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Exploration and selection of native macrofungi with heavy metal tolerance for bioremediation in Jakarta Bay, Indonesia

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Abstract. *Noverita, Ratnaningtyas INI, Hernayanti, Ekowati N, Lestari S. 2025. Exploration and selection of native macrofungi with heavy metal tolerance for bioremediation in Jakarta Bay, Indonesia. Biodiversitas 26: 1940-1955.* The increasing pollution of heavy metals Pb and Cd in Jakarta Bay poses a potential threat to marine biota, endangering human health. Macrofungi, known for their ability to mitigate heavy metal pollution through biosorption, are the focus of this research. The study aimed to collect macrofungi from West Sumatra Forest, selectively identifying species that are adaptive to salinity and heavy metals Pb and Cd, possessing a high Heavy Metal Tolerance Index (IT). Field exploration was conducted using a roaming method across 5 forest areas in West Sumatra, followed by laboratory cultivation and systematic screening. A descriptive analysis of fungal exploration data based on morphological characteristics was conducted. Selection results, measured by colony surface area on various treatment media (PDA, PDA with seawater, PDA with Pb/Cd, and PDA with seawater and Pb/Cd), and IT data were analyzed descriptively using GraphPad version 8 heatmaps. This study found that 94 macrofungal species were successfully isolated from 5 forest areas in West Sumatra, comprising 92 species from Basidiomycota and 2 species from Ascomycota. All isolated species demonstrated the capability to grow on various selection media, with varying degrees of adaptation. Nine species, including *Coprinus comatus*, *Favolus tenuiculus*, *Ganoderma applanatum*, *Lepiota ristata*, *Marasmius* sp. 21, *Polyporus badius*, *Polyporus elegans*, *Polyporus* sp., and *Trametes cubensis*, exhibited optimal growth on all media. Statistical analysis with ANOVA (Analysis of Variance) revealed that four species *C. comatus*, *P. elegans*, *Marasmius* sp. 21, and *F. tenuiculus*, consistently showed superior performance across all test conditions. The Heavy Metal Tolerance Index for Pb and Cd across all species was excellent, with 74% classified as very high, 22% as high, and 4% as moderate. These findings suggest significant potential for utilizing these fungal species in marine bioremediation applications.

Keywords: Biosorption, exploration, macrofungi, selection, Tolerance Index

INTRODUCTION

Jakarta Bay stands as a concerning example of marine ecosystem degradation due to heavy metal contamination, particularly with lead (Pb) and cadmium (Cd) levels that significantly exceed the regulatory limits established by the Minister of Environment Regulation No.51/2004 (Hosono et al. 2011; Barokah et al. 2019). This contamination represents a severe environmental and public health crisis, as these heavy metals progressively accumulate in marine organisms through biomagnification, ultimately threatening human populations that depend on these waters for food and livelihood (Ali et al. 2019).

The health implications of these heavy metals are particularly alarming: Lead exposure can result in severe neurological damage, leading to conditions such as paralysis and permanent vision loss. Cadmium contamination poses equally serious risks, notably causing osteomalacia - a condition characterized by bone softening - and multiple spinal fractures (Oladipo et al. 2018; Purohit et al. 2018). Given the severity of this environmental crisis, various remediation approaches have been implemented, though with limited success. Traditional engineering solutions have included the installation of waste filtration systems at river crossings to intercept pollutants before they reach the bay, deployment of specialized amphibious and spider-type heavy machinery for waste removal, and attempts at ecosystem restoration using green mussel shells. However, these conventional methods have proven inadequate, primarily due to their prohibitive costs and limited effectiveness in addressing the scale of the problem (Purohit et al. 2018). In response to these challenges, researchers have begun exploring biological remediation methods, with particular interest in the potential of macrofungi as a cost-effective and environmentally sustainable solution (Hanafiah et al. 2024). This approach, known as mycoremediation, represents a promising alternative that harnesses the natural ability of fungi to process and neutralize environmental pollutants, including heavy metals.

Mycoremediation encompasses multiple mechanisms to mitigate heavy metal contamination, including biosorption, bioaccumulation, bioprecipitation, bioreduction, and bioleaching (Goutam et al. 2021; Kumar and Dwivedi 2021; Zare et al. 2024). Among these, biosorption stands out as a particularly effective method for addressing heavy metal pollution in aquatic environments. This process utilizes fungal biomass, whether living or non-viable, to adsorb and bind metallic ions from contaminated solutions through passive physicochemical interactions (Ayangbenro and Babalola 2017; Shamim 2018). Its efficiency, cost-

effectiveness, and applicability to diverse water systems have positioned biosorption as a preferred strategy in mycoremediation (Akpasi et al. 2023).

Suseem and Saral (2014) have demonstrated the effectiveness of various macrofungi species in heavy metal biosorption by using the macrofungus *Pleurotus eous* as a biosorbent in the biosorption of solutions containing heavy metals Pb, C, and Ni on a laboratory scale (Suseem and Saral 2014). Furthermore, Kapahi and Sachdeva (2019) used macrofungi *Pleurotus platypus* and *P. tuber-regium* to address pollution from metals Cu, Zn, Fe, Cd, Pb, Ni, and *P. sajor-caju* for Zn (Kapahi and Sachdeva 2019). However, the application of these fungi is limited to terrestrial environments, such as agricultural and mining areas, and has not been reported in marine ecosystems, including Jakarta Bay.

Indonesia's forest areas harbor a diverse array of macrofungal species. For instance, in the Lembah Anai Nature Reserve and Batang Palupuah Nature Reserve, 112 species were found (Noverita et al. 2017). In the Bukit Rimbang Bukit Baling Wildlife Reserve (SMBRBB) in Riau, Sumatra, 138 species were discovered (Noverita et al. 2019). This biodiversity represents a largely untapped resource for developing novel bioremediation strategies.

The process of identifying suitable fungal species for heavy metal biosorption follows a systematic approach, beginning with the collection and isolation of macrofungi from natural habitats, followed by laboratory testing of their growth capabilities in metal-contaminated media, and finally, assessment of their metal tolerance through comparative growth studies (Mohamadhasani and Rahimi 2022).

This research initiative focuses on 2 primary objectives: first, to explore and identify macrofungi from West Sumatra's forests that demonstrate potential for Pb and Cd absorption, and second, to evaluate and select species showing optimal Tolerance Index (IT) values for these metals, laying the groundwork for future applications in marine environment remediation.

MATERIALS AND METHODS

Sample collection and isolation of macrofungi

Sampling and isolation of macrofungi were conducted in 5 forest areas in West Sumatra. The selection and screening of macrofungi were conducted at the Microbiology and Genetics Laboratory of the National University in Jakarta. Metal content was analyzed using atomic absorption spectrophotometry at the Soil Testing Laboratory of the Agricultural Research and Development Agency (BALITTANAH), Bogor, Indonesia.

Field exploration and sampling of macrofungi

Field exploration of macrofungi was conducted directly using a roaming method in various forest areas in West Sumatra. Each discovered fungal sample was initially documented using a camera. It noted its location according to GPS coordinates, morphological characteristics, habitat conditions, and the fungus's life traits (solitary, grouped, parasitic, saprophytic, or symbiotic). For the obtained fungal species, complete fruiting bodies were collected, utilized for herbarium preparation, and isolated for further analysis. The extraction of macrofungi fruiting bodies was done by gently pulling the entire structure with a knife to prevent damage or breakage. Soft-textured samples were immediately placed in sample boxes and labeled with a sample code. Meanwhile, fungi with hard-fruiting body characteristics were placed in brown paper bags and labeled with a sample code (Noverita et al. 2017).

Identification of macrofungi

The obtained macro fungal samples were morphologically identified macroscopically using a magnifying glass, field guidebooks, and field photos to support the identification process (Laessøe 2013; Dighton 2019; Sridhar and Deshmukh 2019). Macroscopic identification parameters include shape, color, and texture of the fruiting body; upper and lower cap shapes; type of hymenophore (gills, pores, teeth, gleba); stipe shape; the presence of a ring and volva. Microscopic identification was conducted using a microscope to observe hyphae and spores. The fungal mycelium was stained with Lactophenol Cotton Blue using the block square technique (slide culture). After the initial identification, a re-identification process was conducted at the Bogoriense Herbarium of the Indonesian Institute of Sciences (LIPI) Research Center for Biology, Cibinong (BRIN).

Production and maintenance of pure cultures of macrofungi

Pure cultures of macrofungi were obtained by isolating the fruiting bodies collected during field research. The isolation of fruiting bodies was performed by aseptically dissecting the inner parts of the stem or cap that were not exposed to the external environment. These parts were then aseptically cut, picked using sterile forceps, and inoculated into a PDA slant medium. The cultures were subsequently incubated at room temperature until colonies developed. For samples with thin sheets, such as *Auricularia* spp. and *Tramella* spp., the fruiting body isolation involved cutting thin sheets approximately +0.5 cm thick, sterilizing them with 70% alcohol, rinsing with sterile distilled water, draining on sterile Whatman filter paper, and inoculating them into slant PDA medium. The cultures were then incubated at room temperature (26-28°C) for approximately one week. Pure cultures obtained from field isolations were re-inoculated onto slant PDA medium and PDA medium in Petri dishes aseptically within a Laminar Air Flow (LAF) to ensure species purity and refresh the species. They were then incubated at room temperature (26-28°C) until colonies developed. The obtained pure cultures were periodically maintained on slant cultures and stored in a refrigerator at 4°C (Noverita et al. 2017).

Macrofungi selection

The selection of macrofungi used in this research was conducted through several simultaneous stages, consisting of:

Ability to grow on PDA medium

Selection to assess the growth ability of macrofungi on PDA medium in Petri dishes was conducted by cultivating 1 cm² pure cultures of 7-day-old fungi using a cork borer into the PDA medium, in duplicate. The cultures were then incubated at room temperature (26-28°C), and their growth was observed daily until the treatment colonies covered the surface of the medium in the Petri dish. Subsequently, the colony area (Enayatizamir et al. 2020) was measured using the colony area measuring technique. The best-performing species exhibiting superior colony growth and the fastest growth rate were selected for further testing in the subsequent stages (Abbas et al. 2014).

Ability to grow macrofungi on seawater-based PDA medium (PDAAL)

Selection to assess the growth ability of macrofungi in seawater with relatively high salinity was conducted by cultivating 1 cm² pure cultures of 7-day-old fungi using a cork borer into PDA medium with seawater solvent at pH 7.2, carried out in duplicates. The cultures were then incubated at room temperature (26-28°C), and the colony growth was observed daily until the treatment colonies covered the surface of the medium in the Petri dish (modified from Enayatizamir et al. 2020). Subsequently, the colony area was measured using the colony area measuring technique (Abbas et al. 2014). In subsequent stages, the best-performing species exhibiting superior colony growth and the fastest growth rate were selected for further testing.

Resistance of macrofungi to heavy metal exposure of Pb and Cd

Fungi demonstrating the best growth ability in the previous selection were cultured on seawater-based PDA medium containing heavy metals. The fungal cultures were tested for their biosorption ability in vitro by separately adding 5 ppm of heavy metals Pb and Cd to the PDA medium. Cultures of 7-day-old fungi were inoculated into the medium in 1 cm² sections using a cork borer, and this stage was conducted in duplicates. The cultures were then incubated at room temperature (26-28°C). Colony growth was observed, and the diameter of the growth rate was measured daily for 7 days using the colony area measuring technique (Figure 1). The species exhibiting the best colony growth and the fastest growth rate were selected for further testing in subsequent stages based on the results.

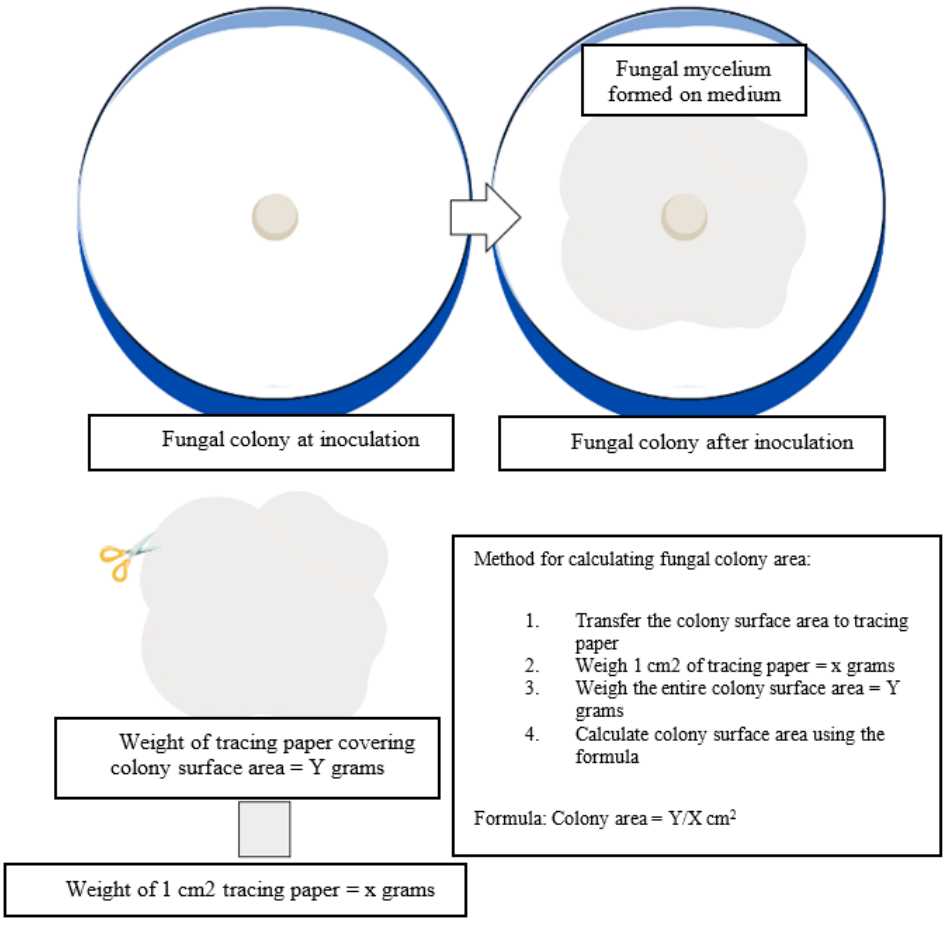

Figure 1. Measurement of colony growth area using the colony area measuring technique (Abbas et al. 2014)

Data analysis

Data obtained from the field exploration of fungi was analyzed descriptively based on macroscopic and microscopic morphological characteristics. The selection results on various treatment media, measured by colony surface area, were statistically analyzed using Analysis of Variance (ANOVA) with the SPSS (Statistical Package for the Social Sciences) software. Additionally, the selection results were also chosen based on the Heavy Metal Tolerance Index (IT) for Pb and Cd using the formula;

$$\text{Tolerance Index} = \frac{\text{The surface area of the macrofungus mycelium in the medium } \textbf{Seawater based PDA medium} \text{ (PDAALPb) or } \textbf{Seawater based PDA medium} \text{ Cd (PDAALCd)}}{\text{The surface area of macrofungus mycelium in PDA medium}}$$

Using the following criteria: Very Low Tolerance Index (0.00-0.39 cm), Low Tolerance Index (0.40-0.59 cm), and Moderate Tolerance Index (0.60-0.79 cm). Furthermore, the data of the Heavy Metal Tolerance Index (IT) for Pb and Cd will be descriptively analyzed using a heatmap with GraphPad version 8. The selected data can be used for further research.

RESULTS AND DISCUSSION

Exploration and isolation results of macrofungi from several forest areas in West Sumatra

The exploration of macrofungi from 5 forest areas in West Sumatra, namely the Gunung Merapi Nature Reserve (TWAGM), Gunung Singgalang Nature Reserve (TWAGSg), Gunung Sago Nature Reserve (TWAGS), Lembah Anai Nature Reserve (CALA), and Batang Palupuh Nature Reserve (CABP), successfully isolated a total of 127 species of macrofungi, cultivated on PDA medium in culture tubes. Out of these, 94 species (Figure 2) exhibited robust growth and were selected for further testing in this study.

Out of the 94 fungal species used in this study, a total of 92 species belong to the phylum Basidiomycota, while two species belong to the phylum Ascomycota. When observed based on their living habits and the substrates on which they grow in the field, the 94 macrofungal species exhibit diverse living habits and substrate preferences. Among them, seven species thrive around plant roots as ectomycorrhizae, 24 species function as saprobes on leaf litter, eight species act as saprobes on decaying wood, 50 species serve as saprobes on dead branches, twigs, and tree trunks, and 5 species acted as parasites on living tree trunks (Figure 3).

Results of macrofungi selection on various growth media

Next, to demonstrate the growth capabilities of 94 macrofungal species isolated from several forest areas in West Sumatra, intended for further use in biosorption of Pb and Cd metals in the seawater of Jakarta Bay, simultaneous selection was conducted on Potato Dextrose Agar (PDA), PDA with seawater (PDA SW), PDA with heavy metal Pb (PDA Pb), PDA with heavy metal Cd (PDA Cd), PDA with seawater and Pb (PDA SW Pb), and PDA with seawater and Cd (PDA SW Cd). All macrofungal species were able to grow on all tested selection media. The determination of growth for these 94 macrofungal species was based on the size of the colony surface area on the medium in Petri dishes after several days of incubation. Each fungal species exhibited different growth rates, with some species starting to cover the surface of the medium in Petri dishes after 3 days of incubation. Therefore, data calculation was based on the colony surface area after 3 days of incubation.

Out of the 94 macrofungal species capable of growing on various selection media, 50 species were chosen based on their relatively good tolerance index values against heavy metals Pb and Cd. The reason for selecting based on the tolerance index is that the chosen fungal species will be used in further research to bioabsorb Pb and Cd metals in the already polluted seawater of Jakarta Bay. A more detailed explanation of each selection result is provided below.

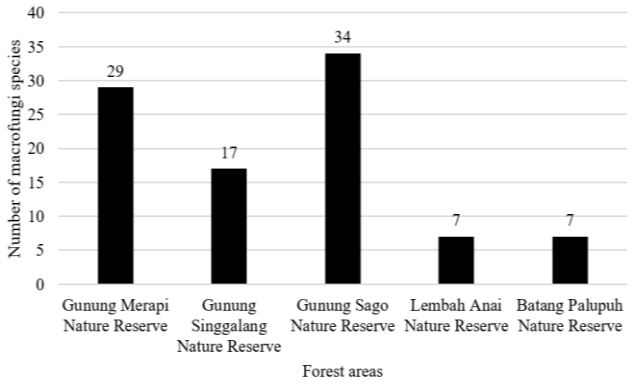

Figure 2. Number of fungal species found at each exploration location

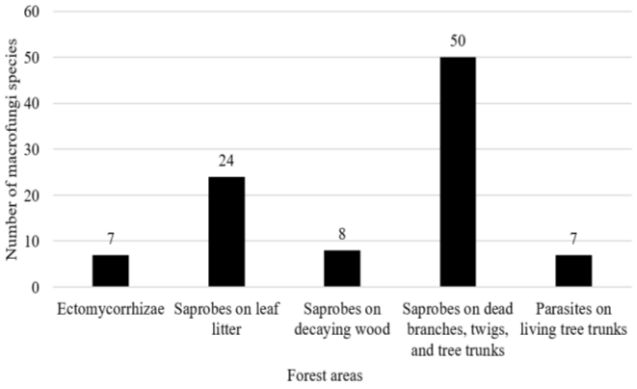

Figure 3. Grouping of fungal species based on living habits and growing substrates in the field

Selection of PDA medium

A total of 50 selected fungal species cultivated in PDA medium with a sufficiently high tolerance index for heavy metals exhibited highly varied growth capabilities. Next, to assess the differences in growth abilities among various macrofungal species based on colony surface area on PDA medium on the third day of incubation, a one-way ANOVA analysis was conducted. The analysis results indicated a significant influence of the fungal species, with a p-value of less than 0.05 ($p<0.05$). Some fungal species exhibited rapid growth, showing colony development after the first day of incubation, whilst a few species had already covered the Petri dish by the third day of incubation. For example, the fungi growth on PDA medium is illustrated in Figure 4 on the first, third, fourth, and fifth days of incubation.

There are noticeable differences in the growth rates among the three example macrofungal species in the PDA medium (Figure 4). *Trametes cubensis*, on the first day after incubation, exhibited an average colony surface area of 0.7747. After the third, fourth, and fifth days of incubation, there was an increase in the colony surface area, albeit not substantial, measuring 3.0769 cm^2, 5.6593 cm^2, and 9.0934 cm^2, respectively. *Polyporus elegans* and *Marasmius* sp. 21 showed faster growth. On the first day after incubation, the colony surface area of *P. elegans* and *Marasmius* sp. 21 was not significantly different from *T. cubensis*. However, after the third day of incubation, they nearly covered the Petri dishes, measuring 53.8351 cm^2 and 50.7473 cm^2, respectively. In comparison, on the fourth day of incubation, their colony surface areas had already covered the entire Petri dish, measuring 61.9506 cm^2 and 64.1758 cm^2. Further statistical analysis using Tukey's test revealed 5 species with significant differences in the mean colony surface area, indicating a significant impact on the largest colony surface area (Figure 5).

The identical letters in Figure 5 indicate that there is a similarity in the mean values of the colony surface area among the five fungal species mentioned. Based on the analysis results, it is evident that the following five fungal species, *Coprinus comatus*, *Favolus tenuiculus*, *Ganoderma applanatum*, *Marasmius* sp. 21, and *Polyporus elegans*, exhibit excellent and rapid growth in the PDA medium compared to other species.

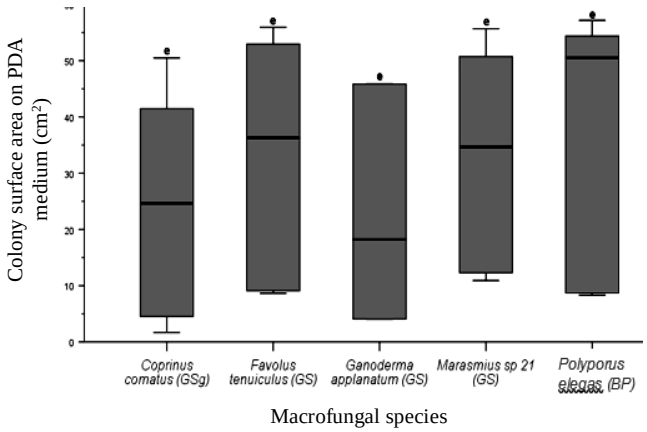

Figure 5. Growth of macrofungal species in PDA medium on the third day of incubation with the largest colony surface area values

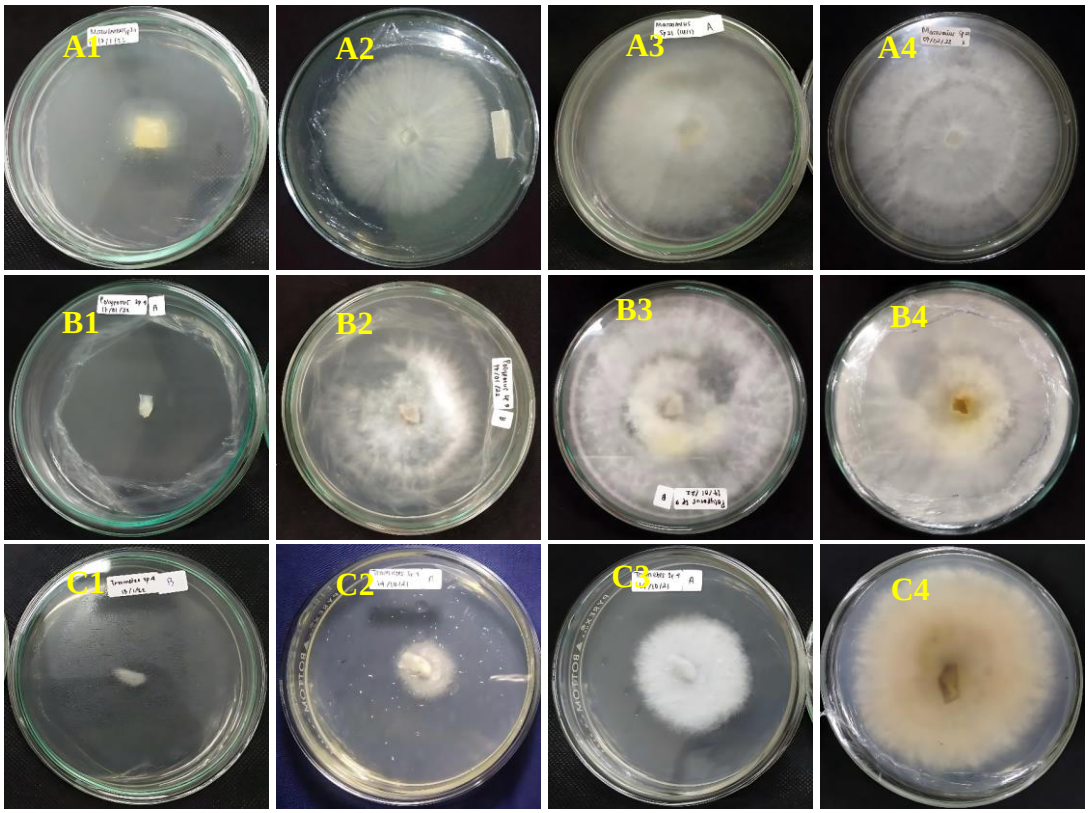

Figure 4. Growth of 3 macrofungal species on PDA medium on the first, third, fourth, and fifth days of incubation; A1-A4: *Marasmius* sp. 21; B1-B4: *Polyporus elegans*; C1-C4: : *Trametes cubensis*

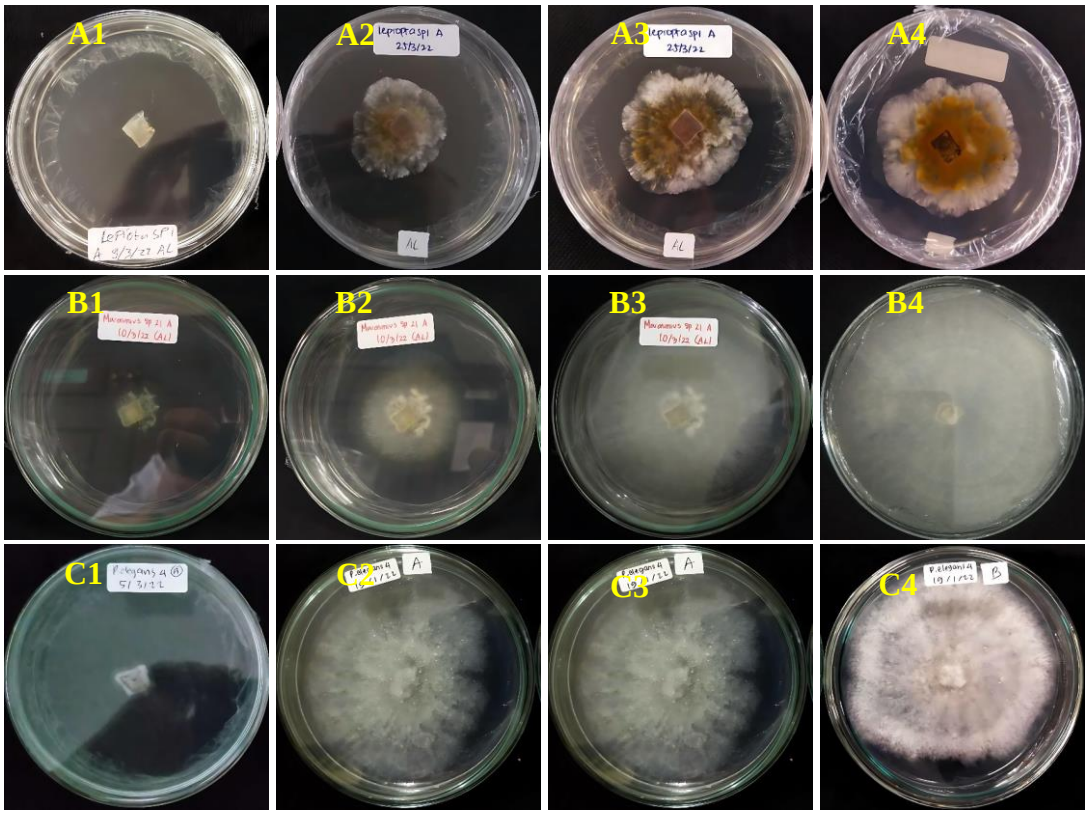

Figure 6. Growth of three example macrofungal species on seawater-PDA medium (PDAAL) on the first, third, fourth, and fifth-day post-incubation; A1-A4 *Lepiota cristata*; B1-B4 *Marasmius* sp. 21; C1-C4 *Polyporus elegans*

Selection of seawater-PDA medium (PDAAL)

The growth ability of the selected 50 fungal species cultivated on the PDAAL medium with sufficiently good heavy metal tolerance indices exhibited varied growth capabilities. Next, to determine the differences in growth abilities among various macrofungal species based on colony surface area on PDAAL medium, a one-way ANOVA analysis was performed. The analysis results revealed a significant influence of different fungal species on the growth of fungal colonies in the PDAAL medium, with a significance value ($p<0.05$).

The growth disparities among the 50 fungal species depended on their ability to adapt to the PDAAL medium with high salt content. Some species did not show growth on the first day after incubation, while others began growing, and by the third day, their colonies had nearly filled the Petri dish. The growth of three example fungi on the PDAAL medium on the first, third, fourth, and fifth day post-incubation is illustrated in Figure 6.

Figure 6 illustrates that fungi capable of adapting to the PDA medium with the addition of seawater, such as *Marasmius* sp. 21, still grow well with normal colony conditions, even though not as fast as in the PDA medium with distilled water. The colony's growth on the third day of incubation has not filled the Petri dish. In contrast, fungal species like *Polyporus elegans* and *Lepiota cristata*, besides exhibiting slower growth, also display distinct colony growth patterns compared to their normal growth. For *L. cristata*, there is a change in color in its colony. This reflects the defense mechanism of these fungi against unfavorable environmental conditions for their growth. Advanced statistical analysisusing the Tukey test reveals five species with significant differences in terms of the largest average values, indicating that these species have a substantial influence on the largest colony surface area (Figure 7).

The same letter labels in the figure indicate similarity in the average surface area of the colonies of the five above-mentioned fungal species. This demonstrates that these five macrofungal species, namely *F. tenuiculus*, *L. cristata*, *Marasmius* sp. 21, *Polyporus badius*, and *P. elegans*, grow exceptionally well on Seawater PDA Medium (PDAAL) compared to other species.

Selection of PDA medium with Lead (PDAPb)

A total of 50 selected species grown on PDAPb medium with a sufficiently high tolerance index showed varying growth capabilities. The one-way ANOVA analysis to assess the growth abilities of different macrofungal species based on the surface area of the colonies in the PDAPb medium indicated a significant influence of different fungal species on the growth of fungal colonies in the PDAPb medium, with a significance value ($p<0.05$). The differences in the surface area of the colonies of these 50 species are related to their ability to grow and adapt in a medium containing heavy metal lead (Pb). Species capable of adaptation can grow rapidly, exhibiting normal-looking colonies, while others still experience growth but at a slower pace, resulting in colonies with a different appearance from their normal state. The growth of three example fungal species on the PDAPb medium is illustrated in

Figure 8, showing successively on the first, third, fourth, and fifth days of incubation.

Figure 8 shows that fungi capable of adapting to PDAPb medium, such as *Marasmius* sp. 21, continue to grow well with a normal colony texture. Fungal species *T. cubensis* and *P. elegans* grow and experience rapid growth, but their colonies show abnormal growth. Advanced statistical analysis using the Tukey test revealed significant differences among species, with the largest average values indicating 7 species that significantly impact the largest colony surface area (Figure 9). The same letter labels in the figure indicate the similarity in the average values of colony surface area for the seven fungal species above. This suggests that these seven macrofungal species (*F. tenuiculus*, *C. comatus*, *G. applanatum*, *L. cristata*, *Marasmius* sp. 21, *P. badius*, and *P. elegans*) grow exceptionally well in the Lead-containing PDA medium (PDAPb) compared to other species.

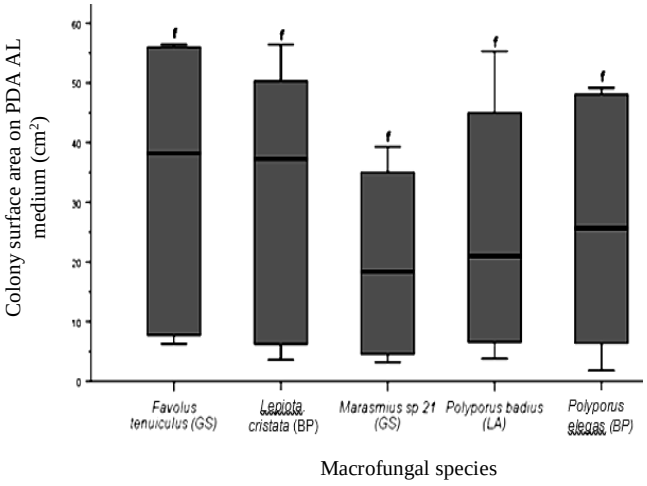

Figure 7. Growth of macrofungal species in seawater PDA Medium (PDAAL) on the third day with the largest colony surface area.

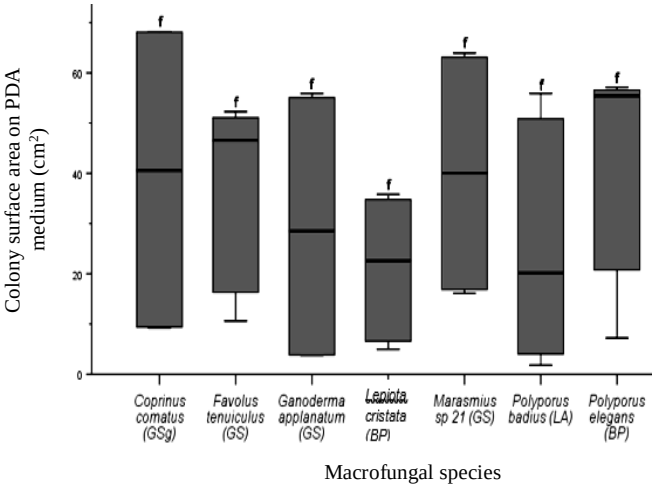

Figure 9. Growth of macrofungal species in lead-containing PDA medium (PDAPb) on the third day of incubation with the largest colony surface area values

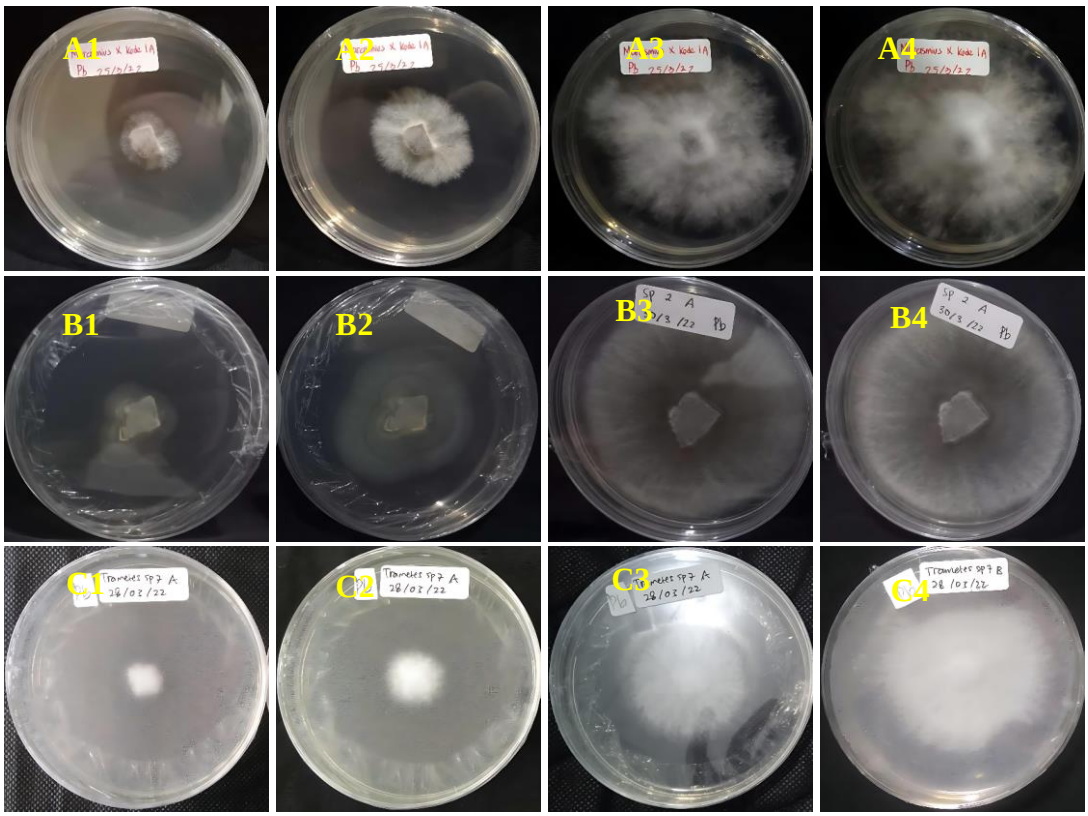

Figure 8. Growth of three example macrofungal species on PDA medium with heavy metal lead (PDAPb) on the first, third, fourth, and fifth days of incubation; A1-A4: *Trametes cubensis*; B1-B4: *Polyporus elegans*; C1-C4: *Marasmius* sp. 21

Selection of PDA medium with Cadmium (PDACd)

A total of 50 selected fungal species, grown on PDACd medium and exhibiting a fairly good tolerance index, demonstrated varying growth capabilities. The results of a one-way ANOVA analysis to assess the growth ability of different macrofungal species concerning the colony surface area on PDA medium with cadmium showed a significant influence of different fungal species on the growth of fungal colonies in the PDA with cadmium medium, with a significance value ($p<0.05$).

The differences in colony surface area among the 50 species depend on the fungi's ability to adapt to the PDA medium containing the heavy metal cadmium (Cd). Species that can adapt will grow rapidly with a normal colony appearance, while others will still experience growth but at a slower rate, displaying colonies that differ from the norm. The growth of three example fungal species on PDACd medium, consecutively from the first, third, fourth, and fifth days of incubation, is shown in Figure 10.

Figure 10 above shows that the three examples of fungal species grown on the PDACd medium exhibit growth characteristics that are almost similar to those grown on the PDAPb medium. The *Marasmius* sp. 21 species grows well with a normal colony texture. The fungal species *Trametes cubensis* and *Polyporus elegans* exhibit rapid growth, but their colonies display abnormal growth patterns. Further statistical analysis was conducted using the Tukey test to identify species with a significant difference in the largest average value on the PDACd medium. Based on this test, six species have a significant impact, with the largest colony surface area (Figure 11). The label with the same letter in the figure indicates the similarity of the average colony surface area values for the 7 macrofungal species listed above. This suggests that the six macrofungal species, *C. comatus*, *F. tenuiculus*, *G. applanatum*, *Marasmius* sp. 21, *P. elegans*, and *T. cubensis*, grow exceptionally well on PDAPb medium compared to other species.

Selection of PDA seawater and Lead (PDAALPb)

A total of 50 selected fungal species grown on PDAALPb medium, exhibiting sufficiently good tolerance indices, showed varying growth capabilities. One-way ANOVA analysis was conducted to determine the growth capabilities of different macrofungal species, based on the colony surface area in the PDAALPb medium, revealing a significant influence of different fungal species on the growth of fungal colonies in the PDAALPb medium with a significance value ($p<0.05$).

The differences in the colony surface area of these 50 species on the PDAALPb medium are highly correlated with their ability to grow and adapt to a medium containing the heavy metal Pb with high salt levels. Some species can grow rapidly with a normal colony appearance, while others still experience growth but at a slower pace, displaying colonies that differ from the norm. The growth of tree example fungal species on PDAALPB medium is shown in Figure 12, including the sequence from the First, Third, Fourth, and Fifth days after incubation.

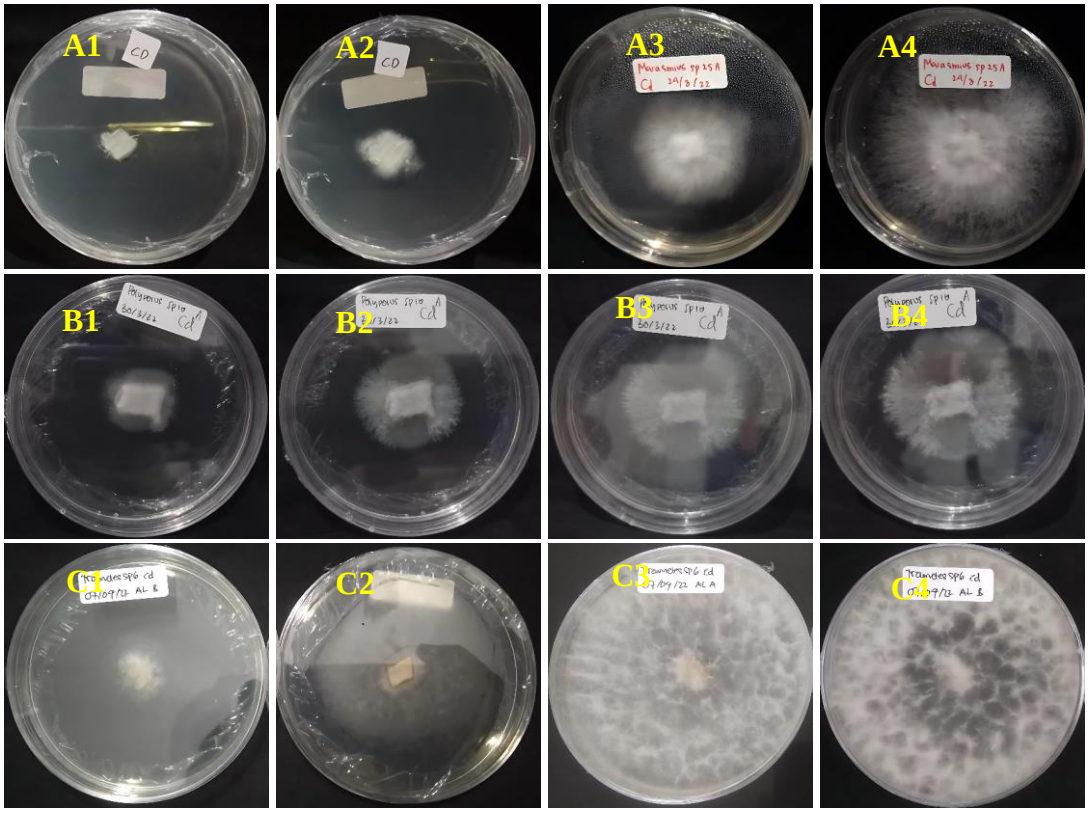

Figure 10. Growth of 3 example macrofungal species on PDA medium with cadmium (PDACd) on the first, third, fourth, and fifth days of Incubation; A1-A4: *Marasmius* sp. 21; B1-B4: *Polyporus elegans*; C1-C4: *Trametes cubensis*

Next, three example species of fungi cultivated on the PDAALPb medium (Figure 12) exhibited robust and rapid growth, filling the Petri dish by the third day of incubation. The colony texture for *Marasmius* sp. 21 remained normal. *T. cubensis* showed a pattern similar to that of other selection media, while *P. elegans* demonstrated colony growth that spread closer to normal. Further statistical analysis using Tukey's test was conducted to highlight species with significant differences in the largest average colony surface area. According to the test, nine species had a substantial impact, with the largest colony surface area (Figure 13).

The same letter labels in the figure indicate the similarity in the average values of the colony surface area for the nine aforementioned fungal species. This suggests that all 9 macrofungal species, C. *comatus*, *F. tenuiculus*, *G. applanatum*, *L. cristata*, *Marasmius* sp. 21, *P. badius*, *P. elegans*, *Polyporus sp.*, and *T. cubensis*, exhibited excellent growth on the PDAALPb medium compared to other species.

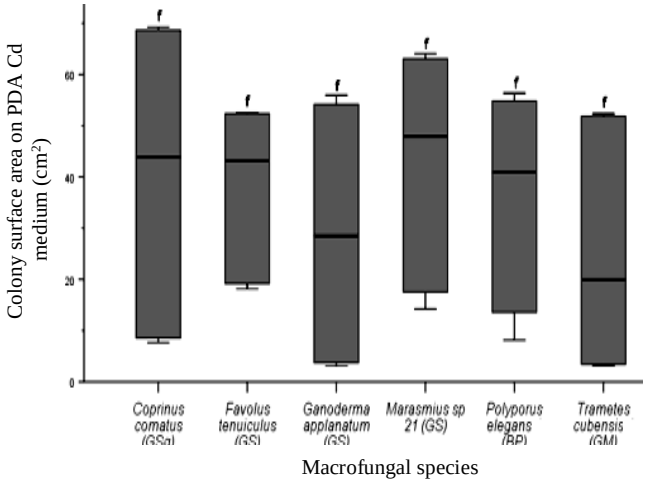

Figure 11. Growth of macrofungal species on cadmium-PDA medium (PDACd) on the third day of incubation with the largest colony surface area values

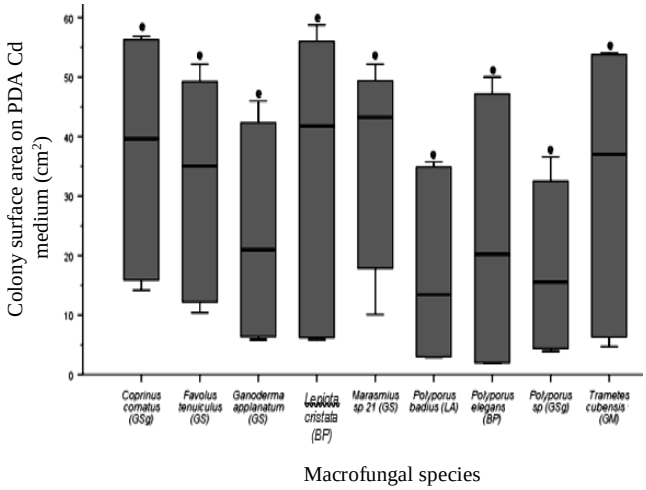

Figure 13. Growth of macrofungal species on PDAALPb medium on the third day of incubation, showing significant differences in colony surface area

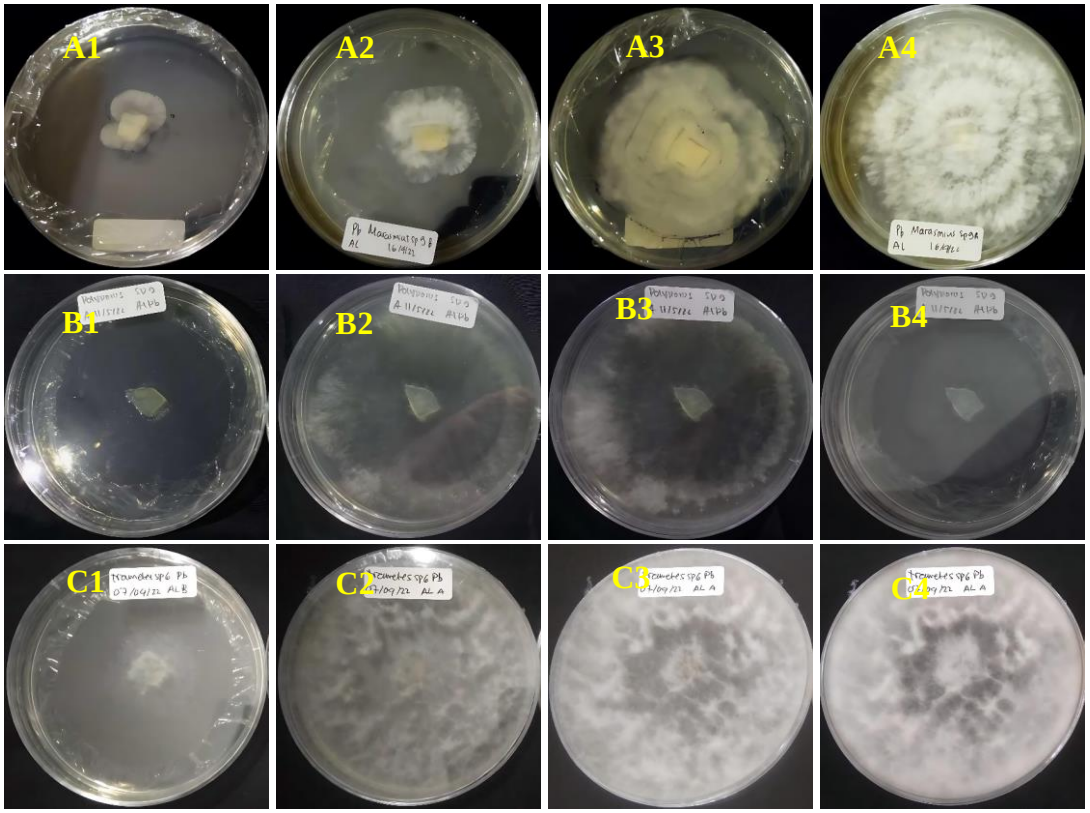

Figure 12. Growth of 3 selected macrofungal species on PDAALPb medium on the first, third, fourth, and fifth days of incubation. A1-A4: *Marasmius* sp. 21; B1-B4: *Polyporus elegans*; C1-C4: *Trametes cubensis*

Selection of PDA seawater and Cadmium medium (PDAALCd)

A total of 50 selected fungal species cultivated on the PDAALCd medium, which exhibited sufficiently good tolerance indices, demonstrated variable growth capabilities. One-way ANOVA analysis was conducted to assess the growth abilities of different macrofungal species based on the colony surface area on the PDA Seawater with Cadmium medium. The results indicated a significant influence of different fungal species on the growth of fungal colonies in the PDA medium containing seawater and cadmium, with a significance level of ($p<0.05$).

Similar to the growth of fungal species on the PDAALPb medium, the differences in the colony surface area of the 50 fungal species on the PDAALCd medium depended on their ability to adapt to the PDA medium containing cadmium and high salt concentrations. Some species exhibited rapid growth with a normal colony appearance, while others experienced slower growth, displaying colonies that differed from the norm. Figure 14 shows several examples of the growth of fungal species on the PDAALCd medium sequentially from the first, third, fourth, and fifth days after incubation. Figure 14 shows that the 3 selected mushroom species grown on the PDAALCd medium exhibit growth characteristics almost identical to those grown on the PDAALPb medium. There is a slight difference in the growth of *Marasmius* sp. 21, which shows slower growth initially (one day after incubation).

Advanced statistical analysis was performed using the Tukey test to highlight species with significant differences in the largest average colony surface area. Based on this test, 8 species exhibited a significant influence on the colony surface area (Figure 15).

The same letter labels in the graph indicate the similarity in the average colony surface area for the eight macrofungal species listed above. This indicates that all eight macrofungal species, namely *Coprinus comatus*, *F. tenuiculus*, *G. applanatum*, *L. cristata*, *Marasmius* sp. 21, *P. badius*, *P. elegans*, and *Polyporus* sp., exhibit excellent growth on the PDAALCd medium compared to other species.

Selection of the best species from all growth media

Based on the selection results of macrofungal species grown in different growth media, 9 macrofungal species exhibited the best growth across all selection media. These species are *C. comatus*, *F. tenuiculus*, *G. applanatum*, *L. cristata*, *Marasmius* sp. 21, *P. badius*, *P. elegans*, *Polyporus* sp., and *Trametes cubensis*. Next, to evaluate the 9 macrofungal species with the best colony surface area across all growth media, a Complete Randomized Factorial Design Analysis (CRFD-A) was conducted. The analysis revealed significant differences in the growth of different macrofungal species across all growth media, with a significance level of ($p<0.05$). Further analysis using Tukey's test revealed that 4 macrofungal species exhibited the largest colony surface area across all selection media, namely *C. comatus*, *P. elegans*, *Marasmius* sp. 21, and *F. tenuiculus* (Figure 16).

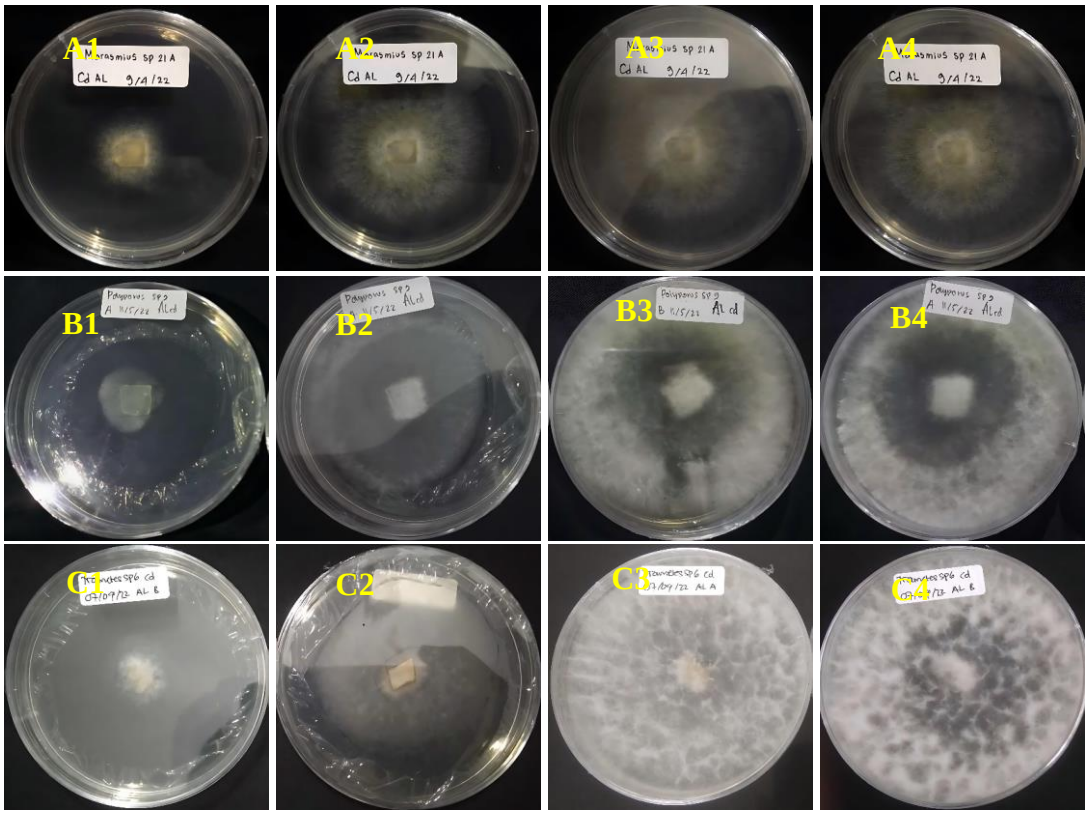

Figure 14. Growth of three selected macrofungal species on PDA Seawater and Cadmium (PDAALCd) medium on the first, third, fourth, and fifth days of incubation. A1-A4: *Marasmius* sp. 21; B1-B4: *Polyporus elegans*; C1-C4: *Trametes cubensis*

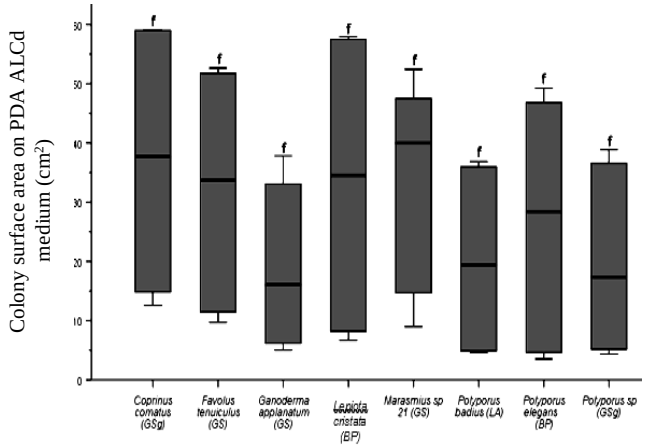

Figure 15. Growth of macrofungal species in PDA seawater Cadmium medium on the third day of incubation with the largest colony surface area

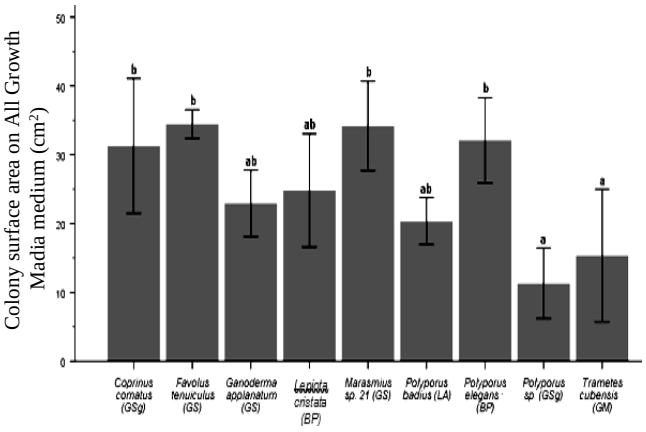

Figure 16. Growth of macrofungal species with the best colony surface area on the third day of incubation in all growth media. The error bars in the diagram represent the standard deviation of colony surface area values across all growth media

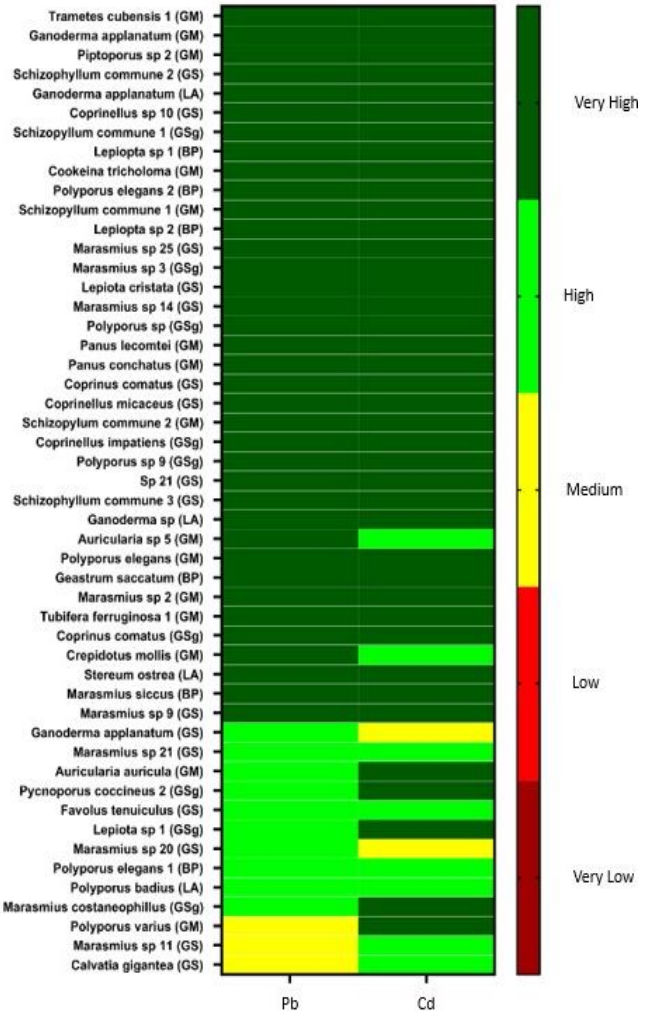

Figure 17. Heat map diagram illustrating the tolerance characteristics of macrofungal species in PDA medium with seawater lead and seawater cadmium based on the tolerance index values. The color gradient in the diagram represents the tolerance characteristics of macrofungal, as determined by the ti values and categorized into 5 levels: very high, high, moderate, low, and very low

Different letter labels in the graph indicate significant differences between variables, while the same letter labels in the graph indicate similarity in the average colony surface area values of the above-mentioned fungal species. This suggests that the fungal species *C. comatus*, *P. elegans*, *Marasmius* sp. 21, and *F. tenuiculus* grew very well in all growth media compared to other species.

Heavy Metal Tolerance Index of macrofungal species

Measurement of the Heavy Metal Tolerance Index was conducted to determine the capability or tolerance of the studied fungal species to heavy metals Pb and Cd added to the PDA medium with seawater (PDAALPb and PDAALCd). The measurement results are presented based on the macrofungi's characteristics in tolerating heavy metals in seawater using a heat map diagram. This diagram illustrates the tolerance characteristics of macrofungi, categorized based on different color gradients: very high heavy metal tolerance (dark green), high (green), moderate (yellow), low (red), and very low (dark red). All of the isolated macrofungal species exhibit diverse tolerance abilities ranging from moderate to very high (Figure 17). This figure shows that the heavy metal tolerance index, especially for lead (Pb) and cadmium (Cd), from the tested 50 fungal species is very good. Approximately 74% are classified as very high, 22% as high, and only 4% as moderate. This indicates that the tested species are highly tolerant to heavy metals Pb and Cd.

A total of 94 macrofungal species were successfully isolated from the West Sumatra Forest Area. These fungal species were most abundant in the Gunung Sago Nature Reserve (TWAGS) and the Gunung Merapi Nature Reserve (TWAGM), with 34 and 29 species, respectively. This distribution is attributed to the dense and diverse vegetation in these 2 forest areas. Abundant litter was found on the forest floor, along with a substantial amount of dead and

decaying wood, providing favorable growing substrates for macrofungi. As a result, the light intensity is relatively low, and the humidity is sufficiently high in these areas. Additionally, both reserves are well-preserved, with no cultivation activities by residents.

Conversely, the Gunung Singgalang Nature Reserve (TWAGSg) exhibits less lush vegetation, as it has been extensively utilized for illegal farming by the local population. The least number of species was discovered in the Lembah Anai Nature Reserve (CALA) and the Batang Palupuh Nature Reserve (CABP). These reserves, characterized by steep and rocky hills, exhibit limited plant diversity, scarce litter, and deadwood, which results in fewer fungal species. This illustrates how environmental conditions significantly impact the diversity and abundance of macrofungi in the forests of West Sumatra.

Macrofungal species found in five forest areas in West Sumatra belong to the phyla Basidiomycota and Ascomycota. The Basidiomycota phylum comprises fungi with large fruiting bodies (basidiocarps), where the basidium serves as the organ for generative spore production (basidiospores) (Jones et al. 2015). On the other hand, the Ascomycota phylum features bodies known as ascocarps, which produce generative spores, or ascospores, within their asci. In the latest fungal classification, based on molecular data, both the Basidiomycota and Ascomycota phyla fall under the Kingdom Fungi, subkingdom Dikarya (Hibbett et al. 2007).

While observing the number of species found, the Basidiomycota phylum dominates, with 93 species (95.88%) identified. The remaining 2 species belong to the Ascomycota phylum: *Cookeina tricholoma* and *Podostroma* sp. 1. This demonstrates that macrofungi are predominantly from the Basidiomycota phylum, with only a small portion belonging to the Ascomycota phylum (Kirk et al. 2013; Noverita et al. 2017, 2018).

Hibbett et al. (2007) further subdivided the Basidiomycota phylum into 3 subphyla: Pucciniomycotina, Ustilaginomycotina, and Agaricomycotina. The Agaricomycotina subphylum is the largest group within the Basidiomycota phylum, and all its species have large fruiting bodies visible to the naked eye. According to Retnowati et al. (2019). The number of macrofungal species from the Basidiomycota phylum is significantly higher compared to their microfungi counterparts, with 829 species for macrofungi and 89 species for microfungi. The discovered macrofungal species originate from the Agaricomycotina subphylum (Widyatmoko 2018; Sun et al. 2024).

The number of macrofungal species from the phylum Ascomycota is typically low in natural environments. It is found in specific orders within the Ascomycota phylum, namely Pezizales, Xylariales, Helotiales, Eurotiales, and Hypocreales. A previous study reported that the macrofungal species from the Ascomycota phylum in Indonesia are very few, including species such as *Elaphomyces tropicalis* sp. nov., *Chlorociboria* cf. *aeruginosa*, *Podostroma* cf. *cornu-damae*, *Onygena* sp., *Morchella* aff. *deliciosa*, *Morchella* sp., *Scutellinia* sp., *Trichaleurina javanica*, *Cookeina speciosa*, *Cookeina tricholoma*, *Phillipsia* sp., *Daldinia* spp., and *Xylaria* spp. (Putra 2021).

Taxonomically, the identified species from the Basidiomycota phylum are predominantly from the order Agaricales, comprising 53 species grouped into families Agaricaceae, Marasmiaceae, Psathyrellaceae, Entolomataceae, Crepidotaceae, Mycenaceae, and Hygrophoraceae. This is followed by the order Polyporales, with 34 species in families Polyporaceae, Ganodermataceae, Fomitopsidaceae, and Stereaceae. The remaining species are from the orders Auriculariales (family Auriculariaceae) with three species, and Boletales (family Sclerodermataceae) with one species. As for the Ascomycota phylum, the identified species belong to the orders Pezizales (family Sarcoscyphaceae), with one species, and Hypocreales (family Hypocreaceae), with one species.

The order Agaricales is characterized by fruiting bodies consisting of 3 main parts: cap (pileus), gills (lamellae), and stem (stipe). These fungi typically live as saprophytes on leaf litter and dead and decaying wood, and some form ectomycorrhizal associations around plant roots. Polyporales, on the other hand, are characterized by large-sized fruiting bodies with pores on the underside of the cap. Species in Polyporales often have a hard texture and are commonly found as saprophytes on dead wood; some are parasitic on living trees. Agaricales and Polyporales are two fungal orders with the highest diversity of genera. A study conducted by (Noverita et al. 2018) in the Thousand Islands Archipelago also revealed that the most encountered species belong to the Agaricales order, accounting for 28 species (44%), followed by Polyporales (Aphylloporales) with 19 species (30%). According to biodiversity status data presented by Retnowati et al. (2019), the two fungal orders with the most recorded species in Indonesia are Polyporales and Agaricales, with 297 and 274 species, respectively, as of 2017.

Macrofungal species successfully cultured on PDA media in the culture tubes were sourced from fruiting bodies growing on various substrates, primarily functioning as saprophytes on leaf litter, twigs, branches, dead stems, and decaying wood. Some fungi live in symbiosis with plant roots, forming ectomycorrhizal associations, while others act as parasites on living plants. According to Begum (2021), especially macrofungi, play a crucial role in nature (Begum 2021).

As saprophytes, these fungi degrade lignocellulose through enzymes they produce, including cellulase, ligninase, and hemicellulase, breaking it down into simpler sugar forms that can be further utilized by surrounding organisms, especially plants, as a source of nutrition. Ectomycorrhiza is a mutualistic symbiosis between fungi and higher plants. In the field, ectomycorrhizal fungi can be observed through their mycelium, which extends outside the plant root cells, enveloping the root surface between the plant-root cortex cell walls (intercellularly) and forming Hartig net tissue. Ectomycorrhiza plays a role in the efficient uptake of mineral nutrients and water, as well as protecting roots from abiotic and biotic stressors. Fungi with parasitic characteristics can cause root and stem rot in plantations and forests, resulting in significant losses for the infected plants. These fungi are also known as white-rot fungi, which can cause wood decay by breaking down lignin

(Bari et al. 2020). Fungi, especially macrofungi, are not only crucial as decomposers of organic compounds in the forest environment but also provide significant benefits to human life, serving as a source of food, clothing materials, and medicine. Additionally, macrofungi play a vital role in environmental bioremediation, particularly in the biosorption of heavy metals. Many species have been applied in metal bioremediation, such as *Polyporus squamosus* and *P. sulphureus* against heavy metals Hg, Pb, Cd, and Cu, and *Pleurotus platypus* and *P. tuber-regium* against heavy metals Cu, Zn, Fe, Cd, Pb, Ni (Kapahi and Sachdeva 2019).

The selection of fungal species on the PDA medium reveals differences in the growth capabilities of each species, despite PDA being a common medium for fungal growth. One factor influencing fungal growth in a growth medium is the nutrient content. Some fungal species can thrive on media with simple nutrients, while others require more complex or additional nutrients to grow. PDA (Potato Dextrose Agar) is a common medium with relatively simple nutrient content used for the isolation and growth of various fungal species. Based on its composition, it is considered a semi-synthetic medium as it is composed of natural ingredients (potato) and synthetic materials (dextrose and agar). Potatoes provide carbon (carbohydrates), vitamins, and energy; dextrose serves as a sugar and energy source, and agar acts as a solidifying agent. Each of these components is essential for the growth and reproduction of fungi.

The results of selecting 50 species grown on PDA medium, subjected to ANOVA followed by Tukey's test, revealed five macrofungal species with significantly larger colony areas compared to the others. These species are *C. comatus, F. tenuiculus, G. applanatum, Marasmius* sp. 21, and *P. elegans*; these five species exhibit rapid growth, producing compact and dense colony textures. This indicates that these species adapt quickly and effectively utilize PDA as a nutrient source compared to other fungal species.

The selection of macrofungal species on PDA medium with seawater solvent (PDAAL) shows that all tested fungal species can grow and adapt to a medium with high salinity, but their growth abilities vary. Some species grow rapidly, while others grow slowly and only exhibit growth after the third day of inoculation. Additionally, some species display colony growth that differs from the norm. The selection of the PDAAL medium aims to obtain macrofungal species that can survive in seawater conditions with high salt content, characterized by high salinity. Salinity is the total amount of dissolved salt material, consisting of inorganic ions such as sodium, chloride, phosphorus, and nitrogen, as well as vitamins, expressed in units of g/kg or parts per thousand (ppt). According to Gunde-Cimerman and Zalar (2014), organisms capable of living in high-salinity environments are referred to as halophilic organisms, and only those that are tolerant can survive in such environments. This is related to 3 physiological problems in high salinity environments, such as in seawater: (i) having relatively low water potential, (ii) containing relatively high ion concentrations, and (iii) having a basic pH.

Salinity is a crucial factor that affects the growth of organisms. If the salinity concentration in the substrate exceeds the concentration inside the cell, it can cause cell damage, hinder growth, or even lead to cessation. Organisms that cannot adapt to high-salinity environments experience shrinkage of their cells' cytoplasm, resulting in cell death. In contrast, organisms that can survive under such conditions continue to grow well. This is closely related to osmotic pressure, which triggers cells to undergo osmoregulation processes. Defines osmoregulation as the process of regulating the fluid concentration of the body or regulating the osmotic pressure of body fluids by cells or living organisms to balance with the osmotic pressure of the environment outside the cell, thereby allowing physiological processes within the cell to proceed normally. The osmoregulation process results in an increased need for energy, as osmoregulation is a metabolic process that requires active ion transport to maintain salt concentration within the cell.

Osmotic and ionic pressure significantly affect the life of fungi in high-salinity conditions (Gunde-Cimerman and Zalar 2014). Research on the mechanism controlling osmotic pressure in fungal mycelium has been conducted since 1967 by Jones and Jennings. The study utilized the fungus *Paradendryphiella salina*, grown in a glucose-triton medium with varying ionic compositions, including potassium, sodium, magnesium, and calcium. The results showed that the addition of potassium ions (K+) to the growth medium resulted in the fungus growing well, with the highest dry biomass. On the other hand, the addition of sodium stimulated fungal dry-weight production at low concentrations but inhibited it at high concentrations. Studies on other fungal species grown in malt extract medium with different ionic compositions also showed that the vegetative growth of all tested fungi was inhibited by sodium. From these findings, it can be stated that the main challenge for fungal growth is the permeability of hyphae to potassium in media with high salinity and the ionic composition of the fungal growth medium (Jones et al. 2022).

Fungi have long been known to be Halotolerant and Halophilic. According to Gunde-Cimerman and Zalar (2014), initially, fungi were only known as contaminants in preserved foods at high concentrations of salt and sugar. Still, since 1975, it has been discovered that fungi are also naturally found in environments with high salt concentrations, including salty lakes. Some examples of fungal species reported to live in high salinity are *Wallemia sebi, W. muriae*, and *W. ichthyophaga*, which belong to the Clade Basidiomycota along with Ustilaginomycetes and Hymenomycetes. *Cladosporium dominicanum, C. psychrotolerans, C. velox, C. spinulosum*, and *C. halotolerans. Debaryomyces hansenii, Aureobasidium pullulans, Hortaea werneckii*, and *Wallemia ichthyophaga* (Gunde-Cimerman and Zalar 2014). Furthermore, Jones et al. (2015) identified 424 marine fungal species (in 251 genera) from the Clade Ascomycota, 94 species (in 61 genera) from the anamorphic fungal group, and 12 species (in 9 genera) from the Clade Basidiomycota.

Out of the 50 species that were able to grow on the PDAAL medium, based on the results of the ANOVA test

followed by the Tukey test, five species exhibited the fastest growth with the largest colony surface area, significantly different from other fungal species. These species are *F. tenuiculus, L. cristata*, *Marasmius* sp. 21, *P. badius*, and *P. elegans*. This indicates that these five species are classified as halotolerant fungi, which are likely capable of surviving in marine environments despite their terrestrial origins. According to phylogenetic studies, many marine organisms have terrestrial ancestors (Hibbett et al. 2007; Amend et al. 2019). Furthermore, Pang et al. (2016) broadly defined marine fungi as "any fungus repeatedly found from a marine habitat and: i) capable of growing and/or sporulating (on substrates) in the marine environment; ii) forming symbiotic relationships with other marine organisms; or iii) proven to adapt and evolve genetically or actively metabolize in the marine environment".

Analysis of 50 fungal species cultivated on lead-supplemented PDA (PDAPb) and cadmium-supplemented PDA (PDACd) media revealed significant variations in colony surface area expansion. While certain species exhibited unaltered growth patterns in the presence of heavy metals, others demonstrated differential responses marked by substantial growth inhibition. Affected strains showed reduced growth rates accompanied by morphological abnormalities, including irregular colony pigmentation (e.g., atypical color shifts) and structural deformities.

Fungal physiology studies indicate that trace quantities of essential heavy metals, such as cadmium (Cd), manganese (Mn), and zinc (Zn), are crucial for metabolic processes. However, supra-optimal concentrations induce toxicity, disrupting cellular functions. This metal stress manifests as suppressed mycelial proliferation, altered morphogenesis, impaired physiological activity, and compromised reproductive capacity. The observed phenotypic aberrations in sensitive species align with these established mechanisms of heavy metal toxicity in fungi (Baldrian 2003).

Lead (Pb) and Cadmium (Cd) are two examples of metals that fall into the category of toxic metals, which do not have biological functions and can interfere with biological processes in organisms. Therefore, to maintain their growth, organisms, in this case, fungi, must have defense systems in their cells. According to Prabhakaran et al. (2016), to survive in an extreme environment for growth, some microbes have defense mechanisms to metabolize and convert harmful heavy metals into less harmful forms, thus producing heavy metal-resistant microbes. Furthermore, Ayangbenro and Babalola (2017) stated that microbes have degradative enzymes to degrade specific contaminants and develop various mechanisms to maintain homeostasis against heavy metals, as well as adapt to such environments (Ayangbenro and Babalola 2017).

The ANOVA test, followed by the Tukey test, revealed that 7 fungal species grown on the PDAPb selection medium exhibited the largest colony surface area. These species are *C. comatus, F. tenuiculus, G. applanatum, L. cristata, Marasmius* sp. 21, *P. badius*, and *P. elegans*. Additionally, 6 fungal species grown on the PDACd medium had the largest colony surface area. These species are *C. comatus, F. tenuiculus, G. applanatum*, *Marasmius* sp. 21, *P. elegans*, and *T. cubensis*. This suggests that the addition of heavy metals, such as Pb or Cd, to the growth medium does not always inhibit the growth of these fungal species; in fact, it can stimulate their growth.

The species mentioned above are not only able to grow on PDA, PDAAL, and PDAPb, as well as PDACd media. Still, they are also capable of growing on PDA medium supplemented with seawater and heavy metals Pb and Cd (PDAALPb and PDAALCd). The growth of these 50 selected fungal species in PDAALPb and PDAALCd media also varies significantly. There are 9 fungal species grown on PDAALPb, and 8 species grown on PDAALCd have the largest colony surface area values, which are significantly different from the colony surface area of other species after conducting ANOVA and Tukey tests. These fungal species are: *C. comatus, F. tenuiculus, G. applanatum, L. cristata*, *Marasmius* sp. 21, *P. badius*, *P. elegans*, *Polyporus* sp., and *T. cubensis* grown on PDAALPb, and *C. comatus*, *F. tenuiculus*, *G. applanatum*), *L. cristata*, *Marasmius* sp. 21, *P. badius*, *P. elegans*, and *Polyporus* sp. has grown on PDAALCd. These fungal species exhibit high tolerance to salinity and the presence of heavy metals, allowing them to thrive on seawater-supplemented PDA media containing heavy metals such as Pb or Cd.

Fungi are capable of thriving in extreme environments, such as varying pH levels, temperatures, and diverse nutritional conditions, as well as exhibiting tolerance to high metal concentrations (Oladipo et al. 2018). The ability of fungi to survive in such extreme environments is attributed to the components that comprise their cell walls. According to Gow et al. (2017), the fungal cell wall is the most crucial part of the fungus's interaction with its environment. Thirty percent or more of the fungal cell's components consist of the cell wall. The primary components of the fungal cell wall are polysaccharides (80%) and proteins (3-20%), with the remainder being lipids, pigments, and organic salts. The fungus's ability to survive and grow is supported by the components of the fungal cell wall that have active sites capable of binding metal ions. These active sites include cross-linking polysaccharides (chitin, chitosan, and glucans), glucuronic acid, galactosamine, glycoproteins, melanin, and phenolic polymers (phenols, peptides, and lemic acid), carboxyl, carbonyl, amino, hydroxyl, phosphate, methoxy, and mercapto.

The mechanism of fungal tolerance to high salt is determined by membrane permeability, ion accumulation in vacuoles, the role of polyols in maintaining turgor in mycelium, the role of calcium and divalent cations in potassium movement, and sodium excretion from the mycelium, the existence of a sodium pump to remove it from the mycelium, and the involvement of ATPase in maintaining the K+/Na+ ratio in glycolysis processes (Jones et al. 2022).

Coprinus comatus, *F. tenuiculus*, *G. applanatum*, *L. cristata*, *Marasmius* sp. 21, *P. badius*, *P. elegans*, *Polyporus* sp., and *T. cubensis* are nine macrofungal species with the best colony surface area across all growth media. The results of the ANOVA and Tukey tests indicate that 4 species exhibit the best colony surface area across all selection media used, namely *C. comatus*, *P. elegans*, *Marasmius* sp. 21, and *F. tenuiculus* (Figure 16).

The tolerance index of the 50 selected macrofungal species in this study to heavy metals Pb and Cd is classified as excellent, ranging from moderate to very high. About 74% are classified as very high, 22% as high, and only 4% as moderate. This means that the tested species are highly tolerant to heavy metals Pb and Cd. When associated with the nine best macrofungal species, *T. cubensis* exhibits a very high tolerance index among macrofungal species (Figure 17).

In conclusion, this study has successfully isolated 94 macrofungal species from 5 forest areas in West Sumatra, with 92 species belonging to Basidiomycota and two species to Ascomycota, demonstrating diverse ecological roles including ectomycorrhizal associations (seven species), saprobic activity on various substrates (82 species), and parasitic relationships (five species). From these isolates, 50 species showed promising growth on various media containing seawater and heavy metals, with four species (*C. comatus*, *P. elegans*, *Marasmius* sp. 21, and *F. tenuiculus*) exhibiting exceptional performance across all test conditions. The heavy metal tolerance analysis revealed outstanding results, with 74% of tested species showing very high tolerance to Pb and Cd, particularly *T. cubensis*, which demonstrated a notably high tolerance index. These findings indicate significant potential for using these fungi in marine environment bioremediation applications, particularly for addressing heavy metal contamination in areas such as Jakarta Bay, while also contributing to our understanding of fungal diversity in Indonesian forest ecosystems.

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