The Mycoremediation of *Escherichia coli* by *Pleurotus ostreatus*, *Stropharia rugosoannulata*, and *Trametes versicolor* in Contaminated Water

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Escherichia coli contamination is a major health concern that can cause adverse health effects like fever and gastrointestinal illness. White-rot fungi produce exoenzymes that degrade pollutants, including *E. coli*, in a process called mycoremediation. The degradation abilities of *Pleurotus ostreatus*, *Stropharia rugosoannulata*, and *Trametes versicolor* have been examined in previous literature, but no studies have compared their abilities to remediate *E. coli*-contaminated water. The purpose of this study was to directly compare the abilities of these fungi to degrade *E. coli* in water. It was hypothesized that white-rot fungi would decrease *E. coli* concentration in water, and *T. versicolor* would remove more *E. coli* than *P. ostreatus* and *S. rugosoannulata*. After culturing fungi with wheat straw, *E. coli*-inoculated water was added to each sample. Initial and final concentrations (CFU/mL) of *E. coli* were determined following serial dilutions. *T. versicolor* treatments resulted in a 52.51% decrease in *E. coli* concentration, while *P. ostreatus* and *S. rugosoannulata* treatments resulted in a 12.90% increase and a 114.21% increase in *E. coli* concentration, respectively. A one-way ANOVA found that results were statistically significant ($F(38103.22, 5147.34) = 7.40$, $p < 0.001$), and a post-hoc Scheffé test was conducted to compare the fungus species. This post-hoc test found significant differences between *T. versicolor* vs. *S. rugosoannulata*, *T. versicolor* vs. control, and *P. ostreatus* vs. control. The results of this experiment suggest that *T. versicolor* can remediate *E. coli*-contaminated water more efficiently than *P. ostreatus* and *S. rugosoannulata*.

Introduction

Escherichia coli is a type of coliform bacteria that can cause adverse health effects such as fever, sore throat, and gastrointestinal illness (Taylor et al., 2015). Although it has many benign strains, others like *E. coli* O157:H7 can be life-threatening. As a matter of fact, in the year 2000, *E. coli* and other pathogenic fecal coliforms caused 40,000 deaths due to blood infections in the United States alone (Pini & Geddes, 2020). These health concerns, along with *E. coli*'s increasing resistance to antibiotics (Russo & Johnson, 2003), call for a method of *E. coli* removal that does not involve antibiotics.

One such method is mycoremediation, which uses fungal mycelia to decontaminate the environment. These mycelia are part of fungi's underground body structure and have the ability to release antimicrobial enzymes that stun and digest bacteria (Pini & Geddes, 2020). For fungi to be able to grow and produce these enzymes, they need to be on a substrate like wheat straw, sawdust, or wood chips (Rhodes, 2014). The mycelia of white-rot fungi excrete enzymes like laccase and manganese peroxidase, which can digest lignin, an organic polymer found in plants, as well as other organic molecules (Rhodes, 2014). These enzymes can therefore be utilized to break down and remove contaminants from the environment, including *E. coli*. *Pleurotus ostreatus*, *Stropharia rugosoannulata*, and *Trametes versicolor* are three species of white-rot fungi that have been studied by researchers due to their ability to produce laccase (Barh et al., 2019; Pini & Geddes, 2020; Taylor et al., 2015). *Pleurotus ostreatus*, commonly known as the oyster mushroom, has been used extensively to remove various contaminants like cadmium and benzo[a]pyrene from water and soil (Kulshreshtha et al., 2014; Rhodes, 2014). On the other hand, *Stropharia rugosoannulata*, often referred to as the garden giant, has been shown to have the capability to degrade contaminants like polycyclic aromatic hydrocarbons (PAHs) and synthetic dyes (Pozdnyakova et al., 2018). *Trametes versicolor*, or the turkey tail fungus, has also been utilized to degrade PAHs and other harmful contaminants like paraquat (Akhtar & Mannan, 2020). Using species of white-rot fungi like these as a natural way of removing *E. coli* from wastewater is less dangerous to aquatic wildlife than other treatment methods, which can form sludge byproducts that then require further sterilization (Pini & Geddes, 2020). Mycoremediation is also more affordable than other methods and has an estimated cost of only about $1000-2000/acre per year (Pini & Geddes, 2020).

E. coli serves as an indicator of fecal surface water contamination, making it an effective organism to use for studying bacteria-removal methods like mycoremediation (Taylor et al., 2015). Fecal contamination harms both humans and the environment, and contact with it can have negative health effects such as earache, blood-infection, and even death (Pini & Geddes, 2020; Taylor et al., 2015). Waterborne illnesses like *E. coli* are also detrimental economically, as they harm the shellfish industry and cause tens of thousands of beach closures annually (Taylor et al., 2015). Water sources in urban areas typically face *E. coli* contamination from untreated sewage, which contains fecal pathogens (Pini & Geddes, 2020). Fecal contamination is a significant public health concern, as *E. coli* is infectious and could contaminate water supplies. The concentration of *E. coli* in contaminated water can be quantified by spreading the water, which is often diluted using a serial dilution, over agar-filled Petri dishes and letting the *E. coli* grow over a course of 24 h. The *E. coli* colonies can then be counted manually and measured using Colony-Forming Units/ milliliter (CFU/mL) (Pini & Geddes, 2020).

In existing scientific literature, researchers have utilized various methods of mycoremediation to test fungi's ability to remove *E. coli* from wastewater. Pini & Geddes (2020) inoculated sterilized water with *E. coli* and added potato dextrose broth to provide a nutrient source. They then grew *Pleurotus ostreatus* in jars full of organic wheat straw and filled said jars with the *E. coli*-inoculated water. Through this experiment, they found that *Pleurotus ostreatus* is able to remove 99.25% of *E. coli* from inoculated water over the course of 96 h. A study conducted by Taylor et. al (2015) took a different approach, using biofilters containing *Stropharia rugosoannulata* to remove *E. coli* from contaminated water. Results showed that filtering *E. coli*-contaminated water through a *Stropharia rugosoannulata* mycofilter leads to an approximately 20% decrease in *E. coli* concentration. Thus, previous research suggests that both *Pleurotus ostreatus* and *Stropharia rugosoannulata* have some capability of remediating *E. coli*-contaminated water. It is also important to note that there has not yet been research published on *Trametes versicolor*'s ability to remove *E. coli* from water, demonstrating the lack of knowledge about *Trametes versicolor* as a tool for degrading *E. coli*.

The purpose of this project was to compare the *E. coli*-remediating abilities of *Pleurotus ostreatus* and *Stropharia rugosoannulata*, two white-rot fungus species that have already been studied by various researchers, to the *E. coli*-remediating capability of *Trametes versicolor*, a species of white-rot fungi that has not yet been tested for its ability to degrade *E. coli*. Although there is no existing literature about *T. versicolor*'s role in remediating *E. coli*-contaminated wastewater, the fact that it is a type of white-rot fungi suggests that it could produce the enzymes necessary to degrade bacteria. Additionally, *T. versicolor* has already been used in previous research to degrade other contaminants in water. Therefore, it was

reasonable to assume that this fungus also has the potential to break down *E. coli*. By directly comparing the ability of each of these three species of fungi to remove *E. coli* from water, this project aimed to find the most effective species of white-rot fungi to use for mycoremediation, which would aid in the search for a natural method of *E. coli* removal.

It was hypothesized that adding white-rot fungi to *E. coli*-contaminated water would cause the concentration of *E. coli* in the water to decrease because of the exoenzymes produced by white-rot fungi that can break down bacteria. It was further hypothesized that *Trametes versicolor* would degrade more *E. coli* than *Pleurotus ostreatus* and *Stropharia rugosoannulata* because of its ability to produce laccase and break down several different types of contaminants like PAHs and paraquat. Each species of fungi was grown in plastic deli cups containing organic wheat straw as a substrate, and each cup was then filled with *E. coli*-inoculated water and incubated for 96 h at 37°C. The *E. coli* remaining in the water of each cup was then quantified by growing the bacterial colonies on agar-filled Petri dishes and counting the number of colonies in each dish to calculate CFU/mL.

Methods

During the course of this experiment, all lab surfaces were disinfected using ethyl alcohol before and after use, and all pieces of lab equipment were either autoclaved, heated using a Bunsen burner, or sterilized with ethyl alcohol. Additionally, all procedures involving living organisms were carried out in a fume hood, and personal protective equipment was used. To prevent contamination before use, all agar plates were sealed with Parafilm and stored in the fridge. The procedures used in this experiment were based on the methods used by Pini & Geddes (2020) in a similar mycoremediation experiment.

264 grams of wheat straw were cut into 3 to 5 centimeter segments. Then, thirty 250-milliliter beakers were filled with approximately 2.20 grams each of wheat straw. These beakers were each covered with aluminum foil and autoclaved at 121°C to sterilize the wheat straw. Next, the sterilized wheat straw inside the 30 beakers was transferred to 30 5.5-ounce plastic deli cups that had been sterilized using an ethyl alcohol bath. This process was then repeated three more times to create a total of 120 wheat-filled cups for the experimental treatments and the control. Afterwards, 60 plug-shaped portions of mycelia-covered agar (~1 centimeter in diameter) were punched out from each purchased fungus plate using a sterilized cut piece of a plastic straw. The fungal plugs were added to the wheat-filled deli cups to inoculate them with fungal mycelium. Thirty cups were inoculated with 2 plugs each of *Pleurotus ostreatus*; 30 cups were inoculated with 2 plugs each of *Stropharia rugosoannulata*; 30 cups were inoculated with 2 plugs each of *Trametes versicolor*; and 30 cups were inoculated with 2 plugs of plain malt extract agar without fungi to serve as the control. After the plugs were added, all the cups were labelled using a permanent marker to indicate the type of fungi with which they had been inoculated. They were then all assigned a number from 1 to 120. Subsequently, the cups were sealed with their lids and placed in drawers for four weeks prior to experimentation to allow the fungal mycelia to grow. During this growth period, the cups were misted twice a week to keep the mycelia moist.

After four weeks, 250 milliliters of potato dextrose broth were prepared using fresh potatoes, dextrose, and distilled water. One potato was peeled and then cut into ~1x2 centimeter chunks. Next, 50 grams of potato pieces were weighed and added to a beaker containing 350 milliliters of distilled water. The potatoes were then boiled for 30 minutes until they became soft. Then, the contents of the beaker were filtered through a muslin cloth to obtain the extract of the potatoes. Afterwards, distilled water was added to the extract until it reached a total volume of 250 milliliters, and 5 grams of dextrose were mixed into the resulting solution. The broth was then autoclaved for 30 minutes at 121°C.

Next, 1 liter of Lennox LB agar was prepared and autoclaved according to manufacturer instructions. The agar was poured into 125 Petri dishes and left to set for 24 h. While the Petri dishes were setting, 5.5 liters of distilled water were inoculated with 1 mL of living nutrient broth containing *Escherichia coli* K-12. To achieve this, the water was divided equally between five flasks, and 200 microliters of *E. coli* broth were added into each flask. 50 milliliter portions of potato dextrose broth were then added into the flasks, and a stir bar was used to mix together the contents of each flask. Afterwards, the *E. coli* solution was incubated for 48 h at 37°C.

After 48 h, a graduated cylinder was used to add 40 milliliters of *E. coli* inoculation solution into each of the 120 experimental plastic cups. Subsequently, a serial dilution was performed on the leftover inoculation solution at various dilution factors, and a micropipette was used to plate 50 microliters of each level of diluted inoculation solution on Petri dishes containing Lennox LB agar. An inoculation loop was used to spread the solution over the agar plates, and the inoculated dishes were incubated at 37°C for 24 h.

The resulting *E. coli* colonies were observed to determine which dilution factor was the most ideal for quantification. Then, the colonies were counted and quantified using the formula for calculating CFU/mL:

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

The number of colonies was multiplied by the dilution factor and then divided by the volume of the sample taken in milliliters to determine the initial *E. coli* concentration of the solution.

The 120 experimental cups were then placed into drawers and were left at room temperature for 96 h. Afterwards, serial dilutions were performed inside test tubes to dilute the 120 portions of experimental *E. coli* solution at a 1:100 dilution factor. Then, 50 microliter portions were taken from each dilution and plated onto 120 Lennox LB agar plates using a micropipette and an inoculation loop. Each of the plates were labelled with a permanent marker to indicate which cup they had received a sample from and then incubated at 37°C for 24 h. Afterwards, the *E. coli* colonies were counted and quantified using the same formula that was used for determining the inoculated water's initial *E. coli* concentration. After all data were collected and recorded, used pipettes and pipette tips were thrown away, and all living organisms were disposed of using bleach. Data were analyzed using a one-way ANOVA and a post-hoc Scheffé test, and the results of the ANOVA were verified using Welch's *t*-test. The experimental design diagram is shown in Figure 1.

Figure 1. *Experimental Design Diagram*

Title of the Experiment
The Mycoremediation of *Escherichia coli* by *Pleurotus ostreatus*, *Stropharia rugosoannulata*, and *Trametes versicolor* in Contaminated Water

Hypothesis
Adding white-rot fungi to *E. coli*-contaminated water would cause the concentration of *E. coli* in the water to decrease because of the exoenzymes produced by white-rot fungi that can degrade pollutants like bacteria. Furthermore, *T. versicolor* would degrade more *E. coli* than *P. ostreatus* and *S. rugosoannulata* because of its ability to produce laccase and break down several types of contaminants like PAHs and paraquat.

Independent Variable Species of white-rot fungus

Levels of Independent Variable	*Pleurotus ostreatus* (2 plugs)	*Stropharia rugosoannulata* (2 plugs)	*Trametes versicolor* (2 plugs)	Malt extract agar without fungi (2 plugs)
Number of Repeated Trials	30	30	30	30

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

Control Group
Malt extract agar without fungi

Constants
Temperature, amount of water, amount of light, amount of fungi added, size of containers, size of Petri dishes, strain of *E. coli* used, type of substrate used, amount of substrate used

Results

Table 1 displays the mean concentrations of *E. coli* prior to and following fungal treatment, as well as the percent change in *E. coli* concentration from before treatment to after treatment.

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

	Pleurotus ostreatus	*Stropharia rugosoannulata*	*Trametes versicolor*	Control (no fungi)
Concentration of *E. coli* prior to fungi treatment (CFU/mL)	35.82	35.82	35.82	35.82
Concentration of *E. coli* after fungi treatment (CFU/mL)	40.44	76.73	17.01	112.07
% change in *E. coli* concentration	12.90%	114.21%	-52.51%	212.87%

This table displays the mean *E. coli* concentrations before and after 96 h of mycelia treatment along with percent change calculated by $\frac{mean\ initial\ E.coli\ concentration}{mean\ final\ E.coli\ concentratio\ n}$

After 96 h, the treatment groups resulted in the following changes in *E. coli* concentration: a 12.90% increase for *P. ostreatus*, a 114.21% increase for *S. rugosoannulata*, a 52.51% decrease for *T. versicolor*, and a 212.87% increase for the control (see raw data in Tables A1 & A2 in the Appendix).

Table 2 shows the mean, range, and standard deviation of the data.

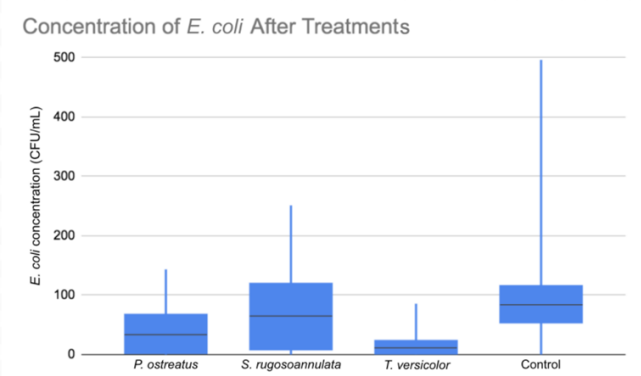
Table 2. *Mean, Range, and Standard Deviation of* E. coli *Concentration (CFU/mL)*

	Pleurotus ostreatus	*Stropharia rugosoannulata*	*Trametes versicolor*	Control (no fungi)
M	40.44	76.73	17.01	112.07
Range	143.40	251.20	85.60	496.20
SD	45.49	81.84	27.00	116.20

This table displays the mean, range, and standard deviation of *E. coli* concentration after 96 h of mycelia treatment.

The *E. coli* concentrations after treatment were compared using a candlestick chart (Figure 2). This chart shows the maximum and the minimum values of each group, as well as the first and third quartiles.

Figure 2. *Candlestick Chart of* E. coli *Concentrations After Treatments*



This chart displays the candlestick plot of *E. coli* concentrations following the four experimental treatments.

At an alpha level of 0.05, there was significant inequality between the mean *E. coli* concentrations after the treatments ($F(38103.22, 5147.34) = 7.40$, $p < 0.001$). Table 3 shows the ANOVA Summary Table, which includes these values. The null hypothesis stated that the means were all equal, and the alternate hypothesis stated that there was at least one inequality.

Based on the information from Table 3, there was sufficient evidence to reject the null hypothesis ($p < 0.001$) and indicate that there is at least one inequality amongst the mean *E. coli* concentrations after each fungi treatment.

Table 3. *One-Way ANOVA Summary Table (α = 0.05)*

Source	*SS*	*df*	*MS*	*F*	*p*	*F crit*
Between	114309.67	3	38103.22	7.40	$1.72 * 10^{-4}$	2.70
Within	468407.63	91	5147.34			
Total	582717.31	94				

This table displays the values calculated using a single-factor ANOVA at $a = 0.05$. $H_0 = \mu_1 = \mu_2 = \mu_3 = \mu_4$; H_a = ALOI

A post-hoc Scheffé test found that significant differences occurred between *T. versicolor* vs. *S. rugosoannulata*, *T. versicolor* vs. control, and *P. ostreatus* vs. control. Table 4 shows the summary table for this test.

Table 4. *Post-hoc Scheffé Summary Table*

Task ($\bar{x}$)	F_s	F'	*Statistically Significant?*
T. versicolor vs. *P. ostreatus*	1.38		No
T. versicolor vs. *S. rugosoannulata*	8.49	8.11	Yes
T. versicolor vs. control	18.95		Yes
P. ostreatus vs. *S. rugosoannulata*	3.25		No
P. ostreatus vs. control	11.12		Yes
S. rugosoannulata vs. control	2.57		No

This table displays the values calculated using a post-hoc Scheffé test.

Discussion

The purpose of this study was to compare the abilities of *P. ostreatus*, *S. rugosoannulata*, and *T. versicolor* to remediate *E. coli*-contaminated water. It was hypothesized that treating *E. coli*-contaminated water with the mycelia of white-rot fungi would cause the concentration of *E. coli* in the water to decrease because of exoenzymes produced by white-rot fungi that can break down bacteria. Furthermore, it was hypothesized that *T. versicolor* would remove more *E. coli* than *P. ostreatus* and *S. rugosoannulata* because of its ability to produce laccase and break down several different types of contaminants like PAHs and paraquat. The results of this study partially supported the first hypothesis and fully supported the second hypothesis, as they suggested that some species of white-rot fungi may inhibit *E. coli* growth rather than breaking down existing *E. coli*. These findings are due to the fact that some of the treatments resulted in an increase in *E. coli* concentration rather than a decrease. The results also demonstrated that, out of the three species of white-rot fungi tested, *T. versicolor* had the greatest capacity to degrade *E. coli* in contaminated water, which supported the second hypothesis.

The mean *E. coli* concentrations of each experimental group showed that *T. versicolor* was the most effective at remediating *E. coli*-contaminated water, followed by *P. ostreatus* and then *S. rugosoannulata* (Table 1). In fact, *T. versicolor* was the only white-rot fungi that caused a decrease in overall *E. coli* concentration, suggesting that the other two fungi may hinder *E. coli* growth rather than decreasing *E. coli* concentration. While the samples treated with *T. versicolor* experienced a 52.51% decrease in *E. coli* concentration, the samples treated with *P. ostreatus* and *S. rugosoannulata* experienced a 12.90% increase and a 114.21% increase in *E. coli* concentration, respectively. *T. versicolor* also had the lowest range and standard deviation (Table 2), demonstrating the consistency of its remediating capability. These values also support the indication that *S. rugosoannulata* has the lowest remediation capability out of the tested species, as its range and standard deviation are the highest out of the tested fungi and are exceeded only by the control group. The "whiskers" on the candlestick plot also provide an indication of the amount of variation among the *E. coli* concentrations of each group, suggesting that *S. rugosoannulata* causes the most variability out of the three fungus species. This indicates that the remediating capability of *S. rugosoannulata* is fairly inconsistent, which implies that it may not be an effective species to use for mycoremediation.

There was a significant difference between the *E. coli*-remediating capabilities of the various white-rot species tested ($F(38103.22, 5147.34) = 7.40$, $p < 0.001$). Because the p value was less than the level of significance ($a = 0.05$), there was sufficient evidence to reject the null hypothesis. A post-hoc Scheffé test (summary shown in Table 4) found differences between the following groups: *T. versicolor* vs. *S. rugosoannulata* ($F_s = 8.49$), *T. versicolor* vs. the control group ($F_s = 18.95$), and *P. ostreatus* vs. the control group ($F_s = 11.12$). Contrarily, the Scheffé test found that there was no significant difference in the remediating capability of *S. rugosoannulata* vs. the control group ($F_s = 2.57$), suggesting that there is not a difference between *S. rugosoannulata*'s ability to remediate contaminated water and plain wheat straw's ability to remediate contaminated water.

Because the assumption of test groups having equal variances was not fulfilled, a Welch's *t*-test was performed to verify the results of the one-way ANOVA. The results of the Welch's *t*-test confirmed the statistical significance of the ANOVA and the Scheffé post-hoc test, as the *p*-value yielded by the Welch's *t*-test ($p = 1.23 * 10^{-6}$) was even smaller than the *p*-value yielded by the one-way ANOVA ($p = 1.72 * 10^{-4}$). This result suggests that the one-way ANOVA was more stringent than the Welch's *t*-test, so the results of the one-way ANOVA were used when analyzing the data collected during the present study.

It was found that the *E. coli* concentrations of the samples treated with *T. versicolor* decreased, while the *E. coli* concentrations of the samples treated with *P. ostreatus* and *S. rugosoannulata* increased. This differs from a recent study conducted by Pini & Geddes (2020), which found that treating contaminated water with *P. ostreatus* led to a 99.25% decrease of *E. coli* colonies after 96 h. However, their study used slightly different methods, including a shaker table, membrane filters, and fewer trials than this current experiment.

This experiment's results also contrast with a study conducted by Taylor et al. (2015) that investigated the ability of *S. rugosoannulata* to remove *E. coli* from synthetic stormwater. They found that the use of *S. rugosoannulata* results in a 90% reduction in thermotolerant coliforms in synthetic stormwater. The results of their experiment directly oppose the findings of this paper, which indicate that *S. rugosoannulata* treatment resulted in an increase of *E. coli* colonies in contaminated water. Regardless, it is important to consider that their study utilized mycofiltration, which relies on a fungal inoculated biofilter rather than the fungal mycelia itself on a substrate.

According to research by Kulshreshtha et al. (2014), mushrooms produce enzymes that allow them to degrade various pollutants like organic and synthetic dyes. Specifically, *P. ostreatus* has been found to be able to degrade oxo-biodegradable plastic. The present study somewhat supports this information, as its results demonstrated that *T. versicolor* was able to degrade pollutants in water. However, *P. ostreatus* resulted in the *E. coli* concentration increasing, suggesting that it did not degrade any pollutants.

The results of this experiment supported a study conducted by Pezzella et al. (2017), which found that *T. versicolor* is able to degrade endocrine disrupting chemicals (EDCs) more efficiently than *P. ostreatus*. This matches the data collected during this experiment, which found that *T. versicolor* was able to degrade *E. coli* more effectively than *P. ostreatus*. It is important to note that Pezzella et al.'s study focused on degrading a different type of pollutant than this study; however, the process of mycoremediation utilizes enzymes like laccase that can degrade a variety of waste and pollutants (Kulshreshtha et al., 2014).

An experiment by Pozdnyakova et al. (2018) focused on the degradative abilities of *S. rugosoannulata*, finding that it is effective in degrading polycyclic aromatic hydrocarbons (PAHs), synthetic anthraquinone dyes, and oil. Their study states that their results demonstrate the degradative potential of *S. rugosoannulata* towards a wide variety of environmental pollutants (Pozdnyakova et al., 2018). However, the results of the present study demonstrated a lack of degradative activity. This could be due to the fact that a different pollutant was tested, but it is once again important to consider that laccase is considered to be able to degrade an array of pollutants (Kulshreshtha et al., 2014).

During the course of this experiment, there were several sources of error that could have affected the results. For instance, due to time constraints and slow fungi shipping times, the experimental groups were not inoculated with fungi at the same time, which caused certain groups to have more growth time than others. Because of the delayed inoculation, the cups containing *T. versicolor* were allowed to grow a week longer than the cups containing *P. ostreatus*, *S. rugosoannulata*, and the control. Additionally, while the fungal mycelia were growing, visible contamination began to appear. Small, black dots were present in many of the *T. versicolor* samples, as well as some of the *S. rugosoannulata* and control samples. Furthermore, there were several other types of contamination, like white and gray growths on the control samples. However, after comparing recorded contamination to data, there did not appear to be any significant correlation between visual contamination and *E. coli* concentration after treatment. Errors could have also taken place during quantification and data analysis; several of the *E. coli* plates contained colonies that could not be counted due to reasons such as falling under the designated size-threshold or being too clustered to differentiate accurately. These plates were excluded from quantification and analysis. Additionally, outliers were included in data analysis because they occurred

due to natural variation. However, this inclusion could possibly have had an impact on results and conclusions. To improve techniques, more time should be allotted for inoculation so that the general timeline of each group can be kept as consistent as possible. Furthermore, sterile cultures should be used, and procedures should take place in an environment that minimizes contamination as much as possible.

To gain more insight into the realm of mycology and to reinforce the results of this study, further research should be performed. Testing other species of fungi could help expand the current literature about mycoremediation, which is still fairly limited. Another option would be to test the same three species of fungi on a different contaminant to see if their degradation ability varies between contaminants. Contrarily, an exact replication of this experiment could be conducted to help validate results and determine if confounding variables played a role in the present study's results and conclusions. This repetition would be beneficial because it is difficult to draw conclusions based off of a singular experiment.

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Appendix A

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

Trial #	*Pleurotus ostreatus*	*Stropharia rugosoannulata*	*Trametes versicolor*	Control (no fungi)
1	6.6§	0	0	14.8
2	§	251.2	0	†§
3	53.4	10.2	0	251.8
4	37.6	91.6	0	62.8
5	121.2	40	§	*†
6	21	121	85.6	10.8
7	31.2	247.4	78.8	98.4
8	4.2	118.4	22.8	†§
9	100.6	113.4	1.2	496.2
10	28.6	0§§	0	195.4
11	143.4†	0§§	0.2	†§
12	§††	128.6	7.2	65
13	16.8††	*†	0	0
14	0	85.2	2.2	237.4
15	0.2	90¶	17.2	§
16	2.2†	*†	†	68.6
17	3.2†	9.4	2	102.2
18	68.8††	7.2	0.8	52**
19	101.4	†	33.4	128.2
20	11.4§	25.2	†	†

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

21	17.4	26.6	0.4	*†
22	0§§	200.8	†	56.6
23	†	†	43.6	87.2
24	0	27.4	0	65.2
25	134.6	0	0	50.2
26	1	0	67.4	§
27	76.8§	†	§	†
28	65	208.4¶	51.2	86.6
29	0.2	†	0	††
30	45	39.6	11.2†††	†

* Colonies were too small to count accurately

† Too many colonies were clustered to count accurately/made it extremely difficult to count

§ Hazy "clouds" were present that made it difficult or impossible to count individual colonies

¶ Plate was split in half, then colonies in one half were counted and multiplied by 2

** Plate was split into quarters, then colonies in one quarter were counted and multiplied by 4

†† Colonies appeared too faint to count accurately/made it extremely difficult to count

§§ Colonies fell below the delineated size threshold of viability

Note: all initial *E. coli* concentrations were 35.8 CFU/mL