



Utilization of Indigenous Agricultural Waste Materials as Biofilters in Contaminated Water

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Abstract

Bioremediation using spent Oyster mushroom (*Pleurotus* sp.) substrate (*Pleurotus* SMS), wood chips, and charcoal offers a cost-effective and environmentally friendly approach to water treatment. However, further study is needed to determine its application in Isabela province due to differences in the composition of mushroom spent substrate in the Philippines compared to those in other countries. Hence, this study was conducted to develop a prototype water treatment system integrated with different agricultural waste materials to treat heavy metals, such as cadmium (Cd) and arsenic (As), and *Escherichia coli* contamination in water. A prototype water treatment system with separate compartments for *Pleurotus* SMS (200 g), wood chips (70 g), and charcoal (180 g) as biosorbents was developed. Separate biofilter experiments for Cd and As, and *E. coli* were performed at various flow rates (1 mL/min, 5 mL/min, and 10 mL/min) and sampling times, and the percent removal and bacterial concentration were calculated. The water treatment system with mushroom spent substrate registered the highest efficiency at the slowest flow rate of 1 mL/min (i.e. 97% for Cd and 95% for As). However, of the two heavy metals, As significantly registered a higher percent removal than Cd, even at faster flow rates, suggesting that the filter had a greater affinity towards As than Cd. The decrease in *E. coli* levels from 154 CFU/mL to an average of 15 CFU/mL over 48 hours of filtration indicates that biofiltration could be a feasible substitute for conventional membrane-based methods. The fabricated water treatment system based on oyster mushroom spent substrate is effective for the removal of pathogens as well as Cd and As from contaminated water. These findings can be used as a basis for a sustainable solution for treating heavy metals from industrial wastewater and simultaneously solving the waste issue in mushroom farms.

Keywords – Arsenic – Cadmium – charcoal – *Escherichia coli* – mushroom spent – mycoremediation – wood chip

Introduction

Water pollution, particularly by heavy metals and pathogens, poses serious threats to the environment, human health, and agricultural productivity. Contaminants such as cadmium (Cd) and arsenic (As) are particularly problematic due to their toxicity, persistence, and ability to bioaccumulate in the ecosystem (Srivastava et al. 2015). Moreover, coliforms in water, such as

Escherichia coli, are the leading cause of gastrointestinal diseases in humans (WHO 2023). The increasing levels of pollutants, including heavy metals, biological oxygen demand (BOD), chemical oxygen demand (COD), total dissolved solids (TDS), and pathogenic bacteria, make water unsuitable for drinking, irrigation, aquaculture, and other uses. The improper discharge of industrial and agricultural effluents into water bodies disrupts the ecological balance, leading to diminished water quality. Foundational studies have shown that using untreated effluents for irrigation negatively impacts plant growth and yield while contributing to the degradation of soil health (Schroeder & Balassa 1963, Nandy & Kaul 1994). With these persistent challenges, there is an urgent need for sustainable and efficient water remediation technologies, particularly in regions like the Philippines, where industrialization, mining, harmful agricultural practices, and population growth have further strained water resources (Magahud et al. 2015, Kapoor & Sing 2021, Diwa et al. 2023, Sabijon et al 2025).

Using living organisms to detoxify polluted environments, bioremediation offers a cost-effective and environmentally sustainable solution for treating water, soils, and sediments contaminated with heavy metals and organic pollutants. Bioremediation techniques can be classified into two main types: (1) in situ bioremediation, which optimizes the activity of native microbial communities to degrade contaminants, and (2) ex-situ bioremediation, which involves the introduction of microbial inoculants to the contaminated site (EPA 1992). In both approaches, the bioavailability of the contaminant, as well as optimal environmental conditions (e.g. temperature, pH, oxygen levels, and nutrient supply), are critical factors for successful remediation.

Among biological agents, fungi are dominant decomposers in nature due to their ability to secrete powerful enzymes that degrade organic matter and adsorb inorganic pollutants (Moore & Chiu 2001). Fungi are also known for their combative growth, extensive biomass production, and intricate hyphal networks, making them ideal candidates for bioremediation (Chiu et al. 2009). Mycoremediation, a branch of bioremediation that employs fungi to degrade or transform environmental contaminants such as industrial effluents, sewage sludge, and heavy metals, is increasingly recognized for its environmental applications, with fungal species—particularly those belonging to the genus *Pleurotus* (Oyster mushrooms)—demonstrating promising capacity for the remediation of heavy metal-contaminated environments (Ashoka et al. 2002, Stamets 2005).

Mushroom cultivation is one of the largest solid-state fermentation industries globally (Moore & Chiu 2001). In the Philippines, oyster mushroom (*Pleurotus* sp.) farming has gained popularity due to its low-cost production using agricultural waste materials such as rice straw and corn cobs (Mina et al. 2020, Quion et al. 2023). The byproduct of this cultivation, known as spent mushroom substrate (SMS), is often discarded despite containing residual nutrients and enzymes capable of further environmental use (Ball & Jackson 1995, Semple et al. 2001). In the province of Isabela, where cultivating this mushroom species has become popular, challenges arose, particularly in managing solid waste from the used Oyster mushroom fruiting bags, besides stocking them. Interestingly, high levels of residual nutrients and enzymes are still left in the spent substrate (Ball & Jackson 1995, Chiu et al. 1998, Semple et al. 2001). Previous studies have demonstrated the effectiveness of SMS in treating heavy metal contamination and coliform presence in water (Ahlawat et al. 2010, Srivastava et al. 2015, Corral-Bobadilla et al. 2019). However, the potential of SMS produced in the Philippines for local applications, such as in Isabela Province, has not been thoroughly investigated.

Given the unique composition of SMS from local agricultural waste, this research explores its potential as a biosorbent for removing cadmium (Cd), arsenic (As), and *E. coli* from water. In this study, spent *Pleurotus* substrate from mushroom farms in Isabela Province was integrated into a prototype water treatment system designed to remove heavy metals. The objectives of the study include fabricating and testing a water treatment system that utilizes spent substrate as a biofilter; evaluating the biosorption capacity of mushroom spent substrate for Cd and As; assessing the biofilter's efficacy in removing bacterial contaminants; and determining the optimum water flow rate to maximize heavy metal removal. Our results provide insights into the applicability of indigenous agricultural waste materials for water remediation, contributing to the solution of two major

environmental challenges: managing agricultural waste and purifying contaminated water. By developing a sustainable and cost-effective water treatment system, this study aimed to address the growing need for clean water in the province of Isabela while promoting the reuse of agricultural byproducts.

Materials & Methods

Design of the prototype water treatment system

The fabricated prototype water treatment system has a 20-L water capacity and utilizes a sump filtration design comprising four distinct compartments where different biosorbents are placed (Fig. 1, Table 1). These compartments are specifically designed to perform various treatment functions.

The first compartment (A) is dedicated to particulate removal. It employs a combination of pebbles and sand to effectively filter out larger suspended solids and debris. The second compartment (B) addresses coliform treatment through the sterilization by wood chips as the water passes through it. These wood chips promote microbial activity that can further reduce coliform levels in the water. The third compartment (C) incorporates pulverized charcoal, which enhances the adsorption of organic compounds and other contaminants, thereby improving overall water quality. Finally, the fourth compartment (D) employs *Pleurotus* sp. spent mushroom substrate (*Pleurotus* SMS), specifically designed for the targeted removal of heavy metals and *E. coli*.

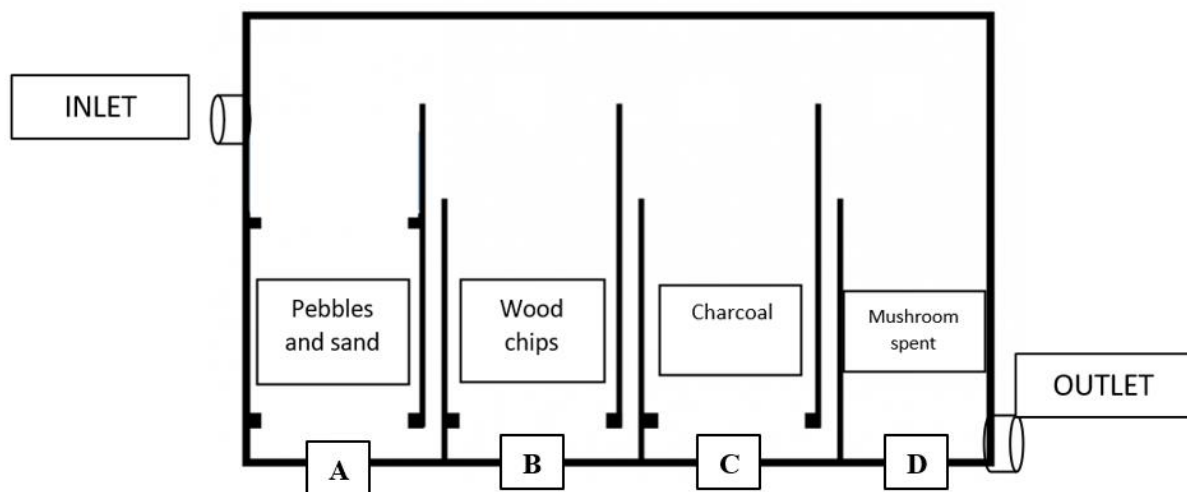


Fig. 1 – Design of the prototype water treatment system using indigenous waste. (A) pebbles and sand, (B) waste wood chips, (C) pulverized charcoal, (D) *Pleurotus* spent mushroom substrate.

Table 1 – Biosorbents used in the prototype water treatment system and their corresponding weight

Biosorbents	Weight (g)
Sand	600
Pebbles	600
Woodchips	70
Charcoal	180
<i>Pleurotus</i> spent mushroom substrate	200

Approximately 20 Kg of *Pleurotus* SMS was sourced from a mushroom farm in San Mateo, Isabela, Philippines. The substrate was pulverized and sieved to achieve a granular size of ≤ 2 mm, facilitating a larger surface area for biosorption. In parallel, Gmelina and Narra wood chips and charcoal, sourced from various furniture shops and public markets in Isabela Province, Philippines,

were washed, pulverized, sieved, and sterilized using the same autoclaving procedure to ensure sterility. These biosorbents were then dried thoroughly to prevent moisture interference during filtration. Before experimentation, all materials were thoroughly washed with deionized water to remove any residual contaminants. The substrate was then sterilized in an autoclave at 121°C and 15 psi for 60 minutes to eliminate any potential microbial interference. Following sterilization, the substrate was dried in an oven at 40°C until it reached approximately 80% dryness, optimizing its physical characteristics for subsequent applications.

Efficacy of the prototype system in heavy metal removal

A G-clamp was employed to generate adjustable resistance, thereby regulating the flow rates through the system. Flow rate is a pivotal parameter in this system, as it directly influences the contact time between the water and the active materials, which is paramount for optimizing the biosorption process. Synthetically contaminated water was formulated as standard solutions of cadmium (Cd) and arsenic (As) with initial concentrations of 1 ppm and 0.5 ppm in distilled deionized water. This controlled preparation facilitated the systematic evaluation of the biosorption capacity of the *Pleurotus* SMS and wood chips in subsequent experiments.

A separate experimental setup was established and triplicated for Cd and As water solutions at different flow rates simultaneously. Initially, 20 L of synthetic contaminated water was filled into a container connected to the inlet, with three distinct flow rate treatments: 1 mL/min, 5 mL/min, and 10 mL/min. These rates were selected to optimize the flow rates at which sulfate reduction, a slow biochemical process in which heavy metals are reduced, works efficiently (Corral-Bobadilla et al. 2019). Once the water from the container is emptied and passes through the biofilter, the first 1-L water sample is collected in the outlet on each replicate. To preserve the water samples, 2 mL of nitric acid was added to each water sample. Subsequently, all treated water samples were stored in a Styrofoam ice box surrounded by ice. For safety and to prevent contamination, gloves, face masks, face shields, and laboratory gowns were worn throughout the experiment. These samples were subsequently transported to the Department of Science and Technology, Regional Standards and Testing Laboratory, Tuguegarao City, Philippines, for heavy metal content analysis using a graphite furnace atomic absorption spectrometer (GFAAS).

Based on the initial and final Cd and As concentrations, the remediation performance of the water treatment system was evaluated by calculating the percentage of heavy metal removal using the following formula:

$$\% \text{ removal} = \frac{\text{initial concentration} - \text{final concentration}}{\text{initial concentration}} \times 100$$

Efficacy of the prototype system in bacterial elimination

An overnight culture of *Escherichia coli* (ATCC 25922) was sourced from the culture collection of the Microbiology and Bio-industry Laboratory. To prepare the primary influent for biofilter testing, a bacterial suspension was generated by inoculating 20 mL of sterile double-distilled water (DH₂O) with a known concentration of *E. coli*. The initial bacterial concentration was accurately quantified using a hemocytometer. Based on this measurement, an 8-liter stock solution was prepared and standardized to a final concentration of 30 cells/mL by diluting appropriate aliquots of the suspension with sterile double-distilled water (DDW). This standardized stock served as the primary influent for the biofilter system, ensuring consistent and controlled conditions for evaluating bacterial removal efficiency.

All materials and solutions used in the preparation were maintained in sterile conditions to minimize the risk of introducing contaminants. A membrane-filtered water sample was utilized as a control to provide a baseline comparison for bacterial elimination efficiency. Effluent and influent samples, each measuring 20 mL, were collected every 12 hours over 48 hours and processed immediately to ensure the accuracy of the results. To assess bacterial concentrations, dilutions were prepared at 10⁵ levels, allowing for effective enumeration of colonies. Subsequently, 100 µL of each

dilution was pipetted onto Luria Bertani (LB) agar plates, which were then incubated at room temperature for 72 hours to facilitate bacterial growth. After the incubation period, bacterial colonies on each plate were counted to determine the concentration of *E. coli* in each sample, expressed as colony-forming units (CFU) per milliliter.

Data analysis

The heavy metal percentage removed at different flow rates (mL/min) in triplicate was presented as mean $\pm$ standard error. Actual values of *E. coli* concentration (CFU/mL) at various water sampling times were given. The Kruskal-Wallis test determined the significant difference between flow rates and sampling times at a 95% significance level.

Results

Heavy metal removal and flow rate optimization

To quantitatively evaluate the remediation performance of the prototype water treatment system, synthetically contaminated water samples spiked with cadmium (Cd) and arsenic (As), with initial concentrations of 1 ppm and 0.5 ppm, respectively, were separately treated at three flow rates: 1 mL/min, 5 mL/min, and 10 mL/min. The results demonstrated that the percentage of heavy metal removal increased at slower flow rates (Fig. 2). At the slowest flow rate of 1 mL/min, the system achieved removal efficiencies of 97% (0.034 ± 0.033 ppm) for Cd and 95% (0.025 ± 0.0005 ppm) for As.

At 5 mL/min, Cd removal dropped to 86% (0.14 ± 0.01 ppm), while As slightly decreased to 0.0795 ± 0.0305 ppm. At the highest flow rate of 10 mL/min, Cd removal decreased to 84% (0.157 ± 0.005 ppm), while As removal remained relatively high at 0.03 ± 0.006 ppm. Statistical analysis using the Kruskal-Wallis test revealed that the differences in Cd removal across flow rates were not statistically significant ($p = 0.0614$). Similarly, variations in As removal were also statistically non-significant ($p = 0.1801$).

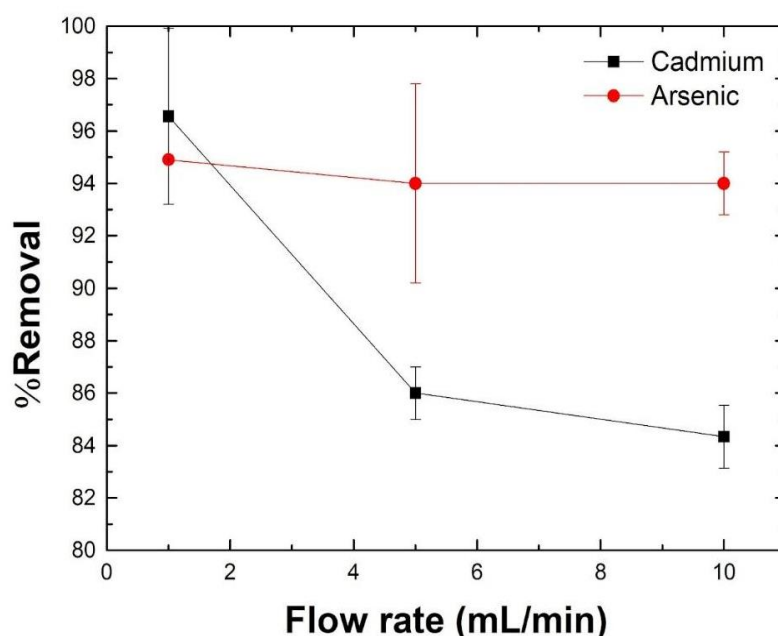


Fig. 2 – Mean ($\pm$ se) percent removal of Cd and As at different flow rates (1 mL/min, 5 mL/min, and 10 mL/min) using the prototype water treatment system.

Bacterial mycofiltration efficacy

This study segment evaluated the efficacy of the fabricated biofilter compared to other filtration systems, specifically pure *Pleurotus* SMS and a membrane filter. The fabricated biofilter,

incorporating *Pleurotus* SMS, demonstrated high efficacy in reducing bacterial contamination, lowering the *E. coli* concentration to an average of 15 cells/mL after 48 hours. Various treatments were assessed, including untreated water, pure *Pleurotus* SMS, and the membrane filter. The results indicated that the fabricated biofilter exhibited the highest performance in bacterial reduction, achieving an average concentration of 15 cells/mL after 48 hours of filtration (Fig. 3). In comparison, pure *Pleurotus* SMS also demonstrated a significant reduction in bacterial counts, with an average of 19 cells/mL after the same duration. Conversely, the membrane filter did not yield any detectable bacterial count, suggesting its effectiveness in eliminating bacterial presence.

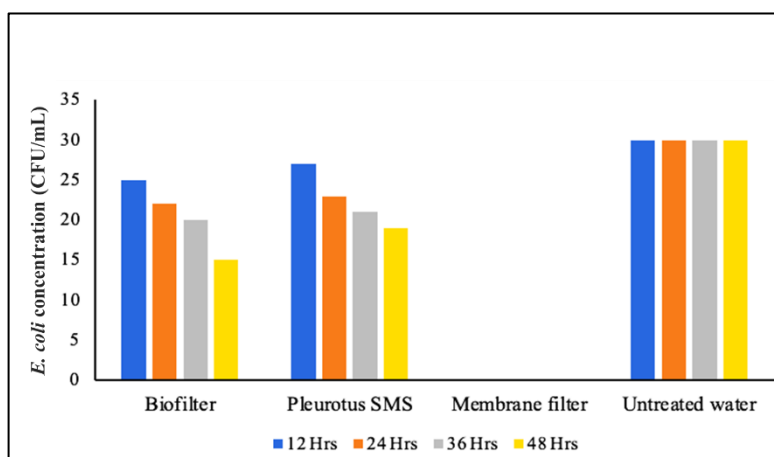


Fig. 3 – *Escherichia coli* concentration (CFU/mL) at different prototype water treatment set-ups over 48 hours.

Discussion

The outcomes of this study demonstrate that *Pleurotus* SMS, wood chips, and charcoal derived from indigenous agricultural waste materials offer a highly effective, eco-friendly solution for the remediation of water contaminated with heavy metals such as cadmium (Cd) and arsenic (As) and putative pathogens such as *E. coli*. The research specifically aimed to develop a sustainable prototype water treatment system that integrates agricultural waste into bioremediation efforts, thus addressing water pollution and waste management challenges. The system's high removal efficiency for heavy metals and *E. coli* aligns with global efforts to develop low-cost, sustainable, and locally accessible water treatment solutions (Ghosh et al. 2018, Bolisetty et al. 2019).

The biofilter experiment results indicate a clear relationship between flow rate and removal efficiency, emphasizing the importance of contact time in the biosorption process. At the lowest tested flow rate of 1 mL/min, the system achieved the highest removal performance: 97% for Cd and 95% for As. This trend reinforces the well-established principle that longer residence time enhances the interaction between contaminants and biosorbent materials, allowing more effective diffusion and binding of heavy-metal ions onto the available active sites in the surface area of the SMS (Corral-Bobadilla et al. 2019).

Slower flow rates allow heavy metals such as Cd^{2+} more time to cross the liquid film boundary, penetrate the biosorbent matrix, and interact with the sorption sites, resulting in higher adsorption efficiency (Chintokoma et al. 2024, Hameed et al. 2024). In contrast, higher flow rates reduce contact time and may lead to incomplete adsorption. The elevated velocity may also contribute to shear-induced desorption of loosely bound Cd, a phenomenon previously observed in packed-bed systems (Sazali et al. 2020). The superior performance of the spent mushroom substrate-based biofilter at low flow rates is consistent with previous findings that highlight the critical role of residence time in optimizing biosorption (Zhang et al. 2021). Longer contact times facilitate stronger and more extensive interactions between metal ions and the biosorbent's functional groups, particularly in complex matrices like wastewater.

Notably, arsenic exhibited consistently higher removal efficiencies than cadmium across all flow rates, with a margin of approximately 10% at the highest flow rate (10 mL/min). This disparity can be attributed to the different chemical properties and behaviors of these metals in aqueous environments. Arsenic, primarily present as anionic species such as H_2AsO_4^- and HAsO_4^{2-} at neutral pH, has greater solubility and mobility, which enhances its interaction with active sites on the SMS surface, particularly with functional groups like carboxyl and hydroxyl groups (Tiwari et al. 2020, Gupta et al. 2025). These physicochemical properties enable arsenic to form rapid and stable complexes with the biosorbent, explaining its consistent removal regardless of flow rate (Sanou et al. 2016).

By contrast, cadmium exists as a cation (Cd^{2+}), which may face stronger competition for sorption sites and be more sensitive to flow-rate-induced changes in residence time. These differences underscore the importance of substrate chemistry and metal speciation in determining removal efficiency. Supporting studies have shown that biosorption performance can vary significantly depending on metal type and the specific affinities of biosorbent functional groups (Tiwari et al. 2020).

In addition to heavy metal removal, the study evaluated the biofilter's efficacy in removing bacterial contaminants, particularly *E. coli*, a key indicator of fecal contamination in water systems (EPA 2012). The SMS-based filter significantly reduced bacterial counts, demonstrating its potential for mycofiltration — a branch of bioremediation where fungi are utilized to remove pathogens and pollutants from water. The reduced *E. coli* from 154 CFU/mL to an average of 15 CFU/mL after 48 hours of filtration suggests that mycofiltration can be a viable alternative to traditional membrane-based systems. This aligns with previous findings, where *Pleurotus* sp. and other fungi demonstrated the capacity to reduce bacterial counts through nutrient competition and potential mycelial predation (Li et al. 2019, Barron 2003). *Pleurotus* SMS as a biofilter could significantly enhance water treatment processes in regions facing heavy metal pollution and bacterial contamination, providing a dual-function filtration system.

The key objective of this study was to assess the viability of utilizing indigenous agricultural waste materials as an effective biofilter for contaminated water. By integrating *Pleurotus* SMS into the water treatment system, this research underscores the potential of agricultural by-products in addressing environmental challenges. Indigenous agricultural waste, such as mushroom spent substrate, wood chips, and charcoal, offers numerous advantages, including local availability, low cost, and biodegradability (Tripathi & Ranjan 2015, Kapahi & Sachdeva 2019, Mwangi et al. 2024). This makes it particularly suited for regions like Isabela, Philippines, where agricultural activities generate significant waste streams that could otherwise pose environmental disposal challenges. The potential for *Pleurotus* SMS to be used as a heavy metal biosorbent offers significant implications for rural and agricultural regions where industrial pollution is a growing concern. The local availability of SMS, often discarded as waste, provides a cost-effective and environmentally sustainable solution for water treatment. This directly contributes to the global drive towards waste valorization, where agricultural by-products are repurposed for environmental remediation (Semple et al. 2001, Chiu et al. 2009). The repurposing of SMS for environmental remediation aligns with current sustainability goals and supports circular economy initiatives in rural agricultural regions (Buswell 2018, Corral-Bobadilla et al. 2019). Moreover, incorporating SMS into water filtration systems could reduce the dependency on synthetic materials, lowering operational costs for water treatment facilities while contributing to the reduction of agricultural waste (Aiduang et al. 2025).

While the results of this study are promising, further research is required to optimize the design, particularly on additional filtration to purify the treated water from the total dissolved solids carried from the biosorbents and the scalability of the prototype water treatment system for field-scale applications. Exploring the efficacy of the common heavy metal contaminations in irrigated and riverine water, such as Nickel and Copper, and other coliform bacteria is also recommended. Future investigations should explore the long-term performance of the SMS biofilter, particularly in real-world applications where water quality may fluctuate due to seasonal and pH changes affecting heavy metal removal efficiency or varying contamination levels. Additionally, the health impacts of using

Pleurotus SMS for water filtration should be assessed to ensure that the biosorbent does not introduce any harmful by-products into treated water (Patil et al. 2020). Scaling the system for broader application, particularly in rural and underserved communities, could provide a sustainable, low-cost solution for improving water quality in areas affected by both heavy metal pollution and bacterial contamination. Collaborative efforts between academic institutions, local governments, and agricultural stakeholders will be essential to ensure the practical implementation of these findings.

The constructed water treatment system, incorporating *Pleurotus* SMS, demonstrates significant potential for removing heavy metals such as Cd and As, as well as bacterial contaminants like *E. coli*. The system's high efficacy, especially at slower flow rates, provides a compelling case for its further development and application in rural regions. The dual function of removing both heavy metals and pathogens highlights the versatility and sustainability of mycofiltration systems based on indigenous agricultural waste. By turning waste into a resource, this approach not only addresses environmental pollution but also promotes sustainable agricultural practices.

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