Mycoremediation of Petroleum Hydrocarbons Using Oyster Mushrooms

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Abstract

This study focused on observing the potential of Oyster mushrooms to remediate polluted substrates by analyzing their growth in the presence of Petroleum Hydrocarbons and assessing nutrient differences. Over six weeks, eighteen bags of sterilized hardwood and soy pellet substrate were observed, comparing uncontaminated controls with and without fungal inoculation, as well as polluted samples with and without fungal inoculation. Qualitative analysis was performed to observe the pH and nutrient contents. The presence of mushrooms improved substrate quality by increasing pH and nutrient content. Oil acted as an unconventional food source, accelerating fungal growth rather than inhibiting it as predicted. These findings focus attention on fungi's resilience and adaptability, suggesting that Oyster mushrooms could play a crucial role in ecological restoration through mycoremediation.

Introduction

Mycoremediation refers to the degradation of hazardous materials or pollutants in environments by relying on the production of enzymes from mushrooms. The chemoheterotrophic nature of fungi makes them a great choice for remediation because they use organic chemicals as their source of energy (Kulshreshtha, Mathur, & Bhatnagar, 2014). Oyster Mushrooms are a white rot fungus, meaning they typically degrade wood. They can also be classified as saprophytic, meaning they decompose dead and decaying matter. To do this, they produce cellulase, oxidase and other ligninolytic enzymes that have the capacity to break down large organic compounds into smaller, more easily digestible compounds (Stamets, 2008).

Petroleum Hydrocarbons are a class of chemicals found in common fuels such as kerosene, gasoline, and motor oil. This is a broad spectrum of large organic compounds that can be

incredibly difficult to break down. These large compounds do not evaporate, nor do they burn very well. They represent the most common environmental pollutants and can be extremely toxic at low levels (*Hawaii Department*, n.d., p. 1).

Methods and Materials

Materials:

- 6 bags of plain soy and hardwood pellet substrate
- 6 bags of Blue Oyster Mushroom inoculated substrate
- 6 bags of Golden Oyster Mushroom inoculated substrate
- 4 packs of Luster Leaf rapid soil test kits
- 2 gallons of spent engine oil

Methods:

The bags inoculated with mushrooms were allowed to fully colonize, meaning the mycelium had a chance to grow throughout the substrate, for about two weeks. Once fully colonized, three of each of the substrate bags were contaminated with 10% concentrations of the spent engine oil. Oil concentration calculation is as follows:

Volume of oil (ml) = [Weight of substrate (Kg) x 1000 x 0.1]/0.8220

Oil was distributed over the top of the substrate blocks and allowed to soak/absorb for one week. Once the oil was absorbed, the substrate bags were placed in fume hoods to mitigate fumes produced by the oil. The bags were cut in an “X” pattern to allow the mushrooms to grow out of the bag. Substrates were sprayed generously with water to retain moisture every few days. The mushrooms were allowed to grow for three weeks.

Once the mushrooms had grown to maturity, soil testing began. Samples from each bag of substrate were taken. Qualitative analysis for the following was performed on all samples: pH, nitrogen (N) content, phosphorous (P) content, and potash (K), compounds containing potassium, content. For the pH tests, soil was added to the fill line on the test chamber, then mixed with distilled water and the Luster Leaf pH indicator tablet. The mixture was shaken vigorously for 1 minute and color was allowed to develop for 1 minute. For the N, P, and K tests, substrate samples were mixed with distilled water in a ratio of 1:5, substrate was allowed to settle for 1 hour and the remaining solution was decanted into the test chambers, respective indicator capsules were mixed with the solution and shaken vigorously and allowed to develop color for 10 minutes (Luster Leaf, n.d.).

Results and Discussion

The soil tests conducted were qualitative and did not produce numerical results. These tests are typically used for gardeners with real soil, while the substrate used in this experiment was a sterilized mix of soy and hardwood pellets made specifically for growing mushrooms. This is not an ideal substrate for growing plants and other soil organisms. The tests used color indicator powders which developed colors that could be identified as one of the following terms, indicating concentrations from highest to lowest: surplus, sufficient, adequate, deficient and depleted.

	Substrate 1	Substrate 2	Substrate 3	Substrate 4	Substrate 5	Substrate 6
Weight	2.634 kg	2.518 kg	2.325 kg	2.946 kg	2.781kg	2.828 kg
Oil Amount	320.4 ml	306.3 ml	282.8 ml	0 ml	0 ml	0 ml
pH	4.5 (very acid)	4.5 (very acid)	4.5 (very acid)	5.5 (acid)	5.5 (acid)	5.5 (acid)

N content	Depleted	Depleted	Depleted	Depleted	Depleted	Depleted
P content	Depleted	Depleted	Depleted	Deficient	Deficient	Deficient
K content	Surplus	Surplus	Sufficient	Deficient	Deficient	Deficient

Contaminated Plain Substrate Results:

Without fungal inoculation, contaminated substrates (1-3) showed extreme acidity with a pH of 4.5. K compounds were presented in surplus while N and P were depleted. Without mushrooms present to metabolize the pollutant, the substrate would have had poor growth conditions for soil organisms.

Noncontaminated Plain Substrate Results:

Without fungal inoculation, noncontaminated substrates (4-6) exhibited a slightly more balanced pH of 5.5 and all nutrients were depleted or deficient. Despite the lack of contamination, this substrate without fungal inoculation gives poor growth conditions.

	Blue Oyster 1	Blue Oyster 2	Blue Oyster 3	Blue Oyster 4	Blue Oyster 5	Blue Oyster 6
Weight	3.792 kg	3.934 kg	4.301 kg	3.862 kg	3.936 kg	3.918 kg
Oil Amount	458.5 ml	478.6 ml	523.2 ml	0 ml	0 ml	0 ml
pH	6.5 (slight acid)	6.0 (acid)	6.0 (acid)	5.5 (acid)	6.0 (acid)	5.5 (acid)
N content	Depleted	Depleted	Depleted	Depleted	Depleted	Depleted
P content	Depleted	Deficient	Depleted	Adequate	Adequate	Deficient
K content	Sufficient	Sufficient	Adequate	Sufficient	Sufficient	Deficient

Contaminated Blue Oyster Results:

For contaminated substrates inoculated with Blue Oyster Mushrooms (1-3), pH was more balanced than plain substrates ranging from 6.0-6.5. N and P remained depleted and deficient, while K content lowered to sufficient/adequate levels.

The mushrooms grew faster and larger than noncontaminated substrates, suggesting that the mushrooms were metabolizing the contaminant for energy and growth.

Noncontaminated Blue Oyster Results:

Blue oysters 4-6 were not contaminated with oil. They had a less balanced pH of 5.5-6.0, but nutrient levels of P and K were higher than their contaminated counterparts.

These mushrooms grew to be healthy, but not as fast or as large as the mushrooms with contaminants present.

	Golden Oyster 1	Golden Oyster 2	Golden Oyster 3	Golden Oyster 4	Golden Oyster 5	Golden Oyster 6
Weight	4.417 kg	4.090 kg	4.593 kg	3.883 kg	3.933 kg	4.041 kg
Oil Amount	537.3 ml	497.6 ml	552.2 ml	0 ml	0 ml	0 ml
pH	6.0 (acid)	6.0 (acid)	5.5 (acid)	6.0 (acid)	5.0 (very acid)	6.5 (slight acid)
N content	Depleted	Depleted	Depleted	Depleted	Depleted	Depleted
P content	Deficient	Adequate	Adequate	Deficient	Adequate	Deficient
K content	Depleted	Depleted	Deficient	Depleted	Deficient	Deficient

Contaminated Golden Oyster Results:

For Golden Oysters 1-3, pH levels were still slightly acidic coming in at 5.5-6.0. N and K were depleted or deficient, yet P was raised to adequate or deficient levels.

Once again, the contaminated mushrooms grew to be much larger than their noncontaminated counterparts.

Noncontaminated Golden Oyster Results:

The noncontaminated Golden Oysters (4-6) had pH levels ranging from 5.0-6.5. The nutrient levels remained mostly depleted or deficient with the exception of P being deficient or adequate.

These mushrooms grew to be healthy but were outgrown by their contaminated counterparts.

Limitations and Recommendations

While this study provides unique and valuable information regarding the relationship between fungi and pollutants, there are many limitations that must be acknowledged. Lab and equipment access was restricted as this research was done at an undergraduate level; this may have impaired the accuracy of analyses and restricted the availability for more extensive testing. Environmental conditions must also be factored into the accuracy of these results as this experiment was done in a controlled laboratory setting using soy and hardwood substrate rather than real soil, which does not replicate real-world conditions. Time and budget constraints also limited the availability of extensive testing and affected the ability to analyze long term effects. Another significant constraint was the inability to utilize gas chromatography with flame ionization detection to analyze the amount of total petroleum hydrocarbons present in the soil. This would have allowed for better analysis of how TPH degrades over time with fungi present.

Future research could address these limitations and restrictions by inoculating pre-polluted soil with fungal samples to better analyze real world conditions. The implementation of quantitative analysis would also be extremely helpful in the analysis of soil nutrients and allow for more precise measurements. Expansion of research by incorporating a larger team could allow for simultaneous testing of all substrates and improve efficiency greatly. Increasing the types of pollutants and differing the concentrations tested would result in a better understanding of how

mushrooms work to adapt to different environments and degrade different compounds. Furthermore, testing the composition of the mushrooms themselves would allow for a better understanding of how the mushrooms react to the contaminants.

Conclusions

This study demonstrates the impact of fungal inoculation on polluted substrates, emphasizing both the adaptability and resilience of Oyster mushrooms. The findings suggest that fungal inoculation could play a crucial role in improving substrate conditions, affecting pH balance, nutrient availability, and overall growth dynamics. In oil-contaminated substrates, fungal activity mitigated extreme acidity while also facilitating nutrient retention and hydrocarbon metabolism, demonstrating the ability of these species to adapt and potentially break down petroleum-based compounds. The accelerated growth observed in contaminated substrates indicates that fungi may exploit pollutants as a metabolic resource, suggesting an intriguing relationship between fungal physiology and environmental contaminants.

Non-contaminated substrates provided cleaner conditions but exhibited slower fungal development, reinforcing the notion that mushrooms are able to metabolize contaminants such as petroleum hydrocarbons for energy. This study shows the potential application of mycoremediation, as fungi show the capability to restore polluted environments by influencing substrate chemistry and nutrient content. Future research integrating quantitative analysis methods, including gas chromatography with flame ionization detection testing, will be essential to assess total petroleum hydrocarbon degradation and confirm the efficacy of fungal-driven pollutant breakdown. These findings emphasize the need for continued exploration into fungal

metabolism, pollutant decomposition mechanisms, and large-scale mycoremediation applications to refine our understanding of fungi's role in environmental restoration.

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