Entry to the Stockholm Junior Water Prize 2023

exSTREAM FUNgi:

A Natural Solution to Protect Watersheds from the Effects of Urbanization

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2. PRELIMINARY MATTERS

2a. Abstract

Rapidly growing populations put pressure on infrastructure and ecosystems and negatively affect water quality. The increase of impervious surfaces, such as parking lots and roads, provide a superhighway for pollutants to enter the water system and destabilize ecosystems. This study focused on the utilization of fungi native to North America that release enzymes to decompose pesticides, hydrocarbons, and waste containing lignocellulose by preventing contamination from entering a watershed system at its origin, which ultimately aids in the protection of ecosystems and water quality. There were two primary components of this experiment. Phase one tested the filtration properties of P. ostreatus and G. frondosa in vitro, and Phase two tested the species' filtration properties in the field, where they could have a significant impact. Both P. ostreatus and G. frondosa reduced soil contaminants in lab and field studies. This solution effectively balances environmental protection efforts with sustainable development by implementing a flexible, innovative, and cost-friendly solution that does not involve a halt in development. Small-scale mushroom sites have real potential to target sources of contamination and act as a barrier to protect the watershed system.

2b. Table of Contents

2c. Key Words

bioremediation · Grifola frondosa · hydrocarbon · lignin · mycofiltration · mycelium · mycoremediation · nitrogen · pesticide · phosphorus · Pleurotus ostreatus · polychlorinated · biphenyls · runoff · sargassum · watershed · white rot fungi

2d. Abbreviations and Acronyms

P. ostreatus (Pleurotus ostreatus) · G. frondosa (Grifola frondosa) · liP (lignin-peroxidase) · MnP (manganese peroxidase) · H_2O_2 (hydrogen peroxide) · ppb (parts per billion) · ppm (parts per million)

2e. Acknowledgements

I am grateful to Martin Rydlo and Stacie Smith, owners of Duntroon Highlands Golf, who permitted soil sampling and planting of test locations on their golf course. I am thankful for the guidance of Liesbeth

Halbertsma, a teacher, who provided invaluable introductions to local environmental groups and helped facilitate connections with Duntroon Highlands Golf.

2f. Biography

Ava Fischer, a grade eleven student from Collingwood, Ontario, is passionate about improving the environment and making positive changes in the world. Her interests include science, astronomy, writing, and mathematics. She actively volunteers with her local watershed trust, where she helps extract phragmites and connects with other environmental enthusiasts. In addition to her environmental volunteer work, Ava contributes to her community by designing and spearheading local food donation bins for seniors in need, sewed reusable masks for local citizens in 2020, and serving as a Government Relations Officer and Team Captain at Future Majority. For two years, she worked with this organization to amplify young voices and advocate for issues affecting young people, especially those related to the environment and climate change. Ava also loves playing basketball competitively and writing songs on the guitar. Living in a small town surrounded by Georgian Bay and the Niagara Escarpment and volunteering with her local watershed have made Ava acutely aware of the decline in local water quality caused by rapid development in her area. These experiences have fueled her passion for preserving and protecting local watersheds and the environment. Ava has participated in four Canada-Wide Science Fairs, earning bronze in 2019, silver in 2021, bronze in 2022, and gold in 2023. She has also published a scientific journal article with the Canadian Science Fair Journal, earned a SHAD scholarship, and will attend a SHAD program in July 2023. With her 2023 Science Fair project, she received the *University of Ottawa Undergraduate Research Scholarship* and the *Youth Science Canada Senior Challenge Award for Environment and Climate Change*. Ava is determined to make a difference in her community and continues to seek opportunities to gain skills and experience. Her passion has inspired her to pursue environmental sciences in her future studies.

3. INTRODUCTION

Rapid population growth puts pressure on infrastructure and ecosystems, negatively affecting water quality. The proliferation of impervious surfaces arising from urban development and construction, such as parking lots and roads, provide a superhighway for pollutants, in the vehicle of runoff, to enter the water system and destabilize ecosystems.

Further, petroleum-based soil pollutants lead to water and oxygen shortages and limit soil nutrient availability. Local indicators of watershed health show the presence of hydrocarbons, pharmaceuticals, harsh cleaning agents, pesticides, fertilizers, and household waste, with increased runoff significantly contributing to the decline of watershed well-being [1]. Industrialization and contaminated waters are closely linked. According to local indicators of watershed health, many local water sources contain harmful contaminants that debilitate ecosystems and harm aquatic life [2].

The proliferation of sargassum, a type of seaweed, in the tropical Atlantic Ocean and Gulf of Mexico has become a pressing concern, with agricultural runoff identified as a key contributor. The fertilizers utilized in agriculture contain high levels of nitrogen and phosphorus, which can cause an overgrowth of algae and seaweed when washed into the ocean [3]. This overgrowth can, in turn, generate large mats of sargassum that can prove detrimental to marine life and shorelines. When these mats wash up on the beach, they release hydrogen sulfide gas that can lead to respiratory problems for humans [4]. Therefore, it is imperative to reduce the amount of agricultural runoff that enters the ocean to prevent the growth of sargassum and protect marine ecosystems and human health.

According to the Office of the Director of National Intelligence for the United States of America, due to rising demand and limited availability, countries worldwide will be more vulnerable to water scarcity during the next two decades. There is a higher risk of illness, slow economic growth, and political instability in countries that do not handle water-related problems effectively. As water supplies become less secure and geopolitical competition heats up, water resources are more likely to be a source of global conflict. Thus, innovative strategies must be employed to protect water sources.

Mycelium-based root systems of fungi isolate and break down soil contaminants through mycoremediation [5]. This mycoremediation process employs fungi to isolate pollutants. Fungi produce oxalic acid, an enzyme that expedites the breakdown of pollutants [5]. P. ostreatus and G. frondosa filter and absorb chemicals to improve hydrocarbon-contaminated soil [6].

This study investigated the use of fungi native to North America that secrete enzymes to break down pesticides, hydrocarbons, and lignocellulose-containing contaminants in soil due to runoff and assessed the potential of these fungi to bioremediate contaminants and mitigate the negative environmental impacts of development on ecosystems.

4. PURPOSE

This project aimed to address and combat the adverse effects of development on watersheds and establish a cost-effective way to protect watersheds where development and population growth are on the rise through the utilization of fungi native to North America that release enzymes to decompose pesticides, hydrocarbons, and waste containing lignocellulose. This approach aimed to prevent contamination from entering a watershed system at its origin, which would ultimately aid in the protection of ecosystems and water quality. The purpose of this experiment was to determine the mycoremediation properties of P. ostreatus and G. frondosa to evaluate their potential to filter contaminants, including those identified by the Blue Mountain Watershed Trust as the greatest threats to the local water system, such as increased contaminated runoff due to development, agricultural pesticides, the aggregate industry, and golf-course maintenance [7]. The first phase of this experiment tested the ability of P. ostreatus and G. frondosa to improve the quality of water samples in a simulated streambed. The second phase of this experiment tested the effectiveness of P. ostreatus and G. frondosa in filtering contaminants through microfiltration from runoff before entering a tributary stream in the watershed system in a field study.

5. Hypothesis

It was hypothesized that the white rot fungi, P. ostreatus and G. frondosa would be successful in the mycoremediation of water contaminated with pollutants to improve water quality in a watershed. Previous studies have found that white rot fungi are among the most efficient lignin-degrading organisms and play a significant role in the global carbon cycle [8]. Extracellular lignin-modifying enzymes with low substrate requirements are released by P. ostreatus and G. frondosa, enabling them to react with various molecules that resemble lignin. Enzymes that break down lignin include lignin-peroxidase (liP), manganese peroxidase (MnP), enzymes that produce hydrogen peroxide (H_2O_2), and laccase [9]. A variety of toxic pollutants, such as polycyclic aromatic hydrocarbons, polychlorinated biphenyls, azo-dyes, pesticides, and trace pharmaceuticals, can be metabolized and broken down by white rot fungi thanks to their unique extracellular nonspecific ligninolytic system and intracellular oxidizing enzymes [10].

6. MATERIALS AND METHODS

6a. PHASE 1: IN VITRO TESTING

Simulated streambed apparatuses were built to replicate natural water flow indoors. 3/16” holes were drilled 1” from the bottom of the middle of the long plastic bin at both ends (one for water entry, one for water exit). Six three-foot lengths of plastic tubing were connected with silicone adherent to the three-foot length of tubing to the bin (this is the front end of the simulated streambed). A 1.5” square of fine plastic mesh was cut, and silicone adhesive was applied to cover the end of the tubing inside the bin. Next, a 6’ length of tubing was attached to the other end of each bin using the adhesive (this returned the water for redistribution - the back end of the simulated streambed). Repeat for each bin. Insert a rubber stopper firmly at the end of both tubes in each of the six bins (two per bin).

Straw was soaked in a plastic pail in diluted household bleach (1 part bleach to 100 parts water, ~ 5.25 ppm sodium hypochlorite) for two minutes. Then, a mycelia-straw substrate was mixed and planted along the "banks" of the simulated streambed. The straw was drained and rinsed using the cold-water faucet sprayer for ten minutes.

One hundred fifty grams of straw was combined with 300 g of mycelium and added to a plastic pail. The mixture was split in half and packed into one side of the first plastic bin. Then the remaining mixture was packed into the other side of the same bin to form the simulated stream bank. The process was repeated to make three simulated streambeds for each species.

Water samples were collected from a local water source, tested for contaminants, and cycled through the simulated streambed apparatus for 25 days. Water contamination levels were tested every three days using water testing strips.

6ai. PHASE 1A: WINTER TESTING

In January 2023, water samples from Pretty River, a major tributary in the Blue Mountain watershed, were tested and cycled through the simulated streambed apparatus.

6aii. PHASE 1B: SPRING TESTING

In April 2023, water samples from a local harbour and ski hill were tested and cycled through the simulated streambed apparatus.

6b. PHASE 2: FIELD TESTING

Straw and 1.4 m x 0.5 m pieces of burlap were sanitized with a diluted sodium hypochlorite solution as described in Phase 1. Mycelia of P. ostreatus and G. frondosa were planted on a golf course at 12 sites between the golf green and tributaries connected to the local watershed system where they have the potential to create a barrier between the golf green and the local watershed system via small tributaries. Approximately one meter long by 30 cm wide trenches were dug in the soil in low-lying areas between the golf green and two small tributaries. The trenches were lined with burlap then 5 cm of prepared straw was placed on the burlap in the trench. Next, a layer about 3 cm thick of mycelia (P. ostreatus or G. frondosa) was mixed with straw, then layered with 3 cm of straw. Another 3 cm layer of mycelia-straw mixture was layered and then covered with a 5 cm layer of straw. An initial soil sample was taken from each site and tested. Then soil testing was conducted every three days. Field testing is ongoing.

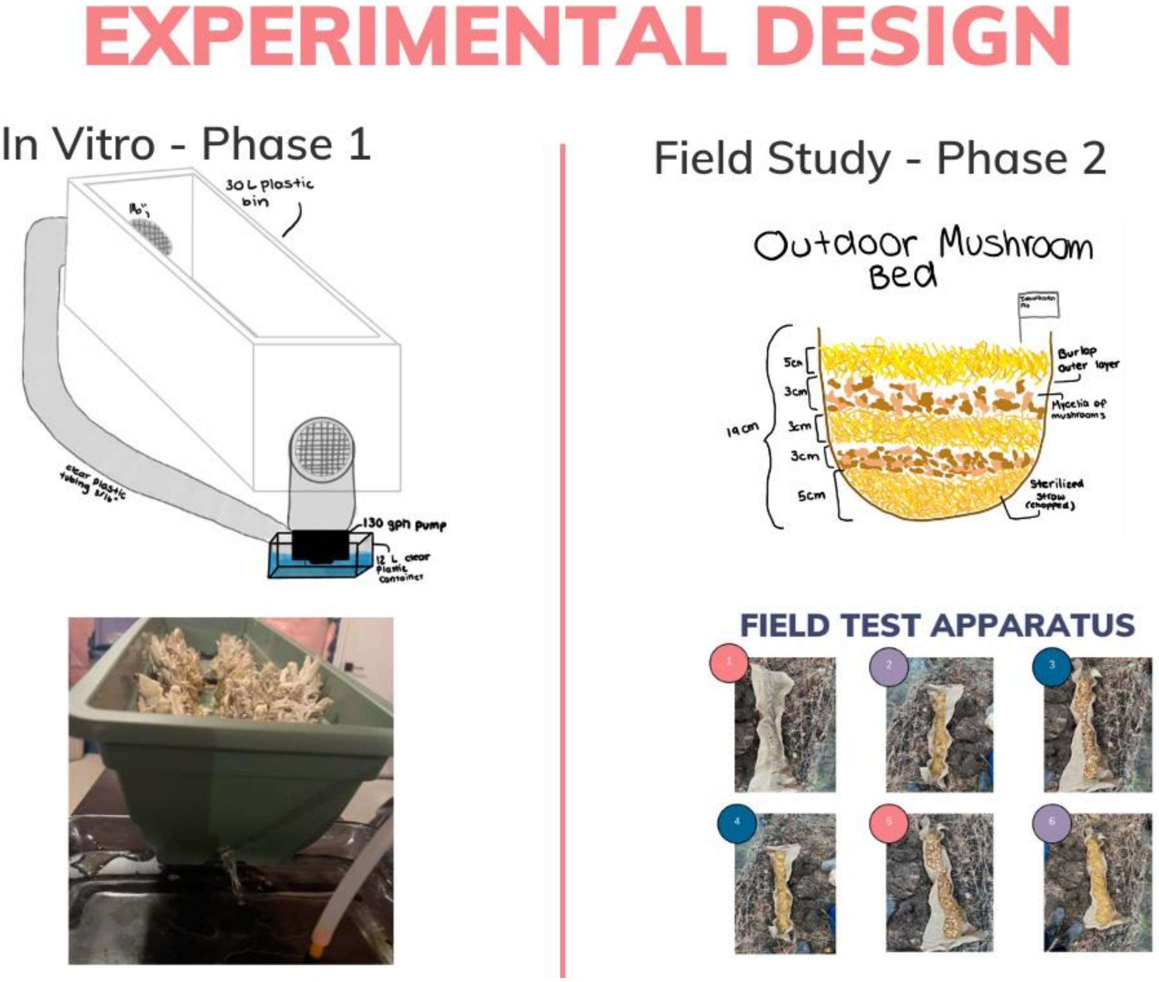

Figure 1 shows the experimental design for Phase 1 and Phase 2.

7. RESULTS

7a. Phase 1: In Vitro Testing

P. ostreatus and G. frondosa trials reduced contaminants from water samples. The mean reduction of 4.3 ppb for lead and 5.7 ppm of phosphorus was notable. Spring samples from the harbour and ski hill showed the highest initial contamination levels.

Filtration most often leveled out by day 19. Typically, phosphorus levels reduced most quickly, and levels of lead lowered more slowly. Lead levels did not drop below 2.5 ppb, except in the ski hill reservoir sample filtered by G. frondosa which dipped to 1.25 ppb.

Across both species of white rot fungi, copper and zinc were the least affected across both white rot fungi species, and nitrate and sulfate were eliminated at the highest rate during the testing phase.

These results are promising, as nitrate was present in all initial soil samples gathered from field testing, and sulfate was detected in high concentrations.

7b. Phase 2: Field Testing

The field study is ongoing as environmental conditions were not conducive to fungi growth until mid-spring. The initial number of contaminants in the sample was greater in the field than in vitro.

A golf course was selected for the field study because the Blue Mountain Trust identified golf course chemicals as a significant threat to the watershed system [7].

Evidence of mycofiltration was identifiable more than three times sooner in the field study.

Preliminary observations indicated that G. frondosa had a higher mean reduction rate (22.5 mg/kg) of nitrogen than P. ostreatus (15.8 mg/kg), further indicating that this species of white-rot fungi would be best placed near agricultural, golf-course sites, or other locations where commercial fertilizers are applied. In addition, in the field, G. frondosa initially showed the most tolerance to and reduced hydrogen sulfide levels sooner in the soil compared to P. ostreatus. Results indicate that P. ostreatus (mean reduction rate 5 mg/kg) is more effective at reducing lead levels than G. frondosa (mean reduction rate 1.66 mg/kg).

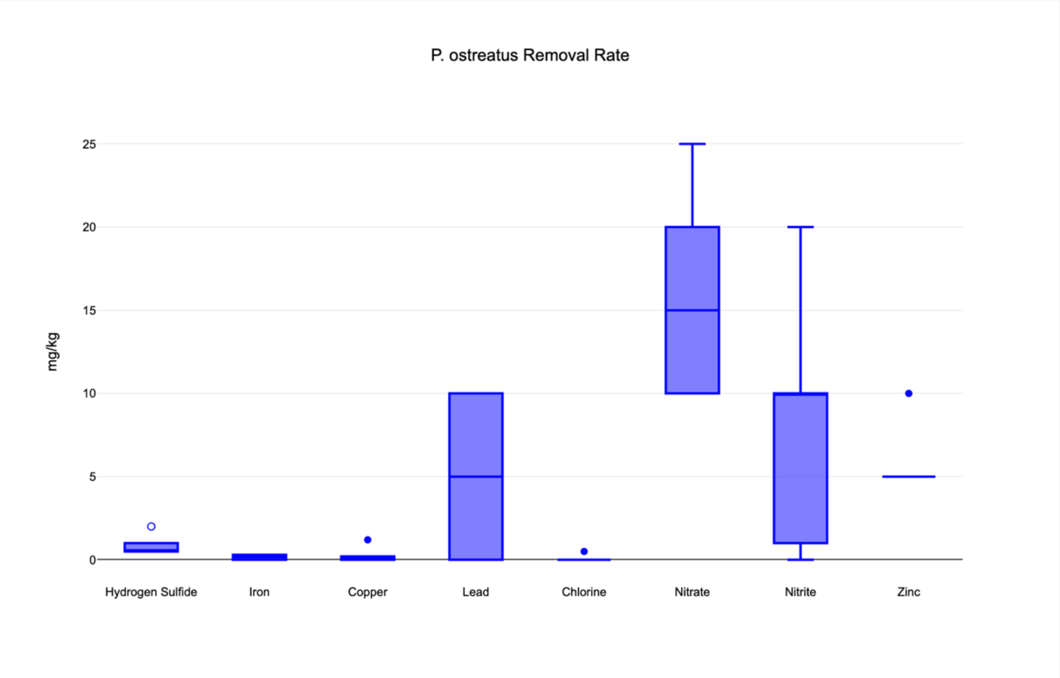

Figure 2 Removal Rate of P. ostreatus in field test

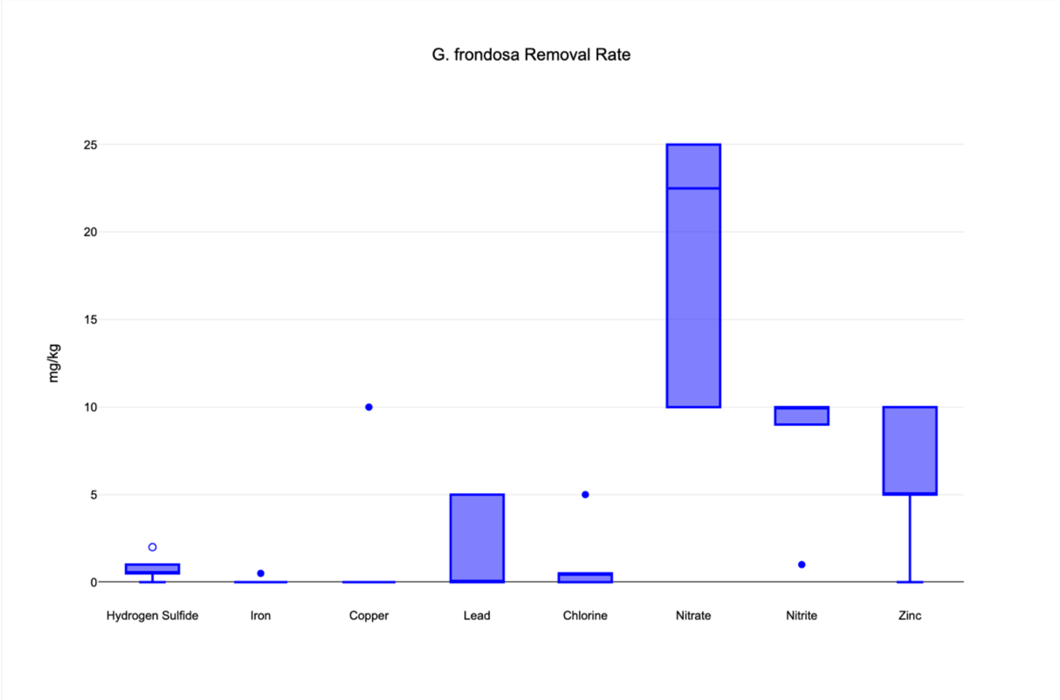

Figure 3 Removal Rate of G. frondosa in field test

6. DISCUSSION

This experiment had some limitations. First, this experiment didn’t assess the role of other organisms in the bioremediation process. Fungi are just one of the primary bioremediators; bacteria and archaea also play a role. In future studies, assessing microbial biomass would be beneficial to further analyze the results. In addition, this experiment didn’t assess methods to dispose of excess biomass. Laboratory testing of both P. ostreatus and G. frondosa after the mycoremediation process to determine the level of contaminants such as heavy metals would impact the disposal method of excess biomass.

This targeted, low-cost micro-solution is capable of diverting contaminants from entering the watershed system. For example, nitrogen is necessary for plant growth; however, excess nitrates can disrupt aquatic ecosystems. This is because it speeds up the process of eutrophication, which results in the overgrowth of aquatic plants and an imbalance in the ecosystem's organisms [11]. This example highlights the importance of just one contaminant studied. The results of this solution are promising, as it led to a reduction of nitrates by 167–200% in vitro and 84% by day 4 in the field study. These findings demonstrate the potential of this solution to address the challenges posed by contaminants in the watershed system.

Few studies have explored the effect of mycofiltration on excess soil nitrogen levels. This issue is especially relevant as excess fertilizers entering the water system contribute to imbalances in the ecosystem, as evidenced on beaches across the Gulf of Mexico and the tropical Atlantic Ocean blanketed in unprecedented amounts of sargassum. This experiment aimed to build upon previous studies that looked at remediation after contamination and investigated the effectiveness of targeted, small-scale mycoremediation as a barrier between contamination sources and a watershed system.

Some studies have focused on the effectiveness of mycofiltration on greywater and stormwater runoff in urban areas, such as Ledford’s “Examining Mycofiltration Efficacy in a First Order Stream” out of Georgia State University. Additionally, some studies, such as Martinez’s “E. Coli Removal by P. ostreatus Mycofilter in Simulated Wet Environmental Pond” from the University of New Mexico, focus more on P. ostreatus’ efficacy in removing bacteria from stormwater runoff ponds.

A substantial body of research exists that focuses on mycofiltration's efficiency in removing hydrocarbons, such as Wu et al.'s 2008 study "Bioremediation of polycyclic aromatic hydrocarbons contaminated soil with monilinia degradation and microbial community analysis." Many of these studies move contaminated soil from a site to a waste treatment site where mycoremediation of the soil occurs.

The solution's flexibility and relative cost-effectiveness are its superpowers. Targeting sources identified as threats to a water system or quickly implementing a remediation solution in an acute environmental pollution scenario makes the solution an imperative tool for sustainable development and environmental protection.

As our cities continue to grow and expand, finding innovative solutions that balance development with the protection of our watershed systems is becoming increasingly important. Urbanization can significantly impact delicate ecosystems, as increased development often leads to higher levels of pollution and other forms of environmental degradation. This experiment focused on developing small-scale, flexible solutions that reduce the quantity of contaminants entering the watershed system through runoff.

Ultimately, the key to successful urban development lies in finding a balance between the needs of growing cities and protecting natural resources, especially water. The simplicity of this solution belies its value as a usable, innovative solution that balances development with nature to create vibrant, thriving communities that are both environmentally sustainable and economically prosperous.

7. CONCLUSIONS

As stated in the hypothesis, the white rot fungi, P. ostreatus and G. frondosa were successful in the mycoremediation process, preventing runoff contaminated with pollutants from entering the watershed system. P. ostreatus and G. frondosa release extracellular lignin-modifying enzymes with low substrate requirements, allowing them to react with a variety of molecules that resemble lignin. The results of the experiment supported the hypothesis. P. ostreatus and G. frondosa were used to filter runoff, successfully removing nitrates, nitrites, some metals, including copper and lead, and other contaminants. The Phase 1 winter samples did not contain zinc or sulfite; thus, it was impossible to determine whether they were effective at removing them from the samples.

A watershed's natural stormwater runoff flow is altered by development which affects water quality. When it rains, large quantities of stormwater runoff from impervious surfaces such as parking lots, streets, and rooftops transport pollutants that the surfaces have absorbed. Increased runoff erodes and threatens to destroy stream channels, and pollutants affect plants and wildlife in rivers, streams, and bays.

Field testing commenced on April 26, 2023, when conditions were conducive to fungi growth. Data collection is ongoing. Based on research and observation, contaminant levels are being measured through soil samples rather than water samples.

This low-cost micro-solution can divert contaminants from entering the watershed system. Establishing ways to protect watersheds where development and population growth are rising would provide cleaner water, protect the environments of growing municipalities, and improve the well-being of Canadian citizens, both environmentally and economically.

Environmental protection efforts can be effectively balanced with sustainable development by implementing innovative approaches such as this solution involving mycoremediation.

A practical plan to scale up this solution using P. ostreatus and G. frondosa to address the contamination from runoff in high-risk entry points of the watershed system needs to address the following considerations:

1. Identify High-Risk Entry Points:
    - Determine the specific streambanks adjacent to golf courses and agricultural land, as well as wetlands collecting runoff from areas with increased impervious surfaces.
    - Conduct a thorough assessment of these locations to prioritize high-risk entry points based on contamination severity and potential impact on the watershed system.
2. Obtain Necessary Resources:
    - Establish sustainable sources of P. ostreatus and G. frondosa mycelium for large-scale production.
    - Acquire basic and inexpensive materials such as straw and burlap to create barriers with the mycelium.

3. Develop Planting and Monitoring Protocols:
    - Devise a systematic approach for planting the mycelium barriers at identified high-risk entry points, ensuring proper coverage.
    - Implement a monitoring system to assess the effectiveness of the mycofiltration solution in reducing soil contaminants, including nitrogen, heavy metals, and hydrogen sulfide levels.
    - Establish regular monitoring intervals and data collection methods.
4. Address Manpower Requirements:
    - Recognize the need for manpower due to the challenging locations of many high-risk entry points.
    - Collaborate with stakeholders, including community volunteers, environmental advocacy groups, and government organizations, to provide the necessary workforce for planting and monitoring activities.
5. Financial Considerations:
    - An awareness and education campaign that highlights the benefits of this low-cost solution, emphasizing its financial benefits for private, municipal, provincial, or federal groups is needed to ensure the solution is sustainable.
    - Seek funding opportunities and partnerships with relevant stakeholders to invest in the scaling process and support long-term implementation.
6. Expand Collaboration and Engagement:
    - Foster partnerships with mycelium producers, environmental advocacy groups, government organizations, and private businesses to leverage their expertise, resources, and networks.
    - Conduct awareness campaigns and educational initiatives to engage local communities and stakeholders, highlighting the benefits of mycofiltration and encouraging their active participation and support.
7. Develop a Systematic Approach:
    - Create a step-by-step plan for upscaling, including site selection, mycelium production, planting, monitoring, and, if necessary, removal processes.
    - Continuously evaluate and refine the approach based on feedback, data analysis, and emerging scientific knowledge.

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