



Biosorption of Pb(II) and Cd(II) by Endophytic Fungi Isolated from *Eichhornia crassipes*: Screening, Identification, and Adsorption Performance

Maswati Baharuddin^{1*}, Husnul Khatimah¹, Ummi Zahrah¹, Siti Fauziah², Sitti Chadijah¹, Fitria Azis¹

¹ Department of Chemistry, Faculty of Science and Technology, Alauddin State Islamic University, Makassar 92118, Indonesia

² Department of Renewable Energy Engineering Technology, Vocational Program, Gorontalo State University, Gorontalo 96128, Indonesia

Corresponding Author Email: bmaswati@gmail.com

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ABSTRACT

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Biosorption is a sustainable and cost-effective approach for removing heavy metals from aquatic environments. This study aimed to screen, identify, and evaluate the adsorption performance of endophytic fungi isolated from *Eichhornia crassipes* for Pb(II) and Cd(II) removal. Experiments were conducted using a single-metal system with synthetic solutions at initial concentrations of 10, 20, and 30 mg/L. Biosorption assays were performed under controlled conditions (pH 7; 25–27 °C) using fungal biomass as the biosorbent. Biosorption performance was analyzed using atomic absorption spectrophotometry (AAS) and expressed as residual metal concentrations (mg/L). *Aspergillus* sp. showed the highest performance for Pb(II), reducing the concentration from 20 mg/L to 0.10 mg/L, followed by *Penicillium* sp. (0.25 mg/L) and *Rhizopus* sp. (1.60 mg/L). For Cd(II), *Aspergillus* sp. reduced the concentration from 30 mg/L to 0.37 mg/L, while *Penicillium* sp. and *Rhizopus* sp. reached 0.65 mg/L and 0.67 mg/L, respectively. These preliminary results suggest the potential of endophytic fungi as promising eco-friendly biosorbents; however, further validation through replicated experiments and statistical analysis is required.

1. INTRODUCTION

Lead (Pb) and cadmium (Cd) are among the most hazardous heavy metals released into the environment as a result of anthropogenic activities, including industrial processes, mining, agricultural runoff, and domestic waste discharge. Cadmium is primarily associated with battery, pigment, and plastic production, whereas lead is commonly derived from smelting activities, mining, and the degradation of lead-based materials [1]. These metals tend to accumulate in industrial wastewater and subsequently contaminate freshwater systems [2, 3]. Although natural processes such as geological weathering contribute to their presence, human activities remain the dominant source of elevated concentrations [4]. The persistence and non-biodegradable nature of Pb and Cd result in severe ecological impacts, including physiological disturbances in aquatic organisms, biodiversity loss, and significant health risks to humans through bioaccumulation and biomagnification in the food chain [1, 5].

To address these challenges, various remediation technologies have been developed, including chemical precipitation, ion exchange, membrane filtration, and adsorption. However, these conventional methods often suffer from limitations such as high operational costs, sludge generation, and reduced efficiency at low metal concentrations. As a result, bioremediation approaches, particularly biosorption, have gained increasing attention as

environmentally friendly and cost-effective alternatives for heavy metal removal. Biosorption utilizes biological materials to bind and remove metal ions through physicochemical interactions, offering advantages such as low cost, high efficiency, and minimal secondary pollution [6, 7].

Among biological agents, fungi have been widely reported as effective biosorbents due to their unique cell wall composition. Fungal cell walls contain biopolymers such as chitin, chitosan, and melanin, which provide abundant functional groups, including carboxyl, hydroxyl, amine, and phosphate, that facilitate metal binding [6, 7]. The biosorption process involves both passive (non-metabolic) mechanisms, such as ion exchange and complexation, and active (metabolic) processes, including intracellular accumulation mediated by compounds such as metallothioneins and phytochelatins [8, 9]. Several genera, including *Aspergillus*, *Penicillium*, and *Rhizopus*, have demonstrated high biosorption capacities for various heavy metals [10, 11].

Despite these advances, studies focusing on endophytic fungi that live symbiotically within plant tissues remain limited, particularly those isolated from aquatic plants. Endophytic fungi may possess enhanced tolerance to environmental stress and metal exposure due to their close association with host plants growing in contaminated habitats. However, research exploring endophytic fungi from a single aquatic plant source, such as *Eichhornia crassipes*, for the simultaneous removal of Pb and Cd under controlled

conditions is still scarce. This gap highlights the need for a systematic investigation into their biosorption potential and comparative performance.

Therefore, this study aims to isolate and identify endophytic fungi from *Eichhornia crassipes* and to evaluate their biosorption performance for Pb(II) and Cd(II) removal using a single-metal system. The study further compares the adsorption efficiency of *Aspergillus* sp., *Penicillium* sp., and *Rhizopus* sp. at different metal concentrations. The findings are expected to provide scientific insights into the development of efficient, economical, and sustainable bioremediation technologies, while also promoting the utilization of locally available aquatic plants for mitigating heavy metal pollution in tropical environments.

2. MATERIAL AND METHODS

2.1 Isolation of endophytic fungi

Plant samples were washed under running water and surface-sterilized with 70% ethanol for 1 minute, followed by 5% sodium hypochlorite (NaOCl) for 5 minutes, and rinsed three times with sterile distilled water. Root, stem, and leaf segments were then cut into small pieces and placed on PDA (Potato Dextrose Agar) medium supplemented with antibiotics to prevent bacterial contamination [12].

2.2 Macroscopic and microscopic identification

Macroscopic identification of fungi was conducted by observing colony characteristics, including shape, elevation, surface texture, and color, after incubation on Potato Dextrose Agar (PDA) at 25-27 °C for 5-7 days. The observations were performed under consistent culture conditions to ensure comparability among isolates [13].

Microscopic identification was carried out using slide culture and staining techniques. A small portion of the fungal isolate was placed on a glass slide, stained with lactophenol cotton blue, and covered with a cover glass. In addition, slide culture preparation was performed to preserve the natural arrangement of fungal structures. The prepared slides were observed under a light microscope at magnifications of 400× and 1000× (oil immersion) to clearly visualize hyphae, conidia, and reproductive structures.

Fungal identification was conducted by referring to standard morphological identification keys and taxonomic descriptions available in the literature, including characteristics of *Aspergillus*, *Penicillium*, and *Rhizopus* species [13].

2.3 Heavy metal biosorption test

Biosorption tests were conducted using fungal isolates cultured in Potato Dextrose Broth (PDB). The fungal biomass was harvested and expressed as dry cell weight prior to use. A known amount of dried fungal biomass was then introduced into incubation containers containing heavy metal solutions prepared from Pb(NO₃)₂ and CdCl₂ at initial concentrations of 10, 20, and 30 ppm (mg/L).

Before inoculation, the pH of each metal solution was adjusted to the desired value using appropriate acid or base solutions. A volume of 2.78 mL equivalent of the fungal culture (based on dry cell weight) was added to each metal-

containing solution. Control samples were prepared in parallel, consisting of metal solutions without the addition of fungal biomass.

All mixtures were incubated in a shaker incubator at 30 °C and 100 rpm for 96 hours. After incubation, the mixtures were separated by centrifugation at 3,000 rpm for 30 minutes. Following centrifugation, the supernatant and the biomass (fungal cells) were clearly separated. The supernatant was then filtered using Whatman No. 41 filter paper, while the biomass residues were collected for further analysis. The residues (biomass) were oven-dried at 105 °C for 1 hour until a constant weight was achieved. All experiments were conducted without replication [14, 15].

This study was designed as an exploratory investigation due to resource limitations, in which each treatment was conducted without biological or technical replication. Consequently, the results presented here should be interpreted as preliminary observations intended to identify general biosorption trends rather than to provide statistically validated conclusions.

2.4 Measurement of biosorption effectiveness

The dried fungal samples were subjected to artificial wet contamination using heavy metal solutions, representing controlled exposure conditions. Following the contamination process, the samples were digested using concentrated nitric acid (HNO₃). The digested samples were then transferred into vials, and the concentrations of lead (Pb) and cadmium (Cd) were analyzed using Atomic Absorption Spectrophotometry (AAS) [14, 15]. This study did not include statistical analysis due to the absence of experimental replication. Therefore, all results were interpreted descriptively, and the findings should be considered preliminary. Further studies with appropriate replication and statistical validation are strongly recommended to confirm the observed trends.

3. RESULTS AND DISCUSSION

3.1 Isolation and identification of endophytic fungi

The isolation of endophytic fungi from the leaves of *Eichhornia crassipes* (water hyacinth) was successfully performed using tissue culture techniques on Potato Dextrose Agar (PDA) medium. The macroscopic morphological observations showed colonies with distinct colors, textures, and growth rates for each isolate, as presented in Table 1.






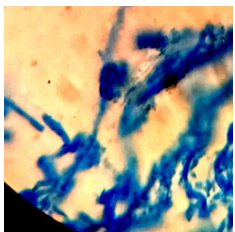
The EC1 isolate displayed irregular colonies with umbonate (domed) elevation, smooth and fluffy surfaces, and a black-and-white coloration. Microscopic examination revealed round conidia with thick walls and septate hyphae, characteristic of *Aspergillus* sp. These results are consistent with the findings of Mawarni et al. [16], who reported that *Aspergillus* sp. colonies typically appear black, with spherical conidia and septate, branched hyphae. Similarly, another study described the macroscopic characteristics of *Aspergillus* colonies on PDA as light to dark green, sometimes turning black with a cream-colored base and a powdery texture [17].

The EC2 isolate, obtained from the roots of water hyacinth, exhibited irregular and flat colonies with a smooth surface, white coloration, and yellow pigmentation. Microscopic observation showed thin-walled, round conidia with septate hyphae, identifying the isolate as *Penicillium* sp. *Penicillium* colonies commonly appear in yellowish-green to bluish-green

shades, often releasing yellow or hyaline exudates. The conidiophores form vesicles, and the conidia are typically

round to elliptical in shape, with fast-growing, flat, wool-like textures [18].

Table 1. Macroscopic and microscopic observation results of the isolates

Isolate	Shape	Observation		Alleged Identification Results
		Macroscopic	Microscopic 100×	
EC1	Irregular umbonate, Fluffy, Black surface, White underside			<i>Aspergillus</i> sp.
EC2	Irregular Flat, Fluffy like cotton, White colony			<i>Penicillium</i> sp.
EC3	Irregular Raised Fluffy like cotton, White colony			<i>Rhizopus</i> sp.

The EC3 isolate exhibited rhizoid-shaped colonies with convex elevation, smooth surface, and grayish-white color. Microscopic examination revealed non-septate hyphae and thin-walled, spherical sporangiospores, identifying the isolate as *Rhizopus* sp. This species is characterized by coenocytic hyphae (multinucleate and aseptate), grayish-white colonies, and the presence of stolons and dark rhizoids [19, 20]. The sporangium is spherical, supported by a smooth sporangiophore opposite the rhizoid, and terminates in a rounded spore head [20].

Microscopic observations reinforced these identifications. *Aspergillus* species showed branched conidiophores ending in vesicles with radially arranged conidia. *Penicillium* species displayed brush-shaped conidiophores with phialides bearing conidia. *Rhizopus* species were characterized by broad aseptate hyphae and spherical sporangia located at the tips of sporangiophores with visible rhizoids [21-23]. These results are consistent with morphological approaches commonly used in fungal taxonomy [24, 25].

3.2 Biosorption efficiency for lead (Pb)

The variation in heavy metal concentration significantly affected the adsorption capacity of the fungi. This phenomenon occurs because the number of active sites on the fungal cell wall is limited, determining the amount of metal ions that can be adsorbed at each concentration level. At lower concentrations, the active binding sites remain abundant, allowing metal ions to interact easily with functional groups such as carboxyl (-COOH), amine (-NH₂), and hydroxyl (-OH). As a result, adsorption efficiency is relatively high since most of the metal ions are captured by the fungal biomass.

Table 2. Absorption of heavy metal lead (Pb) by endophytic fungi

Treatment	Initial Concentration (ppm)	Final Concentration (ppm)	Removal Efficiency (%)
<i>Aspergillus</i> sp.	10	0.12	98.82%
	20	0.10	99.52%
	30	0.43	98.58%
<i>Penicillium</i> sp.	10	0.43	95.74%
	20	0.25	98.75%
	30	1.49	95.05%
<i>Rhizopus</i> sp.	10	0.44	95.63%
	20	1.60	92.02%
	30	2.96	90.12%

The biosorption results of heavy metal lead (Pb) are summarized in Table 2. An apparent inconsistency was observed in the biosorption results of *Aspergillus* sp., where the residual Pb concentration at an initial concentration of 20 mg/L (0.10 mg/L) was lower than that at 10 mg/L (0.12 mg/L). This trend deviates from the typical expectation that higher initial concentrations result in higher residual metal levels. One possible explanation is that the biosorption process reached an optimal interaction condition at intermediate concentration (20 mg/L), where the availability of Pb ions and the accessibility of active binding sites were balanced, leading to more efficient adsorption. At lower concentrations, the driving force for mass transfer may be insufficient to fully utilize available binding sites, whereas at higher concentrations (30 mg/L), partial saturation of adsorption sites may begin to occur, reducing overall efficiency.

In addition, it is possible that supplementary removal

mechanisms, such as precipitation or surface complexation, contributed to the reduction of Pb concentration in solution. These physicochemical processes may occur alongside biosorption, particularly under controlled pH conditions, and can enhance the apparent removal efficiency. However, due to the absence of detailed mechanistic analysis in this study, these explanations remain speculative and should be verified in future studies through more comprehensive experimental design and analytical techniques.

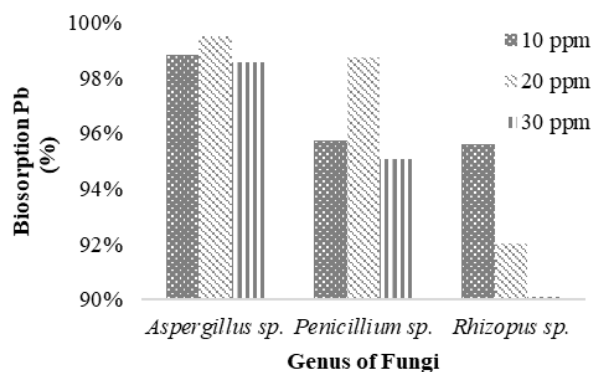


Figure 1. Comparison of lead (Pb) by *Aspergillus sp.*, *Penicillium sp.*, and *Rhizopus sp.*

Based on Figure 1, the highest biosorption efficiency of lead (Pb) was observed in *Aspergillus sp.* at an initial concentration of 20 ppm, reaching 99.52%, whereas the lowest adsorption was recorded in *Rhizopus sp.* at 30 ppm (90.12%). This non-linear trend supports the observation that optimal biosorption may occur at intermediate concentrations, where the interaction between Pb ions and available binding sites is maximized. The superior performance of *Aspergillus sp.* is consistent with previous studies reporting that species such as *Aspergillus niger* exhibit high biosorption capacity for heavy metals, including lead. This capability is generally attributed to the composition of the fungal cell wall, which contains functional groups such as carboxyl and phosphate that act as active binding sites for metal ions. However, considering the exploratory nature of this study and the absence of replication, these findings should be interpreted as preliminary, and further validation is required to confirm the observed trends and adsorption performance [26].

The differences in biosorption efficiency are associated with variations in the structural and chemical properties of each fungal species. *Aspergillus niger* possesses cell walls rich in carboxyl and hydroxyl groups, enhancing its ability to adsorb metal ions [27]. *Penicillium sp.* has complex conidiophores and large colony surfaces that facilitate metal binding. Although *Rhizopus sp.* shows relatively lower efficiency compared to *Aspergillus*, it remains an effective and easily cultivable biosorbent, making it suitable for large-scale bioremediation applications [28].

3.3 Biosorption efficiency for cadmium (Cd)

As shown in Figure 2, the highest cadmium (Cd) biosorption was recorded for *Rhizopus sp.* at 20 ppm, achieving 98.83%, while the lowest was found in *Penicillium sp.* at 10 ppm with 95.03%. Biomass from *Rhizopus oligosporium* was reported to effectively reduce cadmium concentrations by 97.27% within 24 hours [29]. This

efficiency is mainly attributed to the presence of functional groups such as amine, hydroxyl, and carboxyl, which play crucial roles in metal ion binding. Meanwhile, *Penicillium sp.* also shows biosorption potential; *P. oxalicum* was reported to adsorb Cd with a 71% reduction after 96 hours [30]. These findings suggest that *Penicillium* species tend to exhibit lower adsorption capacity compared to *Rhizopus sp.*, possibly due to differences in cell wall structure and the presence of active functional groups involved in biosorption [31]. As shown in Table 3, the biosorption efficiency of cadmium (Cd) varied among the tested fungi.

Table 3. Absorption of heavy metal cadmium (Cd) by endophytic fungi

Treatment	Initial Concentration (ppm)	Final Concentration (ppm)	Removal Efficiency (%)
<i>Aspergillus sp.</i>	10	0.28	97.18%
	20	0.38	98.12%
	30	0.37	98.77%
<i>Penicillium sp.</i>	10	0.50	95.03%
	20	0.56	97.18%
	30	0.65	97.84%
<i>Rhizopus sp.</i>	10	0.12	98.81%
	20	0.23	98.83%
	30	0.67	97.78%

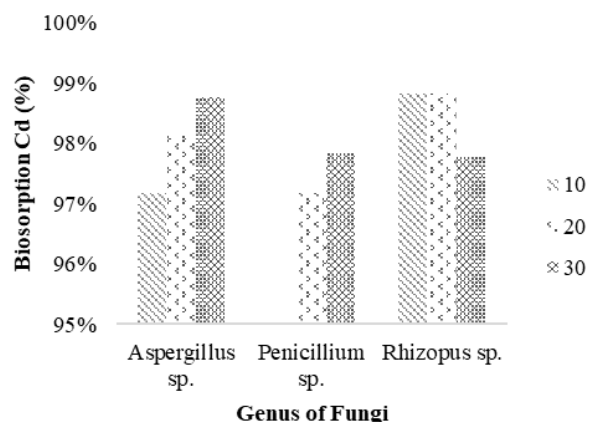


Figure 2. Comparison of cadmium (Cd) by *Aspergillus sp.*, *Penicillium sp.*, and *Rhizopus sp.*

Hydroxyl groups have been identified as the main contributors to enhancing biosorption capacity, as also observed in microbes such as *Bacillus megaterium* and *Rhodotorula sp.* [32]. *Rhizopus sp.* can remove significant amounts of Cd from contaminated media, as reported by Douglas et al. [33].

3.4 Comparative efficiency and bioremediation potential

Based on the biosorption efficiency results obtained through AAS, *Aspergillus sp.* exhibited the highest removal efficiency for Pb, whereas *Rhizopus* showed relatively better performance in Cd removal. This divergence suggests that biosorption in this study is not governed by a uniform mechanism but rather by species-specific surface properties that determine selective metal affinity. Such selectivity indicates that fungal biosorption should be interpreted as a competitive interaction between metal ions and heterogeneous

binding sites, rather than a generalized adsorption process.

A critical limitation of this study is the absence of spectroscopic characterization (e.g., Fourier Transform Infra Red (FTIR)), which restricts direct identification of the functional groups involved. Therefore, any mechanistic interpretation must be treated as inferential rather than confirmatory. Nevertheless, previous studies have consistently demonstrated that fungal biomass contains multiple reactive sites capable of binding heavy metals, supporting the plausibility of the observed biosorption trends [34, 35]. Within this constraint, the present study contributes empirical evidence of biosorption performance but does not claim molecular-level validation.

Importantly, the use of dried fungal biomass strongly indicates that the dominant mechanism in this study is passive biosorption rather than metabolically driven active transport. This distinction is crucial, as passive biosorption is governed primarily by physicochemical interactions such as ion exchange and surface complexation, which are highly dependent on the structural composition of the fungal cell wall [36]. Consequently, differences in biosorption efficiency among *Aspergillus*, *Penicillium*, and *Rhizopus* should be interpreted as a function of surface heterogeneity rather than biological activity.

The higher Pb uptake by *Aspergillus* sp. can be critically linked to its potentially higher density or accessibility of binding sites with stronger affinity toward Pb²⁺ ions. Pb, with its larger ionic radius and higher polarizability, tends to form more stable complexes with available binding groups compared to Cd. In contrast, the relatively higher Cd removal by *Rhizopus* suggests that its cell wall structure may provide binding environments more favorable for Cd²⁺ interaction. This indicates that biosorption efficiency is not solely dependent on the number of binding sites, but also on their chemical compatibility with specific metal ions. Similar species-dependent selectivity has been reported in fungal biosorption studies, emphasizing the role of physicochemical compatibility in adsorption processes [34, 37].

Furthermore, these differences can be critically associated with variations in fungal cell wall composition, particularly the relative proportions of chitin, chitosan, glucans, and other structural polymers. These components create a heterogeneous matrix of binding sites with varying affinities and reactivities. In some fungi, additional compounds such as melanin may further enhance metal binding by introducing extra coordination sites. Therefore, the observed differences between *Aspergillus* and *Rhizopus* are likely rooted in intrinsic structural variability rather than experimental conditions alone [38].

However, without direct compositional or spectroscopic evidence, this explanation remains hypothetical and highlights a key research gap. Future studies integrating FTIR, Scanning Electron Microscope-Energy Dispersive X-ray Spectroscopy (SEM-EDX), or surface chemistry analyses are necessary to validate the proposed mechanisms and to quantify the contribution of specific functional groups. This is particularly important for advancing fungal biosorption from empirical observation toward predictive and design-oriented applications.

Despite these limitations, the present findings remain significant in demonstrating that all three fungal isolates possess practical biosorption potential. Moreover, the relatively faster growth and adaptability of *Rhizopus* suggest an operational advantage in time-sensitive remediation

systems, even if its maximum adsorption capacity is lower than that of *Aspergillus*. This trade-off between adsorption efficiency and growth kinetics should be considered in designing large-scale bioremediation strategies.

In a broader context, fungal biosorption represents a robust and adaptable approach to heavy metal remediation, supported by extensive prior research on fungal tolerance and metal-binding capacity [29, 37]. However, to transition from laboratory-scale feasibility to real-world application, future work must address current limitations by integrating mechanistic validation, process optimization, and scalability assessment.

4. CONCLUSIONS

This study demonstrates that endophytic fungi isolated from *Eichhornia crassipes* are effective biosorbents for Pb(II) and Cd(II) removal under controlled conditions. Based on AAS analysis, *Aspergillus* sp. showed the highest performance for Pb(II), reducing concentrations from 20 mg/L to 0.10 mg/L, while *Rhizopus* sp. exhibited superior Cd(II) removal, reaching 0.67 mg/L from 30 mg/L. These findings reveal species-specific selectivity in metal uptake and highlight the potential of fungal biomass as an eco-friendly alternative for wastewater treatment. However, the absence of spectroscopic characterization limits mechanistic interpretation. Future studies should focus on elucidating adsorption mechanisms and validating the scalability of fungal biosorbents in real environmental systems.

However, it is important to emphasize that these findings are preliminary due to the lack of experimental replication and statistical analysis. Future studies should incorporate rigorous experimental design, including replication and statistical evaluation, to validate these results.

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