that solely inhabits eastern North America. To be used as a filter in developing countries, *Pinus strobus* would need to be dried to limit the degradation of its xylem tissue when exported. Water flows faster by a factor of two orders of magnitude through freshly cut *Pinus strobus* than through dried *Pinus strobus [2]*. This occurrence is likely due to pit membranes becoming clogged when the xylem is dried [2]. The flow rate of water in dried *Pinus strobus* xylem is 0.4 liters per day (L/d), while the average person needs 2 liters of water per day [2]. Therefore, there are many practical limitations in regard to the implementation of filters derived from the xylem tissue of *Pinus strobus* in developing countries.

Several species from the *Pinus* genus are invasive in South America, Africa, and Asia. Invasion by members of the *Pinus* family has resulted in a loss of biodiversity, a reduced amount of water in the ecosystem, and an overall

negative effect on agriculture and concomitantly on the local economy [4]. Notable examples of invasive members of the*Pinus* genus that have caused widespread damage in developing countries are *Pinus taeda* and *Pinus Pinaster* [8]. A filter of waterborne bacteria derived from the xylem tissue of *Pinus taeda*, closely related to *Pinus strobus*, would not need to be dried due to its dispersion and invasion of developing countries in South America, Africa, and Asia. This raises the interesting question of whether or not the invasive *Pinus taeda* can be repurposed to be used as a point-of-use filter of waterborne pathogens or a filter of rainwater.

This study, using inactivated *Escherichia coli*, investigates the xylem tissue of *Pinus taeda* as a point-of-use filter of waterborne bacteria. This investigation predicts that the difference between the bacterial rejection rateof filters derived from the xylem tissue of *Pinus taeda* and those of costly methods of filtration such as boiling and membrane-based filtration will not be statistically significant.

2. Materials & Methods

The materials used in this project were: *Pinus taeda* branches, ⅜ inch PVC tube, 5-minute epoxy, simulated rainwater, L-spreaders, sterile water, *Escherichia coli* stock culture, LB agar plates, parafilm, incubator, and regeneration broth.

In the study, the independent variable was the method of filtration. The dependent variable was the number of bacterial colonies on the nutrient agar after filtration and the percentage rejection of *E.coli*. Both boiling and membrane-based filtration are the positive controls in this experiment as they reject 99.9% of bacteria. Constants in this study include the type of agar, the type of petri dish, the amount of agar, the incubation period, and the incubation temperature.

The xylem filter was constructed by removing 10 sections from a 2 foot-long *Pinus taeda* branch. The sections had the approximate length of 1 centimeter. The bark of the sections was then excised. To complete the construction of the xylem filters, six sections were placed into two ⅜ inch PVC tubes and sealed with epoxy. The *Pinus taeda* branch was provided by Georgian Court University.

To test the efficacy of the xylem filter, the boiling method, and the membrane-based filter, 14 LB (Lysogeny Broth) agar plates were prepared and autoclaved.30 uL of the *Escherichia coli* stock culture was transferred to a sterile tube containing 2 mL of regeneration broth. The bacterial culture was incubated for 24 hours at 37º C (98.6º F).1 uL of the incubated culture was transferred to 11 sterile tubes each containing 20 mL of sterile water.

50 uL of the solution in three tubes, prepared in the previous step, was transferred to three petri dishes labeled “Stock Plate”. The solution was spread using an L-spreader onto the LB agar on the aforementioned petri dishes. The plates were parafilmed, inverted, and then incubated at 37°C for 24 hours.

Three tubes, out of the fourteen that were prepared,were subjected to a hot water bath at 100°C for ten minutes. 50 uL of the solution inthe three tubes was transferred to three petri dishes labeled “Boiling Method”. The solution was spread using an L-spreader onto the LB agar on the aforementioned petri dishes. The plates were parafilmed, inverted, and then incubated at 37°C for 24 hours.

The contents of three tubes, containing E.coli and sterile water, were poured into a membrane-based filter. 50 uL of the filtered water were transferred to three petri dishes labeled “Membrane-Based Filter”. The solution was spread using an L-spreader onto the LB agar on the aforementioned petri dishes. The plates were parafilmed, inverted, and then incubated at 37°C (98.6ºF) for 24 hours.

The contents of three tubes were poured into the prepared xylem filter. 50 uL of the filtered water was transferred to six petri dishes labeled “Xylem Filter”. The solution was spread using an L-spreader on to the LB agar on the aforementioned petri dishes. The plates were parafilmed, inverted, and then incubated at 37°C (98.6º F) for 24 hours.

A similar method was used to test the rainwater collector. Simulated rainwater was made to exceed the regulations set forth by the WHO.

The number of colonies on each plate was counted using a program called “HG Colony Counter” and then recorded. Qualitative data was recorded. Pictures of the plates were taken.

The number of bacterial colonies on the plates after the filtration and incubation period indicates the efficacy of the filtration systems. The filtration systems with the highest efficacy will yield little bacterial colonies on their plates. The filtration systems with the lowest efficacy will yield a “lawn” of bacterial colonies on their plates. Therefore, the mean amount of bacterial colonies of each filtration system along with the standard deviation of those means will be found. The mean amount of bacterial colonies on the “Xylem filtration method” plates will be compared with the mean amount of bacterial colonies on the plates of the positive control “Boiling Method” and the plates of “Common Filtration Method” and the plates of the negative control “No Filtration” using t-tests. T-tests will be used to determine the significance of their differences. Percentage differences will be found between the bacterial colonies on the “stock plate” and the bacterial colonies on the “Xylem filtration method” plates, “Boiling Method”, and “Common Filtration Method” plates to determine the percentage of bacterial rejection.

3. Results

Table 1 displays the average number of colonies on the nutrient agar after the filtration and incubation period along with the standard deviation from the mean. Table 1 also displays the filters’ percentage rejection rates of *E.coli*, which were calculated by finding the percentage difference between the negative control (no filtration) and the experimental groups (xylem filter, membrane based filter, and the boiling method). The percentage rejection rate of bacteria indicates the overall efficacy of the tested filter. The average number of colonies on the LB Agar plates after no filtration and an incubation period was 299 with a standard deviation of 91.9 colonies. The mean value of *E.coli* colonies on the LB Agar plates after exposed to the positive controls (the boiling method and the membrane based filtration) and subjected to an incubation period was 0 with a standard deviation of 0

colonies. Since 0 colonies were found on the aforementioned LB Agar plates, the positive controls exhibited a 100% rejection rate of *E.coli.* The mean value of *E.coli* colonies on the LB Agar plates after xylem point-of-usefiltration and an incubation period was 14.3 with a standard deviation of 24.9 colonies. The xylem filter was calculated to be 95.2% effective at rejecting *E.coli*.The mean value of *E.coli* colonies on the LB Agar plates after xylem rainwater filtration and an incubation period was 12.0 with a standard deviation of 26.8 colonies. The xylem filter was calculated to be 96.2% effective at rejecting *E.coli*.

Table 1. Comparison of *E.coli* growth on agar plates after filtration

Filtration Method	Average Number of Colonies on the plates after Filtration and Incubation(± SD)	Average Percentage Rejection of *E.coli*(± SD)
Xylem Point-of-Use Filter	14.3±24.9	95.2 ± 8.3
Xylem Rainwater Filter	12.0 ±26.8	96.3 ± 8.2
Membrane Based Filter	0±0	100 ± 0
Boiling	0±0	100 ± 0
No Filtration	299±91.9	N/A

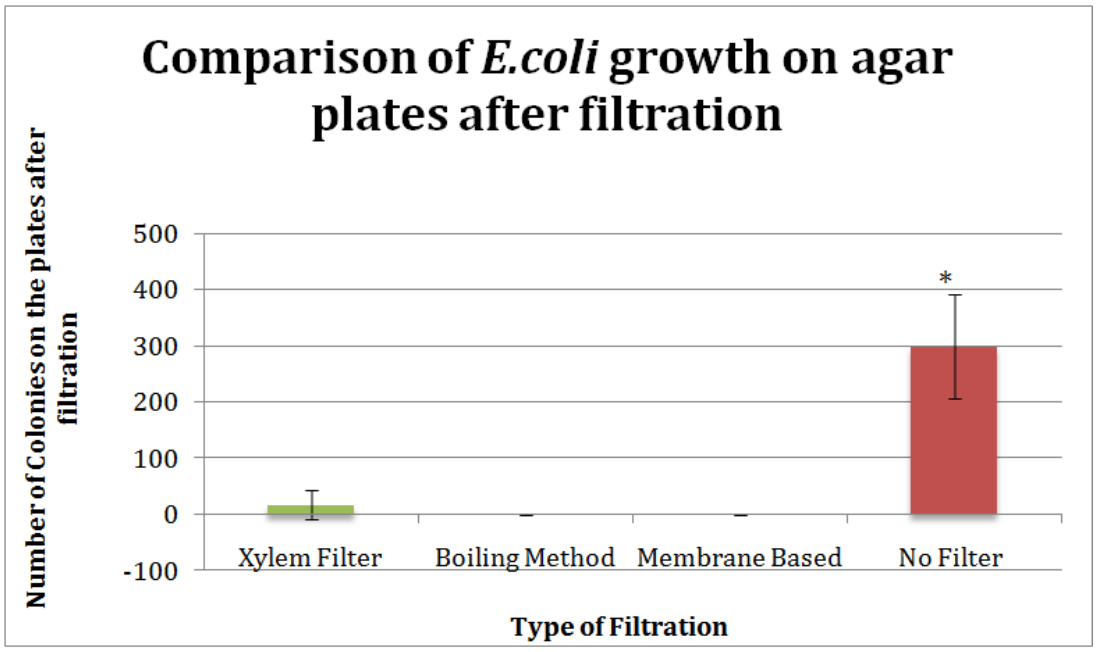

Figure 1. Comparison of E.coli growth on agar plates after filtration

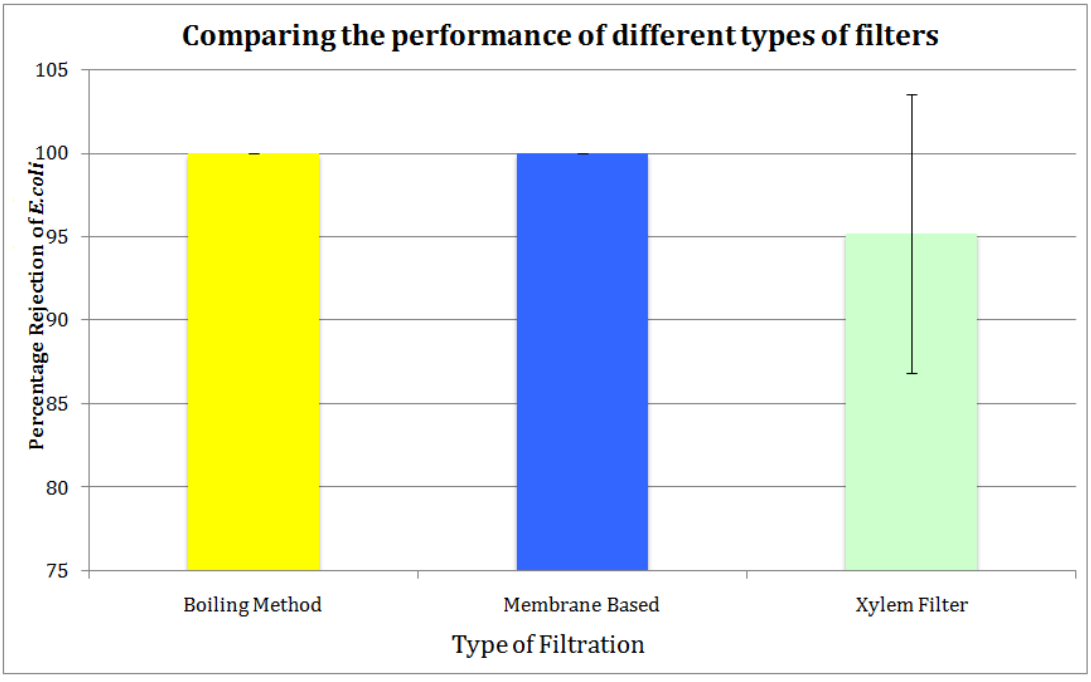

Figure 2. Comparison of the efficacy of different types of filtration

Figure 1 compares the average number of colonies on the nutrient agar after the filtration and incubation period. It also displays the standard deviation, in the form of error bars, from the mean. An asterisk indicates a significant difference between the indicated test group and the xylem filter. Whereas, a p-value less than or equal to 0.05 denotes a significant difference. T-tests were conducted between the xylem filter and the other three test groups to determine if there was a significant difference in terms of bacterial rejection. A two-tailed, type 3 t-test determined that there was no significant difference between the number of colonies on the LB Agar plates after xylem filtration and the number of colonies on the LB Agar plates after exposure to the two positive controls. As indicated by the asterisk, a t-test determined that there was a significant difference between the number of colonies on the LB Agar plates after xylem filtration and the number of colonies on the LB Agar plates after no filtration.

Figure 2 compares the average percentage rejection of *E.coli* after the filtration and incubation period. It also displays the standard deviation, in the form of error bars, from the mean. The percentage rejection of the filters was calculated by finding the percentage difference between the negative control (no filtration) and the experimental groups (listed in the figure above). An asterisk indicates a significant difference between the indicated test group and the xylem filter. Whereas, a p-value less than or equal to 0.05 denotes a significant difference. T-tests were conducted between the xylem filter and the other two test groups to determine if there was a significant difference in terms of bacterial rejection. A two-tailed, type 3 t-test determined that there was no significant difference between the xylem filter's percentage rejection rate of *E.coli* and the positive controls' percentage rejection rate of *E.coli*.

Figure 3 displays the results after the filtration and incubation period. A: Boiling Method; B: Membrane-Based Filtration; C: Xylem Filter; D: No Filtration. As shown in picture A, no bacterial colonies were present on nutrient agar plates after being subjected to the boiling method. This indicates that the boiling method was effective at rejecting *E.coli*. As shown in picture B, no bacterial colonies were present on nutrient agar plates after being subjected to membrane-based filtration. This indicates that membrane-based filtration was effective at rejecting *E.coli*. As shown in picture C, no bacterial colonies were present on nutrient agar plates after being subjected the xylem filter. This indicates that the xylem filter was effective at rejecting *E.coli*. 4 plates out of 6 xylem filter replicates had similar results. As shown in picture D, there were around 200-300 bacterial colonies present on the LB Agar plates after no filtration.

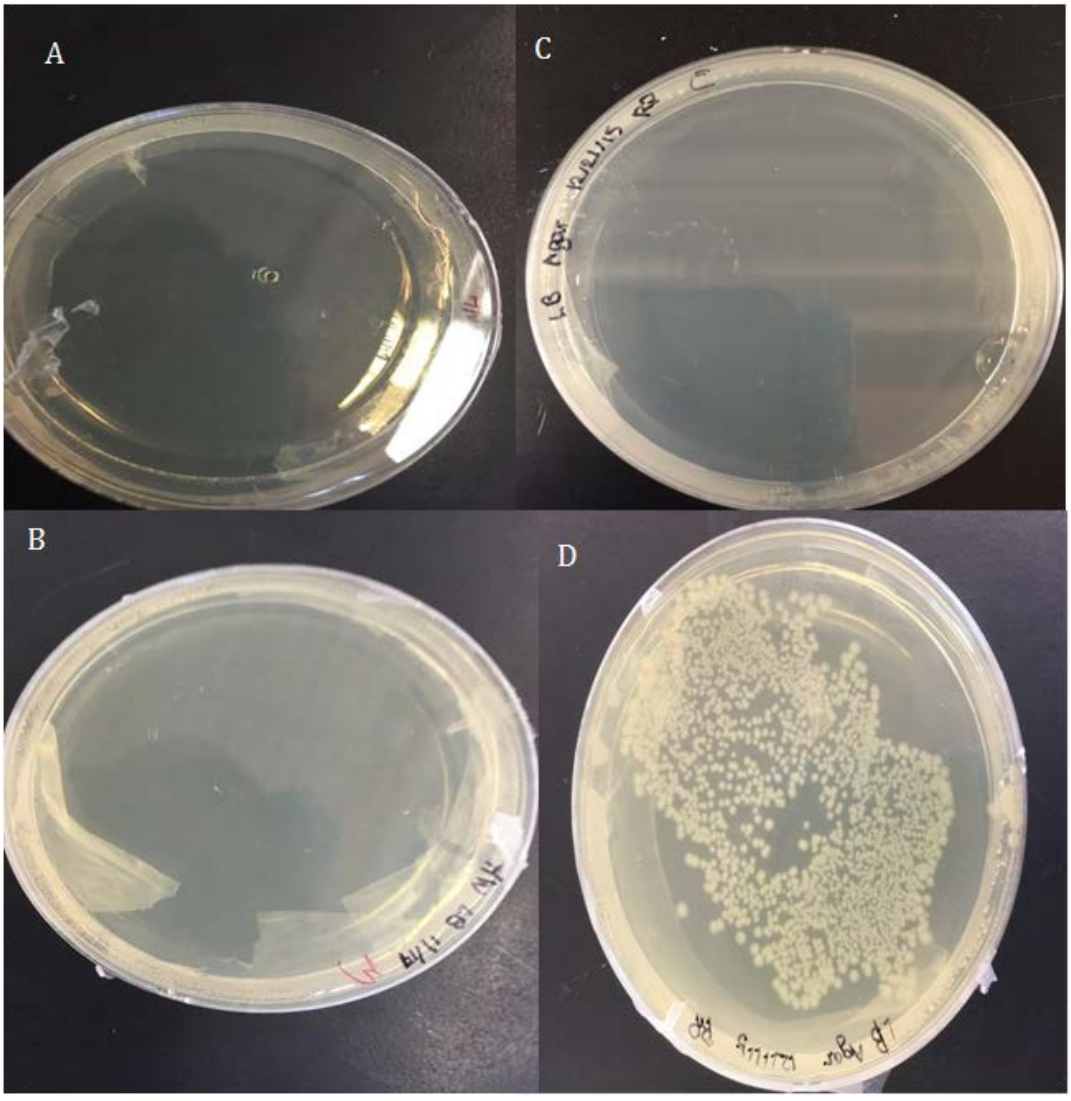

Figure 3. Qualitative Photo-Documentation of Results

4. Conclusion

This study investigated the use of the invasive tree species *Pinus taeda*as a point-of-use filter of waterborne bacteria. It was found through experimentation that the difference between the bacterial rejection rate of filters derived from the xylem tissue of *Pinus taeda* and expensive methods of filtration such as boiling and membrane-based filtration was not statistically significant. Thus, this investigation accepts its hypothesis due to the results of the conducted t-tests.

Although the performance of the xylem filter was determined to not have a significant difference between the performances of the positive controls, it should be made clear that a filter to be widely used should exhibit a bacterial rejection rate exceeding 99.9%. The xylem filter rejected on average 95.2% of *E.coli*. As shown in Figure 2, the xylem filter did on occasion reject 100% of *E.coli*. Thus, more experimentation is needed to definitively determine the bacterial rejection rate of xylem filters derived from *Pinus taeda*.

A t-test determined that the difference between the performance between the xylem filter and the performance of the negative control (no filtration) was statistically significant in terms of rejection of bacteria. As many villages in developing countries have little to no means of filtering waterborne bacteria, the result of the aforementioned t-test supports the notion that xylem filters derived from *Pinus taeda* could assist villages in preventing deaths related to waterborne diseases. Many other technologies can exhibit similar rates of bacterial rejection; however, xylem filters derived from *Pinus taeda*are biodegradable, cheap, and able to be used at the point-of-use and filter rainwater. The xylem filters derived from *Pinus taeda*could assist in solving two problems: the prevalence of waterborne disease-related deaths and the prevalence of invasive pine species in developing countries.

A further study includes definitively determining the bacterial rejection rate of xylem filters derived from *Pinus taeda*. Another further study would test the ability of xylem filters derived from *Pinus taeda*to be used multiple times. A final further study would test if nutritional polysaccharides located in the xylem tissue of *Pinus taeda* flow into the filtered water. The aforementioned study if successful would show that not only does *Pinus taeda* remove waterborne bacteria but it also adds nutritional value to the filtered water.

In summation, this study provides a hopeful alternative to expensive methods of filtration. It hopes to prevent a few of the 3.4 million deaths caused by waterborne diseases, annually. It also hopes to repurpose an invasive species that has damaged local agriculture and economy in several developing countries.

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