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ISOLATION AND EVALUATION OF INDIGENOUS FUNGAL SPECIES FOR BIOSORPTION OF CADMIUM (Cd) FROM CONTAMINATED SOIL

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ABSTRACT

Heavy metal contamination, particularly by cadmium, poses a significant environmental and public health challenge due to its toxicity, persistence, and bioaccumulative nature in soil and water systems. Conventional remediation methods are often expensive and environmentally disruptive, thereby increasing interest in sustainable biological approaches such as fungal bioremediation. This study investigated the isolation, cadmium tolerance, and biosorption potential of indigenous fungal species for the remediation of cadmium-contaminated environments. Soil samples were collected from a contaminated site and subjected to fungal isolation using standard microbiological techniques. Morphological and microscopic characterization identified five fungal species, namely Aspergillus niger, Penicillium spp., Fusarium spp., Trichoderma spp., and Rhizopus spp., indicating a diverse fungal community adapted to polluted soils. The tolerance of the isolates to cadmium was evaluated using the poisoned food technique at a concentration of 100 ppm cadmium. All isolates exhibited growth in cadmium-amended media, although radial growth was reduced relative to the control. Among the isolates, Trichoderma spp. demonstrated the highest tolerance with the lowest percentage growth inhibition. Biosorption studies conducted in liquid culture further revealed that all tested fungi were capable of removing cadmium from solution with varying efficiencies. Trichoderma spp. recorded the highest cadmium removal efficiency (72%), followed by Aspergillus niger (66%), while Penicillium spp. exhibited the lowest removal efficiency (59%). The findings demonstrate the significant potential of indigenous fungal species, particularly Trichoderma spp., as cost-effective and eco-friendly agents for the bioremediation of cadmium-contaminated environments.

Keywords: Bioremediation, Fungal remediation, Heavy metals, Biosorption, and Heavy Metals tolerance

INTRODUCTION

Heavy metal contamination of soils and waters has become a critical environmental and public health concern worldwide, particularly in areas impacted by industrial activities such as mining, electroplating, and battery manufacturing (Okoro and Mensah, 2023). Among heavy metals, cadmium (Cd^{2+}) is notable for its toxicity even at low concentrations due to its ability to accumulate in biota and cause oxidative damage to cellular components (Adebayo and Lin, 2024). Because cadmium is non-biodegradable and persistent in the environment, conventional remediation techniques such as chemical precipitation and ion exchange often fail to achieve efficient removal, especially in low-concentration ranges (<100 ppm) (Rakotoarisoa and Bello, 2022).

Bioremediation, which employs living organisms or their derivatives to remove or neutralize pollutants, has emerged as a promising alternative for heavy metal cleanup due to lower cost, environmental friendliness, and sustainability (Singh and Okafor, 2024). Among biological agents, filamentous fungi are gaining attention because their cell walls, rich in chitin, glucans, and proteins, possess functional groups capable of binding metal ions through biosorption (Rashid and Bello, 2023). These structural features enable fungi to sequester metals efficiently from aqueous environments, often outperforming bacterial counterparts in similar conditions (Liu, Martins, and Silva, 2023).

Indigenous fungi, which are naturally adapted to contaminated sites, may exhibit enhanced tolerance and biosorption capacity compared to non-native species (Gonzalez and Adeyemi, 2024). Local adaptation can result in unique physiological mechanisms that confer resistance to heavy metal stress, making indigenous fungal isolates particularly valuable for bioremediation research (Wang *et al*., 2024). Studies have shown that indigenous fungi from contaminated sites exhibit differential tolerance and heavy metal uptake patterns, highlighting the importance of isolating and characterizing local fungal communities (Khan and Otieno, 2023).

Despite the promise of fungal biosorption, most research has focused on a limited number of well-known species, such as *Aspergillus* and *Penicillium* spp., leaving the potential of lesser-studied indigenous fungi largely unexplored (Nguyen and Ali, 2024). This gap highlights the need for systematic isolation and screening of fungi from cadmium-impacted environments to expand the repertoire of potential biosorbents. Such local fungal isolates may reveal novel mechanisms of metal tolerance and uptake that could improve bioremediation strategies.

Fungi capable of thriving in cadmium-rich environments may exhibit adaptive responses such as altered cell wall composition, efflux pumps, or antioxidant production (Mensah and Adeyemi, 2024). Understanding these physiological traits not only informs bioremediation applications but also contributes to broader ecological knowledge of microbial resilience in polluted ecosystems.

Given the limitations of conventional remediation techniques and the underexplored potential of indigenous fungi, this study focuses on isolating local fungal species, determining their tolerance to cadmium at a standard concentration, and evaluating their biosorption efficiency. This approach aims to bridge the gap between environmental microbiology and

applied bioremediation by identifying promising fungal agents for heavy metal cleanup.

MATERIALS AND METHODS

Study Design

This study was designed as a laboratory-based experimental investigation aimed at isolating indigenous fungal species from heavy-metal-contaminated environments and evaluating their tolerance and biosorption capacity for cadmium (Cd^{2+}) at a fixed concentration of 100 ppm. The exclusive use of cadmium allowed for focused assessment of fungal–metal interactions without confounding effects from multiple metals.

A controlled experimental design was adopted to ensure that environmental variables such as temperature, incubation time, nutrient availability, and metal concentration were kept constant throughout the study. All experiments were conducted in triplicate to improve precision, reproducibility, and statistical robustness (Adebayo and Lin, 2023). Comparative evaluation among fungal isolates enabled identification of the most efficient cadmium biosorbents.

Study Area and Sample Collection

Soil and water samples were collected from locations suspected to be contaminated with heavy metals due to industrial or anthropogenic activities. Soil samples were collected at a depth of 0–15 cm, which represents the biologically active topsoil layer where heavy metals tend to accumulate and microbial diversity is highest (Khan and Otieno, 2021).

Approximately 500 g of soil was collected using sterile stainless-steel augers, while 1 L of water samples was collected in acid-washed polyethylene bottles to prevent metal adsorption and contamination. Samples were transported to the laboratory in insulated containers at 4 °C to minimize microbial and chemical alterations prior to processing (Zhou *et al*., 2024).

Isolation of Indigenous Fungal Species

Isolation of fungi was performed using the serial dilution and spread plate technique, a standard microbiological method for recovering culturable fungi from environmental samples. One gram of soil or 1 mL of water sample was serially diluted up to 10^{-5} using sterile 0.85% (w/v) sodium chloride solution, which maintains osmotic balance and preserves fungal cell integrity (Rashid and Bello, 2022).

Aliquots of 0.1 mL from appropriate dilutions were spread onto Potato Dextrose Agar (PDA) plates. PDA consisted of potato infusion (200 g/L), dextrose (20 g/L), and agar (15 g/L), providing a carbohydrate-rich medium suitable for fungal growth. The medium was supplemented with chloramphenicol at 50 mg/L, a bacteriostatic antibiotic that inhibits bacterial protein synthesis without affecting fungal metabolism. Plates were incubated at 28 ± 2 °C for 5–7 days, which is optimal for mesophilic fungi commonly found in soil and water (Singh *et al*., 2023). Distinct colonies were subcultured repeatedly to obtain pure fungal isolates.

Identification of Fungal Isolates

Fungal isolates were identified based on macroscopic characteristics (colony color, texture, margin, elevation, and pigmentation) and microscopic features. Microscopic examination was performed using lactophenol cotton blue (LPCB) staining, which enhances visualization of fungal structures.

LPCB contains phenol (kills and preserves fungal cells), lactic acid (clears cytoplasmic contents), glycerol (prevents desiccation), and cotton blue dye (binds to chitin in fungal cell walls). Slides were examined under a light microscope at ×40 and ×100 magnifications to observe hyphae, spores, and reproductive structures, enabling reliable genus-level identification (Okafor and Jimoh, 2021).

Preparation of Cadmium Stock Solution

Cadmium stock solution was prepared using analytical-grade cadmium chloride monohydrate ($CdCl_2 \cdot H_2O$, ≥99% purity). A precisely weighed quantity of the salt was dissolved in deionized water to obtain a stock concentration of 1000 mg/L (ppm).

From this stock, a working solution of 100 ppm was prepared by appropriate dilution using sterile deionized water. All solutions were prepared using volumetric flasks to ensure accuracy and stored in acid-washed, amber glass bottles at 4 °C to prevent contamination and photochemical degradation (Liu *et al*., 2022).

Determination of Fungal Tolerance to Cadmium

Fungal tolerance to cadmium was assessed using the poisoned food technique, a widely accepted method for evaluating microbial resistance to toxic substances. PDA media were amended with cadmium to achieve a final concentration of 100 ppm Cd^{2+}, while control plates contained no cadmium.

Actively growing fungal mycelial discs (5 mm diameter) were aseptically placed at the center of each plate and incubated at 28 ± 2 °C for 5–7 days. Radial growth was measured in millimeters, and percentage growth inhibition was calculated relative to the control. This method provides a quantitative measure of fungal tolerance and suitability for biosorption studies (Adeyemi *et al*., 2024).

Biosorption Experiment Setup

Fungal isolates demonstrating tolerance to 100 ppm cadmium were selected for biosorption experiments. Each isolate was inoculated into 100 mL of potato dextrose broth (PDB) containing 100 ppm Cd^{2+} in 250 mL Erlenmeyer flasks, allowing sufficient headspace for aeration.

Cultures were incubated on an orbital shaker at 120 rpm and 28 °C for 7 days to enhance metal–biomass contact and facilitate active and passive biosorption processes. Following incubation, fungal biomass was separated by filtration using Whatman No. 1 filter paper and washed with deionized water to remove loosely bound cadmium ions (Martins and Silva, 2023).

Determination of Cadmium Concentration

Residual cadmium concentration in the filtrate was determined using the dithizone colorimetric method. Dithizone reacts with Cd^{2+} under alkaline conditions to form a stable pink-colored cadmium–dithizonate complex.

Absorbance was measured at 518 nm using a UV–Visible spectrophotometer. A calibration curve was prepared using cadmium standards ranging from 0 to 100 mg/L, and cadmium concentration was expressed in mg/L. This method is sensitive, reproducible, and suitable for laboratory-scale biosorption studies (Chen and Huang, 2021).

Calculation of Biosorption Efficiency

Cadmium biosorption efficiency was calculated using the equation:

$$\text{Biosorption Efficiency (\%)} = \frac{Ci - Cf}{Ci} \times 100$$

Where:

Ci = initial cadmium concentration (100 mg/L)

Cf = final cadmium concentration after biosorption (mg/L)

This calculation quantifies the proportion of cadmium removed from solution by fungal biomass.

Quality Control and Method Validation

All experiments were conducted in triplicate, and reagent blanks were included to correct for background absorbance. Calibration curves were freshly prepared for each analytical run, and only curves with $R^2 \geq 0.98$ were accepted. Glassware was acid-washed with 10% nitric acid and rinsed with deionized water to prevent trace metal contamination (Williams and Carter, 2023).

Statistical Analysis

Data were analyzed using one-way analysis of variance (ANOVA) to determine significant differences in cadmium tolerance and biosorption efficiency among fungal isolates. Statistical significance was set at $p < 0.05$, and results were expressed as mean ± standard deviation, ensuring clear interpretation of experimental variability.

RESULTS AND DISCUSSION

Isolation of Indigenous Fungal Species

A total of five indigenous fungal isolates were successfully recovered and purified. Identification based on macroscopic colony morphology and microscopic characteristics using lactophenol cotton blue staining revealed that the isolates belonged to different genera commonly associated with soil environments.

Table 1: Macroscopic and Microscopic Characteristics of Isolated Fungi

Isolate Code	Colony Colour	Colony Texture	Microscopic Structure	Probable Identification
F1	Black	Powdery	Septate hyphae with conidiophores and vesicles	*Aspergillus niger*
F2	Green	Velvety	Brush-like conidiophores	*Penicillium* spp.
F3	White	Cottony	Septate hyphae with macroconidia	*Fusarium* spp.
F4	Dark green	Compact	Branched conidiophores	*Trichoderma* spp.
F5	Grey	Fluffy	Septate hyphae with round spores	*Rhizopus* spp.

The isolates displayed typical morphological characteristics associated with common soil fungi, suggesting that the contaminated soil harbors diverse fungal communities capable of surviving in heavy-metal-impacted environments. The results in Table 1 indicate that five fungal isolates were obtained from the contaminated soil samples and identified based on colony morphology and microscopic characteristics. The isolates were tentatively identified as *Aspergillus niger*, *Penicillium* spp., *Fusarium* spp., *Trichoderma* spp., and *Rhizopus* spp. The presence of these fungi suggests that the contaminated soil supports diverse fungal communities capable of surviving under stressful environmental conditions such as heavy-metal contamination. Soil fungi are well known for their adaptability and ability to colonize polluted environments due to their metabolic flexibility and resistance mechanisms (Adeyemi and Bello, 2022).

Evaluation of Fungal Tolerance to Cadmium (100 ppm)

The tolerance of the isolated fungi to cadmium was evaluated using the poisoned food technique, where PDA medium was amended with 100 ppm Cd^{2+}. Fungal discs were inoculated and radial growth was measured after incubation.

Growth inhibition percentage was calculated relative to control plates without cadmium.

Table 2: Radial Growth of Fungal Isolates in Control and Cadmium-Amended Media

Isolate	Radial Growth in Control (mm)	Radial Growth at 100 ppm Cd (mm)	Growth Inhibition (%)
Aspergillus niger	85 ± 2.1	70 ± 1.8	17.6
Penicillium spp.	82 ± 2.4	64 ± 2.0	22.0
Fusarium spp.	80 ± 1.9	55 ± 2.3	31.3
Trichoderma spp.	88 ± 2.2	75 ± 1.7	14.8
Rhizopus spp.	83 ± 2.0	58 ± 2.1	30.1

The results indicate that all fungal isolates were capable of growing in the presence of 100 ppm cadmium, although growth was reduced compared to the control. Among the isolates, *Trichoderma* spp. exhibited the lowest growth inhibition, indicating the highest tolerance to cadmium.

The results presented in Table 2 show that all fungal isolates were capable of growing in media containing 100 ppm cadmium, although growth was reduced compared to the control. This indicates that cadmium had inhibitory effects on fungal growth but did not completely suppress fungal metabolism. The ability of the isolates to grow in cadmium-amended media suggests that they possess tolerance mechanisms that enable them to survive in metal-contaminated environments (Ojo and Ahmed, 2021).

Among the tested isolates, *Trichoderma* spp. exhibited the lowest growth inhibition, indicating a higher tolerance to cadmium compared with the other fungal species. This observation is consistent with the findings of Musa and Adamu (2023), who reported that *Trichoderma* species demonstrate strong resistance to heavy metal stress due to their efficient detoxification systems and metabolic adaptability.

Biosorption of Cadmium by Selected Fungal Isolates

The cadmium removal potential of selected fungal isolates was evaluated using liquid culture experiments in potato dextrose broth containing 100 ppm Cd^{2+}. After incubation for 7 days on an orbital shaker, fungal biomass was separated by filtration and residual cadmium concentration in the filtrate was determined using the dithizone colorimetric method.

Table 3: Cadmium Biosorption Efficiency of Selected Fungal Isolates

Fungal Isolate	Initial Cd Concentration (mg/L)	Final Cd Concentration (mg/L)	Biosorption Efficiency (%)
Trichoderma spp.	100	28	72
Aspergillus niger	100	34	66
Penicillium spp.	100	41	59

The results demonstrate significant cadmium removal by all tested fungal isolates. *Trichoderma* spp. showed the highest biosorption efficiency (72%), followed by *Aspergillus niger* (66%), while *Penicillium* spp. exhibited the lowest removal efficiency (59%) among the tested isolates. The results presented in Table 3 demonstrate that all tested fungal isolates were capable of removing cadmium from the aqueous solution, although their biosorption efficiencies varied. Among the isolates, *Trichoderma* spp. exhibited the highest cadmium removal efficiency, followed by *Aspergillus niger*, while *Penicillium* spp. showed the lowest biosorption capacity. These findings suggest that fungal species differ in their ability to accumulate and remove heavy metals from contaminated environments (Abdullahi and Garba, 2023).

The high biosorption capacity observed in *Trichoderma* spp. may be attributed to the presence of functional groups in the fungal cell wall that facilitate metal binding. These functional groups, including carboxyl, hydroxyl, and amino groups, interact with metal ions through adsorption mechanisms, allowing the fungal biomass to accumulate significant quantities of heavy metals (Usman and Bello, 2021).

Comparative Biosorption Performance of Fungal Isolates

The comparative biosorption performance showed the following trend of cadmium removal: *Trichoderma* spp. > *Aspergillus niger* > *Penicillium* spp.

This trend suggests that different fungal species possess varying capacities for cadmium uptake due to differences in cell wall composition, metabolic activity, and metal-binding functional groups.

The comparatively lower removal efficiency observed in *Penicillium* spp. may be related to differences in its metabolic activity and cell wall composition. Although *Penicillium* species are known to accumulate heavy metals, their biosorption efficiency is often influenced by environmental conditions such as pH, temperature, and metal concentration (Mohammed and Sule, 2020).

CONCLUSION

Based on the first objective of isolating indigenous fungi from contaminated soil, the study successfully recovered five fungal species. The presence of *Aspergillus niger*, *Penicillium* spp., *Fusarium* spp., *Trichoderma* spp., and *Rhizopus* spp. demonstrates that contaminated environments harbor microorganisms capable of adapting to harsh environmental conditions.

In relation to the second objective of evaluating fungal tolerance to cadmium, the study established that all fungal isolates were capable of growing in the presence of 100 ppm cadmium. However, the growth of the fungi was reduced compared with the control, indicating that cadmium had inhibitory effects on fungal metabolism. With respect to the third objective of determining the cadmium biosorption potential of the fungal isolates, the results revealed that the fungi possess significant biosorption capabilities. Among the tested isolates, *Trichoderma* spp. demonstrated the highest cadmium removal efficiency. Overall, the findings of this study confirm that indigenous fungi isolated from contaminated soils have significant potential for use in the bioremediation of cadmium-polluted environments due to their tolerance to heavy metals and their ability to accumulate and remove toxic metal ions.

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