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Growth and mycoremediation activity of *Panaeolus antillarum* on lead-contaminated coconut water media

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Abstract. *Bustillos RG. 2026. Growth and mycoremediation activity of* Panaeolus antillarum *on lead-contaminated coconut water media. Asian J Agric 10: g100104. https://doi.org/10.13057/asianjagric/g100104.* This study investigated the mycoremediation potential of lead (Pb) using the coprophilous mushroom *Panaeolus antillarum* cultivated in solid and liquid coconut water media. Mycelial growth performance, including mycelial diameter, dry biomass, and volume loss, was evaluated under different Pb concentrations of 1 ppm, 10 ppm, 100 ppm, and 1000 ppm using coconut water gulaman as solid medium and coconut water as liquid medium. In addition, Pb accumulation in the mycelia was quantified to assess bioaccumulation capacity. Statistical analysis showed that the highest mycelial diameter and thickness were recorded at 1 ppm Pb (77.0 mm), which was not significantly different from the control without Pb (79.50 mm). In contrast, no substantial mycelial growth was observed at 1000 ppm Pb, indicating strong inhibitory effects at high metal concentration. Among all Pb-contaminated treatments, the 1 ppm medium produced the highest mycelial biomass (1.82 g) and volume loss (8.52 mL). Maximum Pb bioaccumulation was significantly recorded at 100 ppm (268 mg/kg), whereas lower accumulation capacity was observed at reduced Pb concentrations, particularly at 1 ppm (11.0 mg/kg). Notably, the detectable Pb content in mycelia grown at 1000 ppm was attributed mainly to passive adsorption rather than active uptake, as excessive Pb levels severely suppressed biomass production. Overall, the observed tolerance to Pb stress and the ability to accumulate Pb indicate that *P. antillarum* exhibits substantial heavy metal resistance and mycoaccumulation potential, supporting its applicability in mycoremediation strategies for Pb-contaminated substrates.

Keywords: Heavy metal, mycelia, mycelial biomass, mycoremediation, *Panaeolus antillarum*

INTRODUCTION

Fungi obtain nutrients directly from the environments in which they grow through absorptive mechanisms across their cell walls. Macrofungi, including mushrooms, are heterotrophic organisms that digest external organic matter and are characterized by the presence of fleshy, spore-bearing fruiting bodies (Sahrawat et al. 2018). Ecologically, mushrooms play a vital role in nutrient cycling by decomposing complex organic compounds into simpler forms that become available to plants and other microorganisms. Beyond their ecological functions, mushrooms have been used since ancient times for culinary purposes due to their distinctive flavors and textures. In recent decades, they have gained increasing attention for their therapeutic potential, leading to their recognition as functional foods or superfoods (Kozarski et al. 2015; Valverde et al. 2015; Bustillos et al. 2025a, b).

Mushrooms possess high nutritional value and are rich in proteins, dietary fiber, vitamins, essential fatty acids, and a wide range of bioactive secondary metabolites, including alkaloids, terpenes, flavonoids, glycosides, and phenolic compounds. These constituents are associated with diverse biological activities, including antimicrobial, anti-inflammatory, antidiabetic, and anticancer properties (Reyes and Nair 2016; Rathore et al. 2017; Bustillos et al. 2024). Such properties have reinforced the importance of macrofungi not only as food and medicinal resources but also as organisms with broad biotechnological relevance.

In addition to their nutritional and pharmacological significance, mushrooms serve as effective biological agents for environmental remediation. Macrofungi produce extracellular enzymes capable of degrading and accumulating a wide range of toxic substances, including heavy metals (Uddin et al. 2020). However, the environments in which these organisms grow are increasingly subjected to contamination from anthropogenic activities such as industrial emissions, mining, smelting, and chrome plating (Musah 2025). These activities introduce persistent pollutants that pose serious risks to both biotic and abiotic components of ecosystems. Among such pollutants, heavy metals such as mercury, beryllium, lead, zinc, arsenic, chromium, and cadmium are considered particularly hazardous due to their persistence, bioaccumulation potential, and toxicity (Engwa et al. 2019). These metals enter terrestrial and aquatic systems through industrial runoff, improper waste disposal, and the extensive use of agrochemicals, where they accumulate in organisms and disrupt physiological processes, ultimately threatening biodiversity and ecosystem stability.

Various physicochemical approaches have been developed to mitigate heavy metal contamination; however, biological alternatives have gained increasing interest due to their cost effectiveness and environmental compatibility. Mycoremediation, which involves the use of macrofungi to detoxify pollutants, has emerged as a promising strategy for reducing heavy metal concentrations in contaminated substrates. Macrofungi are valued not only

for their culinary and medicinal applications but also for their ecological roles as decomposers and bioremediators (Thakur 2020). Several studies have demonstrated the capacity of macrofungi to absorb and accumulate heavy metals, enabling their use as bioindicators of environmental contamination. Species such as *Coprinus comatus* (Dulay et al. 2015), *Russula*, *Lactarius*, *Pleurotus*, and *Agaricus* (Altıntığ et al. 2017), *Lactarius salmonicolor* (Niemiec et al. 2017), and *Pleurotus florida* (Roshandel et al. 2021) have been reported to accumulate significant quantities of heavy metals from polluted substrates. Furthermore, Pleurotus species have shown enhanced remediation efficiency, while Agaricus bisporus has demonstrated the ability to remove Cd(II) and Zn(II) from aqueous solutions (Nagy et al. 2017; Vaseem et al. 2017).

The Philippines, as a tropical country, provides favorable conditions for the growth of diverse macrofungal species that naturally occur on various substrates, including soil, leaf litter, decaying plant residues, and decomposing logs in forested and pasture landscapes, particularly during the rainy season (Kalaw and Albinto 2014; Bustillos et al. 2025a, b). Macrofungal diversity has been well documented in several protected areas, including Mt. Arayat Protected Landscape (Bustillos et al. 2024). Agricultural activities in the foothills of this forest promote the proliferation of coprophilous macrofungi, including *Panaeolus antillarum*, which commonly grows on decomposing dried dung of grazing ruminants. Although this species is not widely consumed in the Philippines due to reports of hallucinogenic effects, it has been reported to possess pharmacological properties. Cultivation techniques for the production of *P. antillarum* mycelia and fruiting bodies have also been developed, facilitating its use in applied research (Bustillos et al. 2014).

Despite the increasing interest in macrofungi in the Philippines, *P. antillarum* remains poorly studied, particularly in relation to its environmental applications. Therefore, the present study aimed to evaluate the mycelial growth performance of *P. antillarum* in lead-contaminated solid and liquid media and to assess its potential for lead mycoremediation. The findings of this study provide baseline information on Philippine strains of *Panaeolus* and highlight their potential use as biological agents for mitigating lead contamination in polluted environments.

MATERIALS AND METHODS

Source of *P. antillarum*

Fruiting bodies of *P. antillarum* were collected from Mt. Arayat Protected Landscape, Arayat, Pampanga, Philippines, under Permit No. III-2023-008 (New) issued in May 2023 by the Department of Environment and Natural Resources, Region III, Pampanga, Philippines. Specimens growing on dried carabao dung were carefully collected, wrapped in brown paper, labeled, and transported to the laboratory for tissue culture revival.

Culture revival and inoculum preparation

Agar blocks measuring approximately 10 mm² by 3 mm were excised from a pure stock culture of *P. antillarum* and aseptically transferred onto sterilized potato dextrose agar in Petri plates. Cultures were incubated at room temperature to promote mycelial growth. Mycelial discs were prepared using a flame-sterilized cork borer with a diameter of 7 mm. Fully colonized cultures were used as the source of inoculum for subsequent experiments.

Preparation of coconut water solid media contaminated with lead

Fresh coconut water (1 L) was obtained from newly cracked coconuts and boiled with 20 g of white gulaman, prepared by grating a gulaman bar as a solidifying agent, and 10 g of sucrose. The mixture was stirred continuously until a homogeneous solution was obtained. The pH was adjusted to 7.0 following the protocol described by Bustillos et al. (2014). The medium was amended with lead sulfate ($PbSO_4$) to achieve final concentrations of 1 ppm, 10 ppm, 100 ppm, and 1000 ppm, while lead-free medium served as the control (0 ppm). For each treatment, 100 mL of medium was dispensed into Erlenmeyer flasks, plugged with cotton, and sterilized in an autoclave at 121°C and 15 psi for 30 minutes. All treatments were prepared in triplicate.

Evaluation of secondary mycelial growth

Sterilized media were poured into Petri plates and allowed to solidify. Each plate was centrally inoculated with a 7 mm mycelial disc and incubated at 30°C to allow mycelial ramification. Mycelial growth was measured daily by recording colony diameter, with final measurements taken after 8 days of incubation. Mycelial density was assessed qualitatively using a scale based on visual observation, categorized as no growth (−), very thin (+), thin (++), thick (+++), and very thick or cottony (++++).

Preparation of lead-contaminated liquid culture media

Stock solutions of lead sulfate were prepared by dissolving 1 g of the salt in 1000 mL of distilled water. Fresh coconut water was used as the liquid culture medium for mycoremediation assessment. Four liters of coconut water were filtered through cheesecloth, after which 900 mL of coconut water was mixed with 100 mL of lead solution to obtain final Pb concentrations of 1 ppm, 10 ppm, 100 ppm, and 1000 ppm. Lead-free coconut water served as the control (0 ppm).

Aliquots of 100 mL of each pre-contaminated medium were dispensed into culture bottles and sterilized in an autoclave at 121°C and 15 Psi for 30 minutes. Following sterilization, mycelial discs were aseptically inoculated into each bottle. Cultures were incubated at 30°C under alternating light and dark conditions to facilitate mycelial growth.

After 10 days of incubation, mycelial mats were harvested, weighed, and air-dried. Volume loss of the liquid medium was recorded. Lead accumulation in the mycelial biomass was quantified using atomic absorption

spectrophotometry. All treatments were conducted in triplicate.

Data analysis

Data were analyzed using analysis of variance. Duncan's multiple range test was applied to determine significant differences among treatments at the 5 percent level of significance for mycelial diameter, mycelial biomass, volume loss, and Pb accumulation. Mycelial density scores were used to assess qualitative differences in mycelial thickness among treatments.

RESULTS AND DISCUSSION

Mycelial growth and density of *P. antillarum*

Mycelial growth of *P. antillarum* decreased progressively with increasing lead concentration in the solid coconut water medium (Table 1). The control treatment (0 ppm) recorded a mean mycelial diameter of 79.50 mm, which was not significantly different from that observed at 1 ppm Pb (77.00 mm), indicating that low lead concentration did not inhibit mycelial expansion. In contrast, significantly reduced mycelial diameters were observed at higher Pb concentrations of 10 ppm and 100 ppm. At 1000 ppm, mycelial growth was almost completely suppressed, with only minimal expansion recorded, indicating that elevated Pb levels severely restricted mycelial development (Figure 1).

Consistent with diameter measurements, mycelial density declined as Pb concentration increased. Very thick or cottony mycelial growth was observed in the control treatment, while dense mycelial growth was still evident at 1 ppm Pb. Treatments with 10 ppm and 100 ppm exhibited progressively thinner mycelia, whereas no visible mycelial growth was recorded at 1000 ppm, confirming the inhibitory effect of high Pb concentration on fungal colonization.

Mycelial growth response in lead-contaminated liquid culture media

The growth performance of *P. antillarum* in lead-contaminated liquid coconut water media showed a similar concentration-dependent response (Table 2). The mycelial dry weight obtained at 1 ppm Pb (1.82 g) was not significantly different from the control treatment (2.03 g), indicating that low Pb concentration did not adversely affect biomass production. In contrast, increasing Pb concentrations resulted in a significant decline in mycelial biomass. The lowest biomass yield was recorded at 1000 ppm Pb (0.18 g), demonstrating strong growth inhibition under high Pb stress (Figure 2).

Analysis of volume loss of the spent medium also revealed significant differences among treatments. The highest volume loss was observed at 1 ppm Pb (8.52 mL), followed closely by the control treatment. Conversely, the lowest volume loss was recorded at 1000 ppm Pb, corresponding with minimal biomass production. These results indicate that lower Pb concentrations supported higher metabolic activity and biomass accumulation, whereas elevated Pb concentrations restricted fungal growth and substrate utilization.

Mycoremediation activity of *Panaeolus antillarum*

The harvested mycelial biomass was analyzed to determine the extent of Pb accumulation across treatments (Table 3). The highest Pb accumulation in mycelia was recorded at 100 ppm Pb, reaching 268 mg/kg, while the lowest accumulation was observed at 1 ppm Pb (11.0 mg/kg). Intermediate accumulation levels were recorded at 10 ppm and 1000 ppm Pb.

Notably, although mycelial growth was severely inhibited at 1000 ppm Pb, the mycelia still accumulated a measurable amount of lead (63.0 mg/kg). This observation suggests that Pb uptake occurred even under conditions of reduced biomass production, likely through surface binding mechanisms. The presence of accumulated Pb across all treatments indicates that *P. antillarum* exhibits tolerance to Pb exposure within a specific concentration range and demonstrates measurable capacity for Pb accumulation, supporting its mycoremediation potential.

Table 1. Mycelial growth diameter and mycelial density of *P. antillarum* after eight days of incubation

Lead concentration	Mycelial growth (mm)	Mycelial density
0 ppm (control)	79.50[a]	++++
1 ppm	77.00[a]	+++
10 ppm	69.62[b]	++
100 ppm	66.92[b]	+
1000 ppm	10.00[c]	-

Note: Means followed by the same letter within a column are not significantly different at the 5 percent level of significance. Mycelial density scale: −: No growth, +: Very thin, ++: Thin, +++: Thick, ++++: Very thick or cottony

Table 2. Mycelial dry weight and volume loss of culture spent of *P. antillarum* grown in coconut water media contaminated with different lead concentrations

Lead concentration	Mycelial dryweight (g)	Volume loss (mL)
0 ppm (control)	2.03[a]	9.66[a]
1 ppm	1.82[a]	8.52[ab]
10 ppm	1.22[b]	5.90[b]
100 ppm	0.60[c]	2.26[c]
1000 ppm	0.18[c]	0.52[c]

Note: Means followed by the same letter within a column are not significantly different at the 5 percent level of significance

Table 3. Lead accumulation in the mycelia of *P. antillarum*

Lead concentration	Lead in mycelia (mg/kg)
1 ppm	11.0[c]
10 ppm	16.0[c]
100 ppm	268.0[a]
1000 ppm	63.0[b]

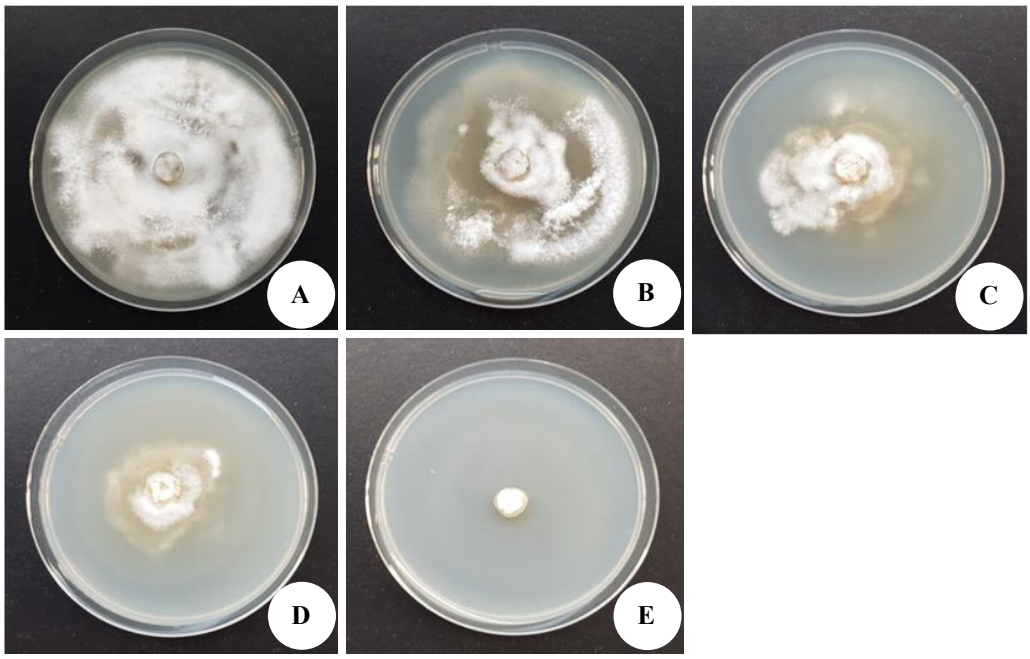

Figure 1. Mycelial diameter and density of *P. antillarum* grown on solid media supplemented with different lead concentrations: A. 0 ppm control, B. 1 ppm, C. 10 ppm, D. 100 ppm, and E. 1000 ppm, seven days after incubation

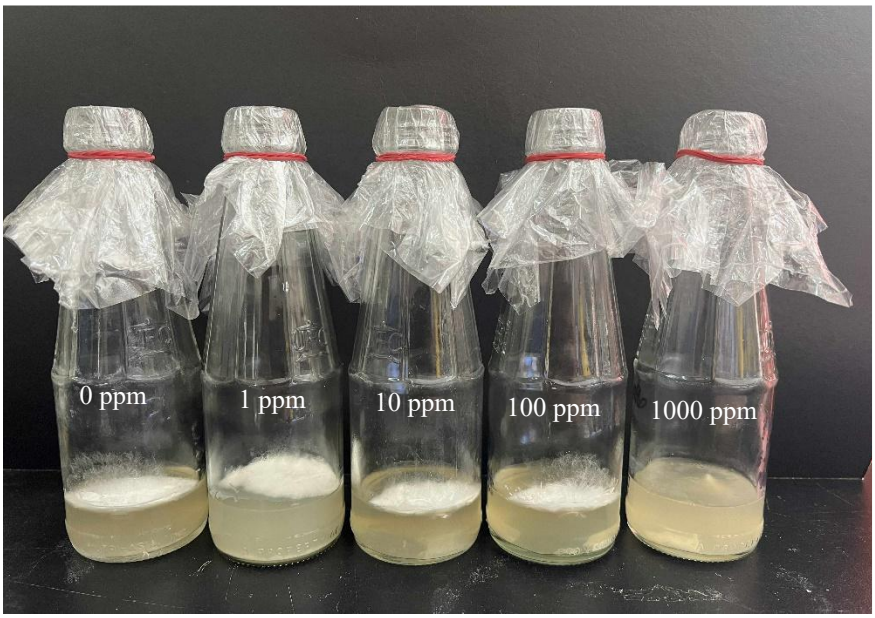

Figure 2. Mycelial growth of *P. antillarum* in liquid coconut water media supplemented with varying lead concentrations, seven days after incubation

Discussion

Macrofungal species exhibit clear substrate preferences that influence their growth, development, and ecological distribution. Their occurrence and physiological performance are strongly affected by the availability and concentration of metallic elements such as manganese, iron, chromium, mercury, copper, aluminum, zinc, arsenic, and nickel in their habitats (Dulay et al. 2015; Niemiec et al. 2017). These elements can function either as essential micronutrients or as toxic stressors, depending on their concentration and chemical form.

Beyond their nutritional and medicinal importance, macrofungi play a key role in ecosystem functioning through organic matter decomposition and nutrient recycling. They naturally grow in environments containing a variety of inorganic elements that are required for normal physiological and morphological development. Over time, macrofungi have gained recognition for their capacity to reduce environmental contamination originating from

agricultural and industrial activities. Pollutants from these sources often accumulate in soil and other fungal habitats, posing long-term ecological risks (Dulay et al. 2015). Several wild macrofungal species have been reported to accumulate substantial quantities of heavy metals, including lead, manganese, iron, chromium, mercury, copper, aluminum, zinc, arsenic, and nickel (Dulay et al. 2015; Niemiec et al. 2017). Among these contaminants, lead is considered particularly hazardous due to its persistence, toxicity, and widespread distribution. As an environmental pollutant, lead threatens both aquatic and terrestrial ecosystems and can adversely affect agricultural productivity and environmental sustainability (Sharma et al. 2025). The uptake of lead by fungi, particularly edible macrofungi, presents potential risks to human health, as prolonged exposure may result in chronic disorders and long-term illness (Ye et al. 2023). Therefore, understanding the mechanisms underlying lead accumulation in macrofungi and developing biological strategies to mitigate its effects are essential for both environmental protection and public health.

Bioremediation using macrofungi has emerged as a viable approach for reducing heavy metal contamination. Several studies have demonstrated the tolerance of macrofungi to toxic compounds, including herbicides and heavy metals. For example, *Polyporus* sp., *Lentinus crinitus*, *Phanerochaete australis*, and *Hypoxylon fendleri* exhibited tolerance to atrazine at concentrations up to 3000 µg/mL (Elias et al. 2025). Similarly, *C. comatus* has been reported to accumulate and reduce heavy metals such as lead, chromium, copper, and cadmium in both mycelia and fruiting bodies (Dulay et al. 2015). Bioaccumulation of barium, chromium, aluminum, and arsenic has also been documented in different parts of *L. salmonicolor* collected from forest ecosystems of the Western Carpathians (Niemiec et al. 2017).

Panaeolus antillarum is a coprophilous macrofungal species that grows on ruminant dung and contributes to nutrient recycling in pasture ecosystems. The nitrogen-rich nature of animal dung supports its growth and facilitates nutrient regeneration for plants, animals, and soil microorganisms. However, studies on this species in the Philippines remain limited, largely due to the reported hallucinogenic effects associated with the genus *Panaeolus*. As a result, these mushrooms have been generally regarded as non-edible and have received limited research attention (Doblin et al. 2019; Hu et al. 2020). Despite this, *P. antillarum* has gained increasing interest due to its pharmacological relevance. Compounds such as psilocybin and its metabolite psilocin have been investigated for their therapeutic potential in treating depression, obsessive-compulsive disorder, anorexia, stress-related conditions, and chronic pain disorders (Carhart-Harris and Goodwin 2017; Reiche et al. 2018). Nevertheless, information on the environmental applications of Philippine strains of *P. antillarum*, particularly in mycoremediation, has remained undocumented.

In the present study, the growth response of *P. antillarum* mycelia in coconut water media contaminated with varying concentrations of lead was evaluated. The effects of lead exposure were evident from both mycelial diameter and density measurements recorded after 8 days of incubation. Mycelial growth decreased markedly with increasing lead concentration, as shown in Table 1 and Figure 1. This growth inhibition at higher lead concentrations aligns with previous findings on *Pleurotus* species grown in submerged culture, where lead resulted in lower mycelial biomass and reduced volume loss compared to other metals such as copper, chromium, and cadmium (Bustillos et al. 2016). In contrast, Zoysa et al. (2020) reported increased mycelial biomass of *Pleurotus ostreatus* under exposure to cadmium, mercury, and lead, although reduced growth was observed in arsenic-treated cultures. These contrasting responses suggest that heavy metal effects on fungal growth are species specific and concentration dependent.

The complete inhibition of *P. antillarum* growth at 1000 ppm Pb indicates a toxicity threshold beyond which physiological processes are severely disrupted. Similar growth suppression has been reported *in Lyophyllum decastes*, where increasing accumulation of cadmium and lead resulted in reduced mycelial growth rates (Huang et al. 2025). Such inhibition may be attributed to the interference of heavy metals with essential enzymes and proteins involved in cell division, metabolism, and hyphal extension (Dulay et al. 2015; Zoysa et al. 2020).

Despite reduced growth at higher lead concentrations, *P. antillarum* demonstrated the capacity to accumulate lead within its mycelial biomass. This finding is consistent with previous reports on heavy metal accumulation in macrofungi such as *P. ostreatus* (Bhatnagar et al. 2021). The ability of macrofungi to absorb heavy metals is largely associated with functional groups present in fungal cell walls, which facilitate metal binding and uptake (Malviya and Jaspal 2023). In this study, maximum lead accumulation was observed at 100 ppm, whereas lower accumulation occurred at 1000 ppm. The reduced accumulation at very high lead concentration may be attributed to growth inhibition and saturation effects that limit cellular uptake capacity. At moderate concentrations, lead uptake is likely dominated by active bioaccumulation involving intracellular transport mechanisms, resulting in higher metal concentrations within the mycelial cells (Magalhães et al. 2022). In contrast, at lower lead concentrations, accumulation appears to occur primarily through biosorption, where lead binds passively to extracellular sites on the fungal cell surface (Singh et al. 2018).

The inverse relationship between mycelial biomass and lead accumulation observed in this study suggests that metal uptake efficiency is influenced by both physiological activity and growth inhibition. These findings support the role of *P. antillarum* as a potential agent for heavy metal removal and provide insight into the mechanisms of metal tolerance and accumulation in coprophilous macrofungi. Further investigation into the biochemical pathways involved in metal detoxification and tolerance is warranted to improve understanding of fungal adaptation to polluted

environments (Srivastava and Srivastava 2023; Daâssi et al. 2025).

Mycoremediation has gained increasing attention as a low-cost, effective, and environmentally sustainable approach to pollution control. The present results suggest that macrofungi, including *P. antillarum*, may serve as biological indicators of lead contamination in soil and other substrates. This species shows promise for use in the rehabilitation of heavy metal-contaminated environments. To enhance its practical application, future studies should focus on evaluating metal uptake under complex environmental conditions, including mixed metal contamination, industrial wastewater, and agricultural substrates. Investigations using pure cultures under controlled conditions, combined with physiological and biochemical analyses, will be essential for elucidating the mechanisms governing lead uptake and tolerance in *P. antillarum*.

In conclusion, this study demonstrated that *P. antillarum* is capable of removing lead from both solid and liquid coconut water substrates. Mycelial growth was significantly influenced by lead concentration, with a clear trend of decreasing growth as lead levels increased. Low lead concentrations supported mycelial development, whereas higher concentrations resulted in substantial growth inhibition. The highest lead accumulation observed at 100 ppm indicates that *P. antillarum* exhibits tolerance to moderate lead exposure while maintaining effective metal uptake capacity. Overall, the findings confirm the lead resistance and notable mycoaccumulation ability of *P. antillarum*, highlighting its potential as a biological agent for the remediation of lead-contaminated environments. This species may also serve as a promising candidate for further studies on the removal of other toxic heavy metals from polluted ecosystems.

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