The Phytoremediation of *Escherichia coli* in Contaminated Water by *Lemna minor*, *Salvinia minima*, and *Azolla caroliniana*

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Pathogenic strains of *Escherichia coli* can cause potentially fatal diseases like hemorrhagic colitis. Phytoremediation is the process in which plants remove contaminants like *E. coli* from the environment. *Lemna minor*, *Salvinia minima*, and *Azolla caroliniana* are three aquatic plant species that have been tested in previous phytoremediation research, but their abilities to expunge *E. coli* from water have not been directly compared. The purpose of this study was to test and compare the abilities of *L. minor*, *S. minima*, and *A. caroliniana* to reduce *E. coli* concentration in contaminated water. It was hypothesized that aquatic plants would decrease the concentration of *E. coli* in water due to the antimicrobial flavonoids they produce, and *L. minor* would kill more bacteria than *S. minima* and *A. caroliniana* because of its fast growth rate and extensive roots. Plants were placed in fertilizer solution, and *E. coli* was added to each sample. Initial and final concentrations (CFU/mL) of *E. coli* in the samples were determined after a serial dilution. *L. minor*, *S. minima*, and *A. caroliniana* resulted in 97.890%, 90.292%, and 99.063% decreases in *E. coli* concentration, respectively. A Kruskal-Wallis test found that results were statistically significant, $H(3) = 51.413$, $p < .001$, and Dunn's pairwise tests found significant differences between *L. minor* vs. control, *S. minima* vs. control, and *A. caroliniana* vs. control. The results of the present study suggest that *L. minor*, *S. minima*, and *A. caroliniana* are equally effective at remediating *E. coli*-contaminated water.

Introduction

Escherichia coli is a Gram-negative type of coliform bacteria, and its pathogenic strains can cause a variety of human diseases that kill more than 2 million people annually (Jang et al., 2017). For example, enterohemorrhagic *E. coli* can cause diseases like hemorrhagic colitis that lead to acute renal failure and death (Jang et al., 2017). These diseases, as well as *E. coli*'s increasing resistance to antibiotics (Russo & Johnson, 2003), indicate a need for a method of reducing *E. coli* contamination that does not involve the use of antibiotics.

Phytoremediation, the use of plants to reduce contamination in the environment, could potentially serve as this method. Aquatic plants produce compounds called flavonoids with potent antimicrobial properties (Hossain et al., 2019), making them likely candidates for serving as a natural method of reducing environmental *E. coli* contamination. These flavonoids can be harnessed to kill bacteria, including *E. coli*, in contaminated water. *Lemna minor*, *Salvinia minima*, and *Azolla caroliniana* are three species of aquatic plants that have been studied for their ability to expunge contaminants, including heavy metals like cadmium, from water (Ceschin et al., 2020; Iha & Bianchini, 2015; Pandey, 2012). *Lemna minor*, which is commonly referred to as duckweed, is an aquatic vascular plant that has been used to remediate organic pollutants, heavy metals, and phenols in wastewater (Ceschin et al., 2020). *Salvinia minima*, or water spangles, is a floating fern that has been shown to have the capability to treat high-strength organic wastewater (Olguín et al., 2007). *Azolla caroliniana*, known colloquially as fairy moss, is a floating plant typically found in ponds that has been studied for its ability to remediate heavy metals in water (Pandey, 2012). These three species of aquatic plants exhibit the potential to serve as treatments for *E. coli*-contaminated water.

E. coli is the most common indicator bacteria used in wastewater quality studies, and its presence in water indicates fecal contamination by a warm-blooded animal, making it a useful tool for studying water treatment methods like phytoremediation (Alexandros & Akratos, 2016; "Bacteria and *E. coli* in Water," n.d.). According to the United States Food & Drug Administration (2019), *E. coli* is carried by wildlife, wildstock, and humans. *E. coli* contamination spreads when feces containing the bacteria come into contact with food or water (United States Food & Drug Administration [USFDA], 2019). Pathogenic contamination in water is a significant health concern worldwide, as ingesting contaminated water or food grown using contaminated water can cause a variety of potentially fatal infectious diseases, including diarrhoeal diseases (Gutierrez-Gines et al., 2021).

E. coli in water can be quantified by spreading the contaminated water, which is typically diluted using a serial dilution, over agar plates and allowing the *E. coli* colonies to grow (Han, 2021). The *E. coli* colonies on the plates can then be counted manually, and *E. coli* concentration can be calculated using the formula for Colony-Forming-Units per milliliter (CFU/mL) (Pini & Geddes, 2020).

Existing literature about phytoremediation heavily focuses on the remediation of heavy metals and toxic chemicals from water and soil. Little is known about the role of plants in the remediation of *E. coli*, especially in contaminated water. Although *L. minor* has been tested for its ability to remove fecal bacteria from waste material (Papadopoulos et al., 2011), the use of *S. minima* and *A. caroliniana* to phytoremediate fecal contaminants has not yet been studied. The present study aims to directly compare the abilities of *L. minor*, *S. minima*, and *A. caroliniana* to expunge *E. coli* from contaminated water in order to determine which species is the most effective at remediating waterborne pathogens.

Literature Review

Conventional Water Treatment Methods

Currently, wastewater is usually treated using a combination of physical, chemical, and biological treatment methods (Pini & Geddes, 2020). However, the use of these methods comes with several drawbacks. Advanced oxidation processes like ozonation consume a large amount of energy and have high maintenance needs, making them relatively expensive (Alexandros & Akratos, 2016). A more affordable and commonly used method of wastewater treatment is chlorination, but adding chlorine to water with organic matter can form carcinogenic byproducts like haloacetic acids that kill aquatic wildlife (Alexandros & Akratos, 2016; Pini & Geddes, 2020). Other byproducts produced as a result of conventional treatment methods include sludge byproducts, which are formed when using microorganisms to break down contaminants (Pini & Geddes, 2020). Sludge byproducts, which contain pathogens, must be disinfected to ensure that they do not contaminate natural waterways (Pini & Geddes, 2020). The disadvantages of existing treatment methods emphasize the need for an affordable, eco-friendly form of treating pathogen-contaminated water.

Phytoremediation Mechanisms

In phytoremediation, plants are utilized to remove contaminants from the environment. According to the United States Environmental

Protection Agency (2012), there are several processes by which plants can reduce environmental contamination, including the storage of contaminants within plant roots, stems, or leaves, the conversion of contaminants to less harmful chemicals, and the conversion of contaminants to vapors that are released into the air. An article by Gutierrez-Gines et al. (2021) mentioned that the roots of *M. robusta* and swamp *L. scoparium* produce exudates that may explain the plants' ability to remediate bacteria in soil. The authors of the article also stated that *P. colorata*, *Kunzea ericoides*, and *L. scoparium* produce secondary metabolites like leptospermone and various mono- and sesquiterpenes that may explain the plants' antimicrobial activity. Similarly, Pooja et al. (2020) stated that aquatic plants produce secondary metabolites that can be antibacterial and inhibit pathogenic growth. They claimed that seaweeds can produce bioactive compounds like terpenes that potentially have antimicrobial properties (Pooja et al., 2020). These studies suggest that *L. minor*, *S. minima*, and *A. caroliniana* have the ability to remediate *E. coli*-contaminated water due to the ability of aquatic plants to produce compounds with antibacterial properties.

Remediation of Pathogens in Soil and Water by Plants

An experiment conducted by Gutierrez-Gines et al. (2021) found that leaf extracts from *Metrosideros robusta* and *Pseudowintera colorata* had a bactericidal effect against *Staphylococcus aureus* and *Burkholderia cepacia*. Additionally, the study found that the use of swamp *Leptospermum scoparium* and *M. robusta* in irrigated pots resulted in a 90% reduction in *E. coli* after 14 days (Gutierrez-Gines et al., 2021). These results demonstrate that both the extracts of medicinal plants and the plants themselves can be used to treat *E. coli* contamination.

The use of aquatic plants to expunge pathogens from water has also been researched, but most studies focus on the use of *Eichhornia crassipes* (water hyacinth) and *Pistia stratiotes* (water lettuce), two species of invasive plants that can cause severe environmental damage if left to grow unsupervised (United States Department of Agriculture [USDA], n.d.). In a study conducted by Hossain et al. (2019), it was found that ethyl acetate extracts of *E. crassipes* inhibited the growth of *Salmonella typhi* and *S. aureus*. According to the study, previous research has also shown that extracts of *P. stratiotes* exhibited antibacterial activity on "a few pathogenic bacteria" (Hossain et al., 2019, p. 10).

These studies and perspectives reveal a gap present in current scientific literature, as existing research regarding the phytoremediation of *E. coli* has either tested the use of plants to treat contaminated soil or studied species of aquatic plants other than the ones tested in the present study. Although *L. minor* has been studied for its ability to remove fecal bacteria from septage (Papadopoulos et al., 2011), neither *S. minima* nor *A. caroliniana* have been tested for their potentials to remediate pathogen-contaminated water. Furthermore, there have been no studies directly comparing the capabilities of *L. minor*, *S. minima*, and *A. caroliniana* to remove *E. coli* concentration from contaminated water.

The purpose of this research was to test and compare the abilities of *L. minor*, *S. minima*, and *A. caroliniana* to reduce the concentration of *E. coli* in contaminated water. To do this, the research question "How do various aquatic plants affect the concentration of *E. coli* in contaminated water?" was asked. It was hypothesized that adding aquatic plants to *E. coli*-contaminated water would result in the concentration of *E. coli* in the water decreasing because of the flavonoids produced by the plants that can kill microbes. It was further hypothesized that *L. minor* would kill more bacteria than *S. minima* and *A. caroliniana* because of its fast growth rate and its ability to eliminate contaminants like organic pollutants and heavy metals from water. Samples of each aquatic plant species were placed into plastic deli cups containing distilled water and water-soluble fertilizer. *E. coli* broth was then added to each cup, and the initial concentration of *E. coli* in the cups was quantified by plating the contaminated water on Petri dishes containing Lennox LB agar and counting the number of colonies in each dish to calculate CFU/mL. The cups were left at room temperature for a week, and the *E. coli* remaining in the water of each cup was quantified.

Methods

Over the course of the experiment, all lab surfaces were disinfected using ethyl alcohol before and after use. All pieces of lab equipment were either autoclaved, heated using a Bunsen burner, or sterilized with ethyl alcohol. Additionally, all procedures involving *E. coli* were carried out in a fume hood. To prevent contamination before use, all agar plates were sealed with Parafilm and stored upside down in the fridge. This storage method reduced the likelihood of condensation from the lids of the Petri dishes dripping onto the surface of the agar. The methods used in this experiment were based on methods from phytoremediation studies by Barchanska et al. (2019), Singh & Balomajumder (2021), and Sudiarto et al. (2019), as well as mycoremediation studies by Pini & Geddes (2020) and Han (2021).

250 mL of potato dextrose broth were made using potatoes, dextrose, and distilled water. One potato was peeled and cut into cubes measuring ~1x1 cm. 50 g of potato cubes were measured using a scale and added to a beaker containing 350 mL of distilled water. Then, the potato cubes were boiled for 30 min until they became soft. The contents of the beaker were squeezed through a muslin cloth to draw out the potato extract from the boiled potatoes. Distilled water was added to the obtained potato extract until the total volume reached 250 mL, and 5 g of dextrose were stirred into the resulting solution. The potato dextrose broth was autoclaved for 30 min at 121°C and placed in the fridge to avoid contamination.

1 L of *E. coli* solution was created by inoculating distilled water with nutrient broth containing *E. coli* K-12. The strain K-12 was chosen because it is non-pathogenic and does not produce toxins ("Non-pathogenic *Escherichia coli* Strains," n.d.). 200 µL of *E. coli* nutrient broth and 50 mL of potato dextrose broth were added to a beaker containing 1 L of distilled water. Potato dextrose broth was included in the solution to serve as a nutrient source for *E. coli* (Pini & Geddes, 2020). The solution was mixed using a sterile stirring rod and incubated for 36 h at 37°C.

2 L of Lennox LB agar were prepared and autoclaved according to manufacturer instructions. Lennox LB agar was chosen because it contains the nutrients necessary for bacterial growth, including nitrogen and amino acids ("LB Agar, Lennox," 2011). The agar was then poured into 130 Petri dishes, which were left to harden for approximately 20 minutes at room temperature. The plates were then sealed with Parafilm, put back into their sterile plastic sleeves, and stored upside down at 4°C until use to avoid contamination from condensation.

16 L of fertilizer solution were made by adding 8 g of water-soluble fertilizer to 16 L of distilled water. One hundred twenty-five 5.5 oz plastic deli cups, which had been sterilized using an ethyl alcohol bath, were each filled with ~ 120 mL of fertilizer solution. Then, each species of aquatic plant was rinsed with distilled water, and 5 g portions of the plants were added to ninety-three of the deli cups. *A. caroliniana* was added to thirty-one cups, *S. minima* was added to thirty-one cups, and *L. minor* was added to thirty-one cups. Thirty-one of the cups were left without plants to serve as a positive control, and one additional cup was left without plants to determine initial *E. coli* concentration. This was modified after the methods from a study by Singh & Balomajumder (2021), which grew *E. crassipes* in 1 L pots filled with 5% Hoagland's nutrient solution. Plants were not placed in distilled water for an acclimatization period prior to being added to the cups due to plant die-off in a previous, unsuccessful attempt.

After the plants were added to all of the cups, each cup was labeled using a permanent marker and labeling tape to indicate the species of plant they contained. The cups within each plant species and the control group were then each assigned a number from 1 to 30, and the thirty-first cup of

each group was labeled "NB" to indicate that they would not receive any *E. coli* solution in order to serve as a negative control. The additional cup without plants was labeled "SD" to indicate that it would be used for a serial dilution. Afterward, the cups were left underneath a fluorescent grow light for 24 h.

After 24 h, a micropipette was used to add 1 mL of *E. coli* solution to each of the experimental cups, excluding the cups labeled "NB." A serial dilution was then performed on the solution inside the cup labeled "SD" at various dilution factors. 50 µL of each dilution were plated on agar plates containing Lennox LB agar. A sterile inoculation loop was used to spread the diluted solution over each agar plate, and the inoculated plates were sealed with Parafilm and left upside down for 72 h at room temperature to allow the *E. coli* colonies to grow.

Following the 72 h growth period, the *E. coli* colonies on the plates were observed to determine the optimal dilution factor for colony quantification. The colonies were then counted manually with a clicker and quantified using the formula for calculating CFU/mL:

$$\text{CFU/mL} = \frac{\#\ of\ colonies * dilution\ factor}{volume\ of\ sample\ taken\ (mL)}$$

To calculate CFU/mL, the number of colonies was multiplied by the dilution factor and divided by the sample's volume in mL.

The 124 experimental cups were left under a fluorescent growth light for 168 h. Subsequently, a 1:2000 dilution was performed on the solution in each cup, and 50 µL aliquots from each dilution were plated on agar plates containing Lennox LB agar using a sterile inoculation loop. The inoculated plates were sealed with Parafilm and left upside down for 72 h at room temperature. The *E. coli* colonies in each plate were then counted using a clicker and quantified using the formula that had been used to calculate the initial concentration of *E. coli*. After the data were collected and recorded, used micropipette tips were thrown away, and all living organisms were placed in 10% bleach solution for 24 h. SPSS was used to conduct a Kruskal-Wallis test and post-hoc Dunn's pairwise tests. Statistical tests were chosen based on final sample sizes being uneven and the assumptions of normality and equal variances not being fulfilled. The experimental design diagram is shown in Figure 1.

Figure 1. *Experimental Design Diagram*

Title of the Experiment
The Phytoremediation of *Escherichia coli* in Contaminated Water by *Lemna minor*, *Salvinia minima*, and *Azolla caroliniana*

Hypothesis
Adding aquatic plants to *E. coli*-contaminated water would result in the concentration of *E. coli* in the water decreasing because of the flavonoids produced by the plants that can kill microbes. Furthermore, *L. minor* would kill more bacteria than *S. minima* and *A. caroliniana* because of its fast growth rate and its ability to eliminate contaminants like organic pollutants and heavy metals from water.

Independent Variable Species of aquatic plant

Levels of Independent Variable	*L. minor* (5 g)	*S. minima* (5 g)	*A. caroliniana* (5 g)	Fertilizer solution with no added plants
Number of Repeated Trials	31	31	31	31

Dependent Variable
Concentration of *E. coli* in water (Colony-Forming Units (CFU)/mL)

Control Group
Fertilizer solution without aquatic plants

Constants
Temperature, pH level of water, amount of fertilizer solution, concentration of fertilizer solution, amount of light, mass of plants added to each trial, size of cups, size of Petri dishes, strain of *E. coli* used, type of agar used

Results

Table 1 includes the mean concentrations of *E. coli* in the contaminated water before and after treatment by aquatic plants. Additionally, the table shows the percent change in *E. coli* concentration from before treatment to after treatment.

After 168 h, there was a 97.880% decrease in *E. coli* concentration in the group treated with *L. minor*, a 90.292% decrease in *E. coli* concentration in the group treated with *S. minima*, a 99.063% decrease in *E. coli* concentration in the group treated with *A. caroliniana*, and a 25.118% decrease in *E. coli* concentration in the group not treated with any plants. Out of the 3 species of plants tested, *A. caroliniana* resulted in the greatest % decrease in *E. coli* concentration, and *S. minima* resulted in the lowest % decrease in *E. coli* concentration.

Table 2 shows the mean, range, and standard deviation of the *E. coli* concentrations following plant treatment. *A. caroliniana* resulted in the lowest final mean *E. coli* concentration, range, and standard deviation. Other than the control, *S. minima* resulted in the greatest final mean *E. coli* concentration, range, and standard deviation.

Figure 2 displays a candlestick chart comparing the *E. coli* concentrations of the trials following the plant treatments.

Table 1. *The Effect of Plant Treatment on the Concentration of* E. coli *K-12 in Water*

	L. minor	*S. minima*	*A. caroliniana*	Control (no plants)
Concentration of *E. coli* prior to plant treatment (CFU/mL)	4.920E+07	4.920E+07	4.920E+07	4.920E+07
Concentration of *E. coli* following plant treatment (CFU/mL)	1.038E+06	4.776E+06	4.611E+05	3.684E+07
% change in *E. coli* concentration	-97.890%	-90.292%	-99.063%	-25.118%

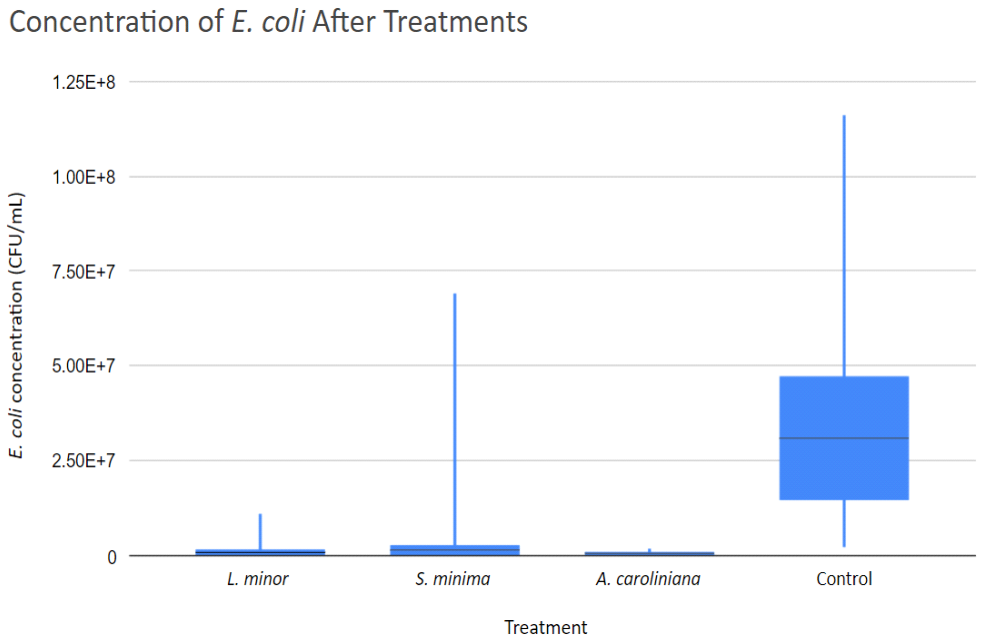
Note. This table shows the mean *E. coli* concentrations before and after 168 h of aquatic plant treatment along with percent change calculated by $\frac{mean\ final\ E.coli\ concentration\ -\ mean\ initial\ E.coli\ concentration}{mean\ initial\ E.coli\ concentration} * 100\%$

Table 2. *Mean, Range, and Standard Deviation of* E. coli *Concentrations (CFU/mL) in Water*

	L. minor	*S. minima*	*A. caroliniana*	Control (no plants)
M	1.038E+06	4.776E+06	4.611E+05	3.684E+07
Range	1.096E+07	6.904E+07	1.600E+06	1.140E+08
SD	2.246E+06	1.333E+07	4.012E+05	3.383E+07

Note. This table shows the mean, range, and standard deviation of *E. coli* concentrations after 168 h of aquatic plant treatment.

Figure 2. *Candlestick Chart of* E. coli *Concentration Following Treatments*



Note. This figure displays the candlestick chart of *E. coli* concentrations after the three plant treatments and the control treatment.

The candlestick chart shows the minimum, first quartile, median, third quartile, and maximum of the data in each group. As shown in the chart, *A. caroliniana* resulted in the lowest median, third quartile, and maximum *E. coli* concentrations, while *S. minima* resulted in the highest median, third quartile, and maximum *E. coli* concentrations out of the plant species tested. Additionally, in the chart, the *S. minima* plot has longer "whiskers" than the *L. minor* and *A. caroliniana* plots.

At an alpha level of 0.05, there was at least one significant inequality between the mean *E. coli* concentrations of the trials following treatment, $H(3) = 51.413$, $p < .001$. Table 3 shows the Kruskal-Wallis Test Summary Table. The null hypothesis stated that all mean ranks were the same, and the alternative hypothesis stated that there existed at least one inequality.

Table 3. *Kruskal-Wallis Test Summary Table (? = 0.05)*

Total N	Test Statistic	*df*	*p*	Conclusion
92	51.413*	3	4.00E-11	Statistically different

Note. This table shows the values calculated using a Kruskal-Wallis test at *?* = 0.05.
*Adjusted for ties

The values in Table 3 demonstrated that there was sufficient evidence to reject the null hypothesis ($p < .001$), indicating that there was at least one inequality within the mean *E. coli* concentrations following the plant treatments.

Dunn's pairwise tests found statistically significant inequalities between *L. minor* vs. control, *S. minima* vs. control, and *A. caroliniana* vs. control. The summary table for the Dunn's pairwise tests is shown in Table 4.

Table 4. *Post-hoc Dunn's Pairwise Tests Summary Table (? = 0.05)*

Group 1	Group 2	*p*	Adjusted *p**	Statistically Different?
L. minor	*S. minima*	2.17E-2	1.30E-1	No
L. minor	*A. caroliniana*	8.62E-1	1.00	No
L. minor	Control	1.15E-10	6.91E-10	Yes
S. minima	*A. caroliniana*	4.87E-2	2.92E-1	No
S. minima	Control	1.20E-5	7.00E-05	Yes
A. caroliniana	Control	3.55E-9	2.13E-8	Yes

Note. This table shows the values calculated using post-hoc Dunn's pairwise tests at *?* = 0.05.
*Adjusted by the Bonferroni correction for multiple tests

Discussion

The purpose of this study was to test and directly compare the abilities of *L. minor*, *S. minima*, and *A. caroliniana* to remediate water contaminated by *E. coli*. It was hypothesized that using aquatic floating plants to treat *E. coli*-contaminated water would result in the concentration of *E. coli* in the water decreasing because of the flavonoids produced by aquatic plant roots that can kill microorganisms. Additionally, it was hypothesized that *L. minor* would kill more *E. coli* than *S. minima* and *A. caroliniana* because of its fast growth rate and its ability to eliminate contaminants like heavy metals from water. The results of this experiment supported the first hypothesis but failed to support the second hypothesis. The first hypothesis was supported because all three species of aquatic plants resulted in *E. coli* concentration decreasing in the experimental trials. Contrarily, the second hypothesis was not supported because the mean *E. coli* concentration after plant treatment was lowest in the group treated with *A. caroliniana* rather than *L. minor*. Moreover, the results of the Dunn's pairwise tests suggested that there was not a significant difference in the amount of bacteria killed by the three species of aquatic plants, further failing to support the second hypothesis.

Based on the mean *E. coli* concentrations of the water prior to and following plant treatment (Table 1), *A. caroliniana* was the most effective at killing *E. coli* present in the water, followed by *L. minor* and *S. minima*. The *A. caroliniana* treatment resulted in a 99.063% decrease in *E. coli* concentration, the *L. minor* treatment resulted in a 97.890% decrease in *E. coli* concentration, and the *S. minima* treatment resulted in a 90.292% decrease in *E. coli* concentration. In addition to having the lowest final mean *E. coli* concentration, the *A. caroliniana* treatment had the lowest range and standard deviation (Table 2), suggesting that its ability to remediate *E. coli* in water is relatively consistent. Contrarily, out of the three plant species tested, *S. minima* had the greatest final mean *E. coli* concentration, as well as the greatest range and standard deviation. This is depicted visually by the candlestick chart found in Figure 2. The length of the "whiskers" on the chart portrays the amount of variation in the final *E. coli* concentrations in each treatment group, which suggests that *S. minima* treatment has the most variability out of the tested plant species. This implies that *S. minima* may not be a suitable choice for phytoremediation despite being relatively effective at killing *E. coli* in water.

There was at least one significant difference between the remediation capability of *L. minor*, *S. minima*, *A. caroliniana*, and the control group with no added plants, $H(3) = 51.413$, $p < .001$. The p value was less than the level of significance ($a = 0.05$), so there was sufficient evidence to reject the null hypothesis. Post-hoc Dunn's pairwise tests found statistically significant differences between *L. minor* vs. control ($p < .001$), *S. minima* vs. control ($p < .001$), and *A. caroliniana* vs. control ($p < .001$). On the other hand, the Dunn's pairwise tests found that there were no significant differences between *S. minima* vs. *A. caroliniana* ($p = .29$), *L. minor* vs. *A. caroliniana* ($p = 1.00$), or *L. minor* vs. *S. minima* ($p = .13$). This suggests that there is no significant difference between the three tested plant species' effectiveness at remediating *E. coli*-contaminated water.

The results of the present study support the results of an experiment conducted by Hossain et al. (2019), which found that ethyl acetate extracts of *E. crassipes* inhibit the growth of *S. typhi* and *S. aureus*, and ethyl acetate extracts of *Spirodela polyrrhiza* have antimicrobial effects against *S. aureus*. Both the present study and the study by Hossain et al. (2019) suggest that plants have some form of antimicrobial activity. However, it is important to note that the experiment done by Hossain et al. used different bacteria, different plants, and ethyl acetate extracts of plants rather than the plants themselves.

An article by Dhir (2020) states that aquatic plants like *Typha latifolia* and *E. crassipes* have the capability to effectively remove pathogens like *E. coli* and *S. aureus* from wastewater. According to Dhir (2020), this removal occurs through root filtration and the production of toxic root exudates. These pieces of information are supported by the data collected during this experiment, which suggest that *L. minor*, *S. minima*, and *A. caroliniana* can remove *E. coli* from contaminated water. Although it should be noted that Dhir's research focused on plant species that are different from the ones tested in the present study, phytoremediation typically occurs through similar mechanisms that rely on the plants' roots.

The data collected in the present experiment also support the results of an experiment conducted by Papadopoulos et al. (2011), in which *L. minor* was grown in a pond containing septage and was able to remove 99.65 ± 1.46% of *E. coli* in the warm season and 99.33 ± 3.03% of *E. coli* in the cold season. The results of both the study by Papadopoulos et al. and the present study suggest that *L. minor* has the ability to remove *E. coli* from contaminated water.

An experiment by Prosser et al. (2016) found that soil treated with kanuka and manuka experienced a 90% reduction of *E. coli* after 5 and 8 days, respectively. These findings are supported by the data collected in the present experiment, which suggest that plants can reduce *E. coli* concentration in contaminated water. However, it is important to consider that Prosser et al.'s study involved contaminated soil, not contaminated water.

According to an article by Pooja et al. (2020), aquatic plants produce secondary metabolites that can be antibacterial and inhibit pathogenic growth. They state that aquatic plants like seaweeds can produce bioactive compounds like terpenes that potentially have antimicrobial properties (Pooja et al., 2020). This information is supported by the results of the present study, which indicate that *L. minor*, *S. minima*, and *A. caroliniana* may have the capability to excrete antimicrobial compounds that kill *E. coli* present in contaminated water.

Throughout experimentation, there were several sources of error and limitations that could have potentially affected the results of the study. The positive control group experienced a 25.118% decrease in *E. coli* concentration despite not being treated with any plants. This indicates a flaw in experimentation and may have occurred due to the addition of distilled water to the trials that was intended to compensate for water lost through evaporation. Similarly, colonies were observed in the plates inoculated with solution from the negative control trials, indicating that contamination was present within the experiment. Although it is possible that these errors affected results, all of the experimental groups were subjected to the same conditions, meaning that the comparisons between groups should have remained unaffected. Contamination was also observed in several of the plates inoculated with solution from the experimental groups (Table A1 in the Appendix). However, plates with contamination were excluded from data analysis in order to minimize their effects on results. Pest snails were found in some of the trials, but there did not appear to be a significant correlation between the presence of pests and final *E. coli* concentration when comparing the recorded occurrences of pests and collected data. Plants were obtained fully-grown from an online retailer specializing in outdoor ponds, meaning that the age of the plants in each group was not kept constant. Still, the approximate mass of plants added to each trial was kept consistent in each group. It is also important to note that the methods used in the experiment did not account for outside factors such as the variations in light intensity and weather throughout the day, which could have affected experimental results.

Errors may also have occurred during colony quantification and data analysis. There were several plates that were excluded from quantification and data analysis for various reasons, including having too many clustered colonies to count accurately (see Table A1 in the Appendix). Several plates from the positive control were excluded due to having too many colonies clustered together to count, which may have reduced the mean *E. coli* concentration of the control group. Furthermore, outliers were included in data analysis to account for natural variation. This inclusion may have had an effect on the results of the experiment and the conclusions made.

To reduce the likelihood of errors occurring and to address the limitations of the methods, several procedural improvements can be made. Sterile plant cultures should be used to grow the plants prior to experimentation, reducing the possibility of contamination and ensuring that all plants are the same age. A grow light with a day-night cycle and some form of air circulation should be used in order to simulate real-world conditions. Additionally, plants should be left in an area where contamination is minimized.

The results of the present study suggest that *L. minor*, *S. minima*, and *A. caroliniana* have the potential to be used as treatments for water contaminated by *E. coli*. Using these plants to create constructed wetlands could serve as an affordable and environmentally friendly alternative to common water treatment methods like ozonation and chlorination, making wastewater treatment more sustainable and thus benefiting wildlife living near contaminated areas. Furthermore, these plants could be used to treat environmental *E. coli* contamination found in large bodies of water like freshwater lakes and ponds. This is significant because aquatic plants are relatively accessible and inexpensive, which would help people living in developing countries who may not be able to afford more expensive treatment methods like advanced oxidation. The present study's results also suggest that phytoremediation could potentially be an alternative to antibiotic treatment; researchers looking to develop methods of water treatment that do not involve antibiotics could expand on these findings by testing the ability of aquatic plants to remediate contaminated water in various conditions, such as in different weather conditions.

To further explore the potential of using phytoremediation to reduce microbial contamination in the environment and to validate the results of the present study, further research should be conducted. Testing other aquatic plant species would expand current knowledge of phytoremediation's application to microbial contamination, which remains limited. The same plant species could be used to remediate water contaminated by another contaminant, which would test whether the remediative ability of the plants varies depending on the type of contamination. Another option would be to test the effect of outside factors (amount of light, pH of water, etc.) on the ability of aquatic plants to remediate water contaminated by *E. coli*. An exact replication of the present study could also be conducted, which would help validate the results yielded by this experiment.

References

A citizen's guide to phytoremediation [Fact sheet]. (2012, September). United States Environmental Protection Agency. Retrieved November 7, 2021, from https://semspub.epa.gov/work/HQ/189975.pdf

Alexandros, S. I., & Akratos, C. S. (2016). Removal of pathogenic bacteria in constructed wetlands: Mechanisms and efficiency. *Phytoremediation*, 327-346. https://doi.org/10.1007/978-3-319-41811-7_17

Bacteria and E. coli *in water*. (n.d.). United States Geological Survey. Retrieved November 7, 2021, from https://www.usgs.gov/special-topic/water-science-school/science/bacteria-and-e-coli-water?qt-science_center_objects=0#qt-science_center_objects

Barchanska, H., Plonka, J., Jaros, A., & Ostrowska, A. (2019). Potential application of *Pistia stratiotes* for the phytoremediation of mesotrione and its degradation products from water. *International Journal of Phytoremediation*, *21*(11), 1090-1097. https://doi.org/10.1080/15226514.2019.1606780

Ceschin, S., Crescenzi, M., & Iannelli, M. A. (2020). Phytoremediation potential of the duckweeds *Lemna minuta* and *Lemna minor* to remove nutrients from treated waters. *Environmental Science and Pollution Research*, *27*(13), 15806-15814. https://doi.org/10.1007/s11356-020-08045-3

Dhir, B. (2020). Effective control of waterborne pathogens by aquatic plants. *Waterborne Pathogens*, 339-361. https://doi.org/10.1016/B978-0-12-818783-8.00017-7

Gutierrez-Gines, M. J., Alizadeh, H., Alderton, E., Ambrose, V., Meister, A., Robinson, B. H., Halford, S., Prosser, J. A., & Horswell, J. (2021). Phytoremediation of microbial contamination in soil by New Zealand native plants. *Applied Soil Ecology*, *167*, 104040. https://doi.org/10.1016/j.apsoil.2021.104040

Han, M. (2021). The mycoremediation of *Escherichia coli* by *Pleurotus ostreatus*, *Stropharia rugosoannulata*, and *Trametes versicolor* in contaminated water. *Journal of the South Carolina Academy of Science*, *19*(2). https://scholarcommons.sc.edu/jscas/vol19/iss2/9/

Hossain, J., Khan, A., & Uddin, M. A. (2019). Antimicrobial efficacy and phytochemical analysis of three aquatic plant species in Bangladesh. *Bangladesh Journal of Microbiology*, *35*(1), 7-11. https://doi.org/10.3329/bjm.v35i1.39797

Iha, D. S., & Bianchini, I. (2015). Phytoremediation of Cd, Ni, Pb and Zn by *Salvinia minima*. *International Journal of Phytoremediation*, *17*(10), 929-935. https://doi.org/10.1080/15226514.2014.1003793

Jang, J., Hur, H.-G., Sadowsky, M.J., Byappanahalli, M.N., Yan, T., & Ishii, S. (2017). Environmental *Escherichia coli*: Ecology and public health implications-a review. *Journal of Applied Microbiology*, *123*(3), 570-581. https://doi.org/10.1111/jam.13468

LB agar, Lennox [Fact sheet]. (2011, October 19). ThermoFisher Scientific. Retrieved April 21, 2022, from https://tools.thermofisher.com/content/sfs/manuals/IFU452322.pdf

Non-pathogenic Escherichia coli *strains biological agent reference sheet (BARS)*. (n.d.). Cornell University Environment, Health and Safety. Retrieved April 28, 2022, from https://ehs.cornell.edu/research-safety/biosafety-biosecurity/biological-safety-manuals-and-other-documents/bars-other/non-pathogenic-escherichia-coli

Olguín, E. J., Sánchez-Galván, G., & Pérez-Pérez, T. (2007). Assessment of the phytoremediation potential of *Salvinia minima* Baker compared to *Spirodela polyrrhiza* in high-strength organic wastewater. *Water, Air, and Soil Pollution*, *181*(1-4), 135-147. https://doi.org/10.1007/s11270-006-9285-9

Pandey, V. C. (2012). Phytoremediation of heavy metals from fly ash pond by *Azolla caroliniana*. *Ecotoxicology and Environmental Safety*, *82*, 8-12. https://doi.org/10.1016/j.ecoenv.2012.05.002

Papadopoulos, F. H., Tsihrintzis, V. A., & Zdragas, A. G. (2011). Removal of faecal bacteria from septage by treating it in a full-scale duckweed-covered pond system. *Journal of Environmental Management*, *92*(12), 3130-3135. https://doi.org/10.1016/j.jenvman.2011.08.008

Pini, A. K., & Geddes, P. (2020). Fungi are capable of mycoremediation of river water contaminated by *E. coli*. *Water, Air, & Soil Pollution*, *231*(2). https://doi.org/10.1007/s11270-020-4464-7

Pooja, K., Rani, S., Rana, V., & Pal, G. K. (2020). Aquatic plants as a natural source of antimicrobial and functional ingredients. *Functional and Preservative Properties of Phytochemicals*, 93-118. https://doi.org/10.1016/B978-0-12-818593-3.00003-8

Prosser, J. A., Woods, R. R., Horswell, J., & Robinson, B. H. (2016). The potential in-situ antimicrobial ability of myrtaceae plant species on pathogens in soil. *Soil Biology and Biochemistry*, *96*, 1-3. https://doi.org/10.1016/j.soilbio.2015.12.007

Russo, T. A., & Johnson, J. R. (2003). Medical and economic impact of extraintestinal infections due to *Escherichia coli*: Focus on an increasingly important endemic problem. *Microbes and Infection*, *5*(5), 449-456. https://doi.org/10.1016/s1286-4579(03)00049-2

Singh, N., & Balomajumder, C. (2021). Phytoremediation potential of water hyacinth (*Eichhornia crassipes*) for phenol and cyanide elimination from synthetic/simulated wastewater. *Applied Water Science*, *11*(8). https://doi.org/10.1007/s13201-021-01472-8

Sudiarto, S. I. A., Renggaman, A., & Choi, H. L. (2019). Floating aquatic plants for total nitrogen and phosphorus removal from treated swine wastewater and their biomass characteristics. *Journal of Environmental Management*, *231*, 763-769. https://doi.org/10.1016/j.jenvman.2018.10.070

United States Department of Agriculture. (n.d.). *Aquatic invasives: Aquatic plants*. National Invasive Species Information Center. Retrieved November 7, 2021, from https://www.invasivespeciesinfo.gov/aquatic/plants

United States Food & Drug Administration. (2019, March 28). *Escherichia coli (E. coli)*. United States Food & Drug Administration. Retrieved April 13, 2022, from https://www.fda.gov/food/foodborne-pathogens/escherichia-coli-e-coli

Appendix

Table A1. *Concentration of* E. coli *(CFU/mL) After Plant Treatment; Raw Data*

Trial #	*L. minor*	*S. minima*	*A. caroliniana*	Control (no plants)
1	0.00	2.20E+6§	4.40E+5	4.71E+7
2	1.48E+6§	3.76E+6§	3.20E+5	3.52E+7
3	1.04E+6	1.16E+6§	1.60E+5	2.12E+6
4	1.60E+5§	9.60E+5	1.32E+6	4.80E+6§
5	0.00**	2.08E+6§	†	§
6	8.00E+4**	4.00E+4	4.00E+5	2.30E+7*
7	**	3.20E+5	2.00E+5	1.44E+7*§
8	0.00	7.20E+5	†	§
9	4.00E+4	2.40E+5	†	§
10	9.60E+5§**	1.84E+6	3.60E+5**	§
11	†§¶	1.36E+6	**	4.69E+7*
12	1.04E+6§	1.44E+7	**	*¶
13	0.00	1.10E+7	**	1.87E+7
14	1.20E+5	2.96E+6	2.80E+5	1.68E+7*§¶
15	§	2.32E+6§	4.80E+5	§
16	†	3.60E+5	2.80E+5	§
17	9.20E+5	6.40E+5§	4.40E+5	4.64E+7*
18	4.40E+5	†	†§	1.05E+8¶
19	4.00E+4	7.20E+5	†§	1.90E+7*
20	2.20E+6¶	§	†	1.85E+7
21	2.60E+6§	9.56E+6	5.60E+5	1.06E+8
22	1.36E+6¶	0.00	1.60E+5	1.26E+7*
23	1.40E+6	4.00E+4	§	7.16E+7¶
24	0.00	5.20E+5	4.40E+5	1.82E+7
25	0.00	†	1.20E+5	1.00E+7
26	0.00	7.20E+5	3.20E+5	¶
27	8.00E+4	1.04E+6§**	2.00E+5	1.16E+8
28	1.10E+7§	2.40E+5	†	1.19E+7
29	†§	7.60E+5	5.60E+5	4.12E+7††
30	§	6.90E+7¶	1.72E+6§	2.48E+7

* Colonies were present that fell below the designated size threshold of viability
† Contamination was present in the plate
§ Strangely-shaped (non-circular) colonies were present that made it difficult or impossible to count individual colonies
¶ Many colonies were clustered in a way that made it difficult or impossible to count individual colonies
** Colonies were clustered around the edge of the plate
†† Colonies were present that appeared faint, making it difficult or impossible to count individual colonies

Note. initial *E. coli* concentration was 4.92E+7 CFU/mL for all trials