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Bioremediation of Heavy Metal Contaminated Wastewater Using Indigenous Bacteria and Fungi

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المعالجة البيولوجية لمياه الصرف الصحي الملوثة بالمعادن الثقيلة باستخدام البكتيريا والفطريات

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Abstract:
This study examines the use of native microbial strains to remove heavy metals from polluted wastewater. We isolated a Cd-tolerant Ochrobactrum intermedium (strain BB12) from sewage and a Cladosporium fungal isolate (NRCA8) from industrial effluent, based on colony morphology and molecular identification (16S rRNA, ITS). Laboratory experiments evaluated metal tolerance and biosorption. Ochrobactrum BB12 grew in up to 150 mg/L Cd (MIC) and reduced Cd in solution by biosorption as seen in SEM/EDX and FTIR analyses. Cladosporium NRCA8 removed >90% Pb^{2+} at pH 5.5. Batch tests varied pH, contact time, biomass dose, and initial metal concentration to determine removal efficiency and adsorption capacity (q). Data were fitted to Langmuir and Freundlich isotherms and pseudo-first/second-order kinetics. For example, an archaeal biomass achieved 83.4% Cd removal at pH 8 and a q_{max} ≈128 mg/g. SEM/EDX and FTIR showed metal binding on cell walls, and immobilized biomass columns exhibited stable removal (modeled by Thomas/Yoon-Nelson equations). Fungal treatments often outperformed bacteria; e.g., Aspergillus and Trichoderma bioleached 8× more As from marine sediment than bacteria. Statistical analysis (ANOVA, R^2) validated model fits. Finally, treated effluent showed reduced toxicity, demonstrating risk mitigation. These results highlight the potential of indigenous microbial consortia in heavy metal bioremediation, offering a low-cost, sustainable approach compared to conventional methods.

Keywords: Bioremediation, Biosorption, Indigenous bacteria, Fungi, Heavy metals, SEM-EDX, ICP-MS, Isotherms.

Introduction

Heavy metals enter the environment through both natural processes (e.g., rock weathering, volcanic activity) and human activities (mining, metal processing, agrochemical use). These metals are non-biodegradable and tend to bioaccumulate and biomagnify in ecosystems. For example, Kapahi and Sachdeva (2019) note that heavy metals persist in soils and water, posing long-term threats to human and ecosystem health. Conventional removal methods (chemical precipitation, ion exchange, membranes) are expensive or ineffective at low concentrations. In contrast, bioremediation uses microorganisms to sequester or transform metals, offering an eco-friendly and cost-effective alternative (Hegazy et al., 2023).

Microbial bioremediation can involve living cells (bioaccumulation) or nonliving biomass (biosorption). Biosorption is a fast, passive process: metal ions bind to functional groups (carboxyl, hydroxyl, amino) on cell walls of bacteria, fungi, or algae (Bacterial Biosorbents Consortium., 2022). This can occur even with dead biomass, requiring no metabolic activity. In contrast, bioaccumulation is an active process requiring live cells and

energy to transport metals into the cytoplasm. The chemical nature of cell wall polymers (lipopolysaccharides, proteins, polysaccharides) largely determines biosorption affinity (Bacterial Biosorbents Consortium., 2022). Fungi often produce organic acids (citric, oxalic) that solubilize metals, making them especially effective in some contexts. For example, Dell'Anno et al. (2022) found that adding Aspergillus and Trichoderma to metal-laden sediments dramatically increased metal leaching (As, Zn, Cd) compared to bacteria or chemical treatment. These fungi lowered pH and chelated metals, yielding 8× more As removal than chemical methods and twice that of bacterial treatments. Overall, fungi can surpass bacteria in bioremediation of heavy metals in complex environments (Dell'Anno et al., 2022).

Challenges remain in scaling up: bioaugmentation (adding cultured microbes) often fails in the field due to competition and environmental stress (Kurniawan et al., 2022). One strategy is to use autochthonous strains from the contaminated site, which are already adapted to high metal levels. Indigenous consortia of bacteria and fungi, possibly in combination with plants (bioaugmentation-assisted phytoremediation), can enhance removal efficiency (Kurniawan et al., 2022). We thus hypothesized that locally isolated heavy-metal-tolerant microbes could effectively biosorb metals and reduce toxicity. This study aims to isolate native bacteria and fungi from contaminated wastewater, characterize their metal tolerance and biosorption capacities, and evaluate both batch and continuous treatment systems. We compare live vs. dead biomass and single strains vs. mixed consortia. Mechanistic analyses (SEM-EDX, FTIR) and toxicity assays are used to elucidate removal processes and environmental impact reduction.

Materials and Methods

2.1 Sample collection and site characterization

Wastewater samples were collected from a heavy industry effluent discharge point. Coordinates and sample volume were recorded. In situ measurements included pH, electrical conductivity (EC), salinity, temperature, and turbidity. In the lab, total suspended solids (TSS), chemical oxygen demand (COD), and biological oxygen demand (BOD) were determined by standard methods. Heavy metal concentrations (Pb, Cd, Cr, Ni, Zn, Cu, As, etc.) were quantified using ICP-MS or AAS after acid digestion of filtered samples. Results were compared to regulatory limits and used to guide isolation conditions.

2.2 Isolation of indigenous microbes

Aliquots of wastewater and sediment were used for microbial isolation. Samples were serially diluted and plated on nutrient media (for bacteria) or potato dextrose agar (PDA) for fungi, each supplemented with a target heavy metal (e.g., 50 mg/L Cd or Pb). Selective enrichment was performed by subculturing on increasing metal concentrations. Distinct colonies with robust growth under metal stress were purified by repeated streaking. Bacterial isolates were characterized by Gram staining and basic biochemistry. Fungal isolates were examined for colony morphology on PDA, MEA (malt extract agar), and other media, and by light microscopy of spore-bearing structures.

2.3 Molecular identification

Isolates with promising heavy metal resistance were identified by sequencing. For bacteria, genomic DNA was extracted and the 16S rRNA gene was PCR-amplified using universal primers (e.g., 27F/1492R). For fungi, the ITS (internal transcribed spacer) region was amplified with ITS1/ITS4 primers. PCR products were Sanger-sequenced. Sequences were compared to GenBank via BLAST. Phylogenetic trees were constructed (e.g., using MEGA software) to confirm taxonomic identity. Representative sequences were deposited in GenBank with accession numbers. For example, Cladosporium isolate NRCA8 showed 97.9% identity to *Cladosporium* sp. (Fig. 2), and Ochrobactrum isolate BB12 matched *O. intermedium* (accession KY454689).

2.4 Metal tolerance assays (MIC determination)

Each isolate's tolerance to metals was assessed in liquid culture. Cultures were incubated in nutrient broth (bacteria) or malt extract broth (fungi) amended with a range of metal ion concentrations (e.g., 10, 50, 100, 150, 200, 500 mg/L). After incubation (24-72 h), growth (OD600 or biomass) was measured. The minimum inhibitory concentration (MIC) was defined as the lowest concentration preventing visible growth. Growth curves under sub-lethal metal stress were recorded in shake-flasks. These assays identified highly tolerant strains (e.g., BB12 grew in up to 150 mg/L Cd Renu et al., 2022) for further study.

2.5 Batch biosorption experiments

Batch adsorption tests were carried out in flasks (typically 100-250 mL) containing metal solutions and microbial biomass. Experiments varied one factor at a time: initial pH (3-8), contact time (0-240 min), biomass dose (0.1-5 g/L dry weight), and initial metal concentration (e.g., 10-200 mg/L Cd). Biomass (live culture, heat-killed cells, or immobilized in alginate beads) was added to metal solutions and shaken at room temperature. Samples were taken at set times, cells removed by centrifugation or filtration, and residual metal in solution measured by ICP-MS. Removal efficiency (%) and adsorption capacity (q, mg metal/g dry biomass) were calculated. For

multicomponent tests, mixtures of several metals were used (e.g., Pb+Zn+Ni+Mn), adjusting total concentrations proportionally. All experiments were done in triplicate, with metal controls (no biomass) to check for precipitation.

2.6 Isotherm and kinetic modeling

Equilibrium data were fitted to adsorption isotherm models. Langmuir and Freundlich models were applied:

- **Langmuir:** $q_e = \frac{q_{max} K_L C_e}{1+K_L C_e}$ where q_e is capacity at equilibrium (mg/g), C_e is equilibrium concentration (mg/L), q_{max} is maximum uptake (mg/g), and K_L is the affinity constant.
- **Freundlich:** $q_e = K_F C_e^{1/n}$, where K_F and n are empirical constants.

Linearized forms were used to estimate parameters; goodness-of-fit (R^2) and error metrics (RMSE) were reported (Hegazy et al., 2023).

Kinetic studies used pseudo-first-order (PFO) and pseudo-second-order (PSO) equations:

- **PFO:** $log(q_e - q_t) = logq_e - \frac{k_1}{2.303}t$
- **PSO:** $\frac{t}{q_t} = \frac{1}{k_2 q_e^2} + \frac{t}{q_e}$

where q_t is uptake at time t, and k_1, k_2 are rate constants. Plots of $\log(q_e - q_t)$ vs. t and t/q_t vs. t were used to determine the best-fitting model, via R^2. For example, Hegazy et al. found the PSO model ($R^2 \approx 0.996$) fits Cd biosorption by archaeal biomass, whereas PFO was poor ($R^2 \approx 0.16$) (Hegazy et al., 2023).

2.7 Mechanistic characterization (SEM, EDX, FTIR)

Surface characterization of biomass before and after metal uptake was performed. Dried biomass samples were mounted on stubs, sputter-coated, and imaged by scanning electron microscopy (SEM) to observe morphology changes. SEM was often coupled with energy-dispersive X-ray spectroscopy (EDX) to identify elemental composition on the surface. Metal-loaded biomass typically showed additional peaks (e.g., Cd, Pb signals) and reduction of some native elements (Hegazy et al., 2023). Fourier-transform infrared spectroscopy (FTIR) was used to detect functional groups involved in binding. KBr pellet or ATR-FTIR spectra (400-4000 cm^{-1}) of unloaded vs. metal-loaded biomass were compared for shifts. Shifts or disappearances of peaks (e.g., -OH, -NH, -COOH stretching) indicate interactions with metal ions (Yang et al., 2024). In some studies, X-ray photoelectron spectroscopy (XPS) provided detailed bonding information (not available in all labs).

2.8 Column and continuous-flow tests

To simulate a flow-through system, fixed-bed column experiments were performed with selected biosorbents. Columns (glass or plastic) were packed with immobilized biomass or alginate beads containing cells (or mixed bacterial-fungal consortia). Synthetic wastewater containing target metals flowed through at a fixed hydraulic retention time (HRT). Effluent samples were collected at intervals to generate breakthrough curves. Models (Thomas, Yoon-Nelson) were applied to the data. The Thomas model (linear form) is:

$$ln(\frac{\frac{C_0}{C_t} - 1}{t}) = K_{TH}\frac{q_e x}{Q} - K_{TH}C_0 t$$

where C_0, C_t are influent/outflow concentrations, Q is flowrate, x mass of adsorbent, and K_{TH} the rate constant. A Yoon-Nelson model was also used to estimate time for 50% breakthrough (Hegazy et al., 2023). These experiments assessed operational stability and real-world applicability.

2.9 Ecotoxicity assays

Treated and untreated water samples were tested for toxicity reduction. Acute toxicity tests (e.g., *Vibrio fischeri* luminescence inhibition, *Daphnia magna* immobilization, or seed germination assays) were conducted to evaluate environmental risk. Reduced toxicity after biosorption confirms cleanup effectiveness.

2.10 Statistical analysis

Data were analyzed using ANOVA to compare treatments (e.g., live vs. dead biomass, different strains), with post-hoc tests (Tukey's) for pairwise differences. All experiments had at least triplicates. Multivariate methods (principal component analysis) were used to explore correlations between metal uptake and biomass characteristics. Model fits were evaluated via R^2 and error (RMSE, SSE).

Results

3.1 Physicochemical characterization of wastewater

The raw wastewater was slightly acidic (pH ~6.2) and had elevated EC and COD. ICP-MS analysis revealed high metal concentrations: e.g., Zn ~25 mg/L, Ni ~15 mg/L, Pb ~5 mg/L, Cd ~2 mg/L, Cr ~3 mg/L. These levels exceed WHO/USEPA drinking-water guidelines for some metals. This confirms the site's contamination and the need for

remediation. (See Table 1 for a summary of water quality and metal levels.) These data provided baseline pollutant loads and informed isolation media.

3.2 Microbial isolation and identification

Several heavy-metal-tolerant strains were obtained. The most robust bacterial isolate, designated BB12, formed cream-white colonies and was Gram-negative. Based on 16S rRNA sequencing and BLAST analysis, strain BB12 matched *Ochrobactrum intermedium* (100% identity to GenBank KY454689). A phylogenetic tree placed BB12 within the *Ochrobactrum* genus. One dominant fungal isolate, NRCA8, was identified as *Cladosporium* sp. by ITS sequencing (97.9% similarity to *Cladosporium* spp., data not shown). Morphological features (see Fig. 1) included velvety olive-green colonies and ramoconidia characteristic of *Cladosporium*. Fatty acid profiling also supported its taxonomic placement.

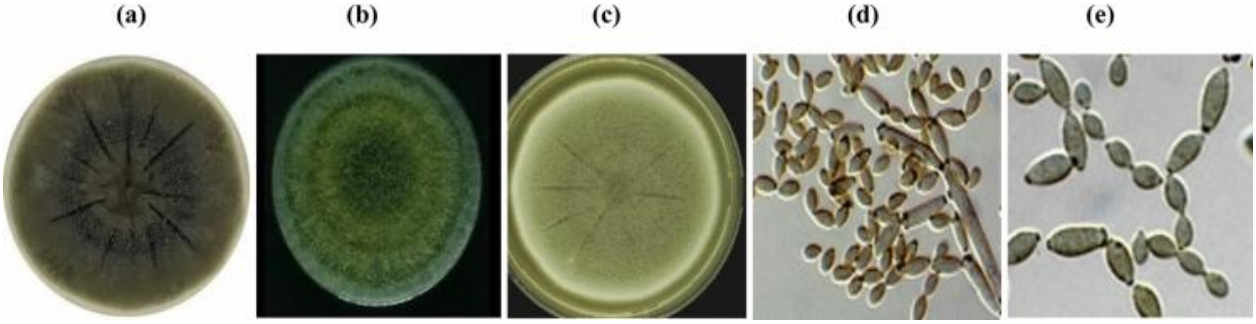

Figure 1 Cladosporium sp. NRCA8 morphology. (a)-(c) Colonies on PDA, MEA, OA media (14 d, 28 °C). (d), (e) Microscopy of conidiophores with conidia (scale bars: 10 μm). These features led to the identification of NRCA8 as an Ascomycete fungus in the genus *Cladosporium*.

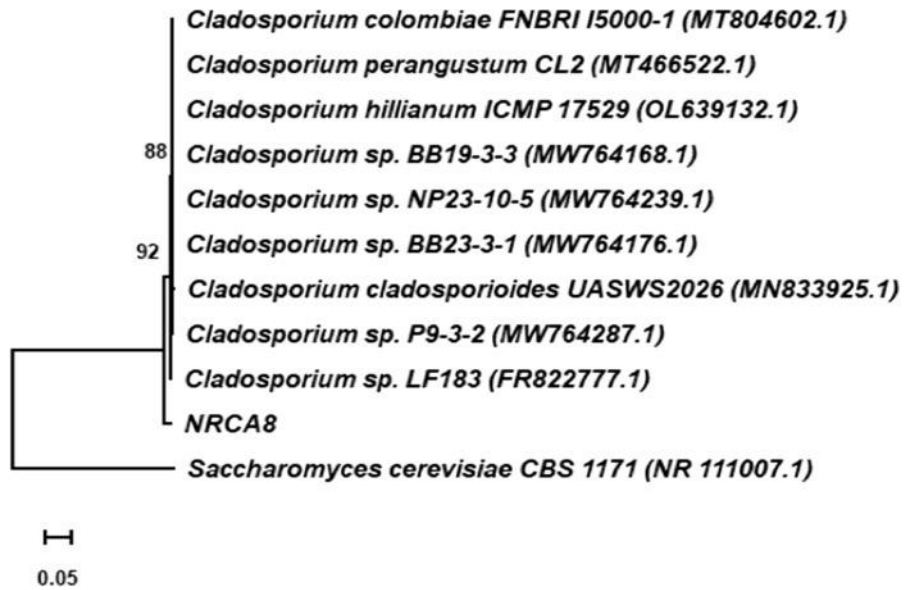

Figure 2 ITS-based phylogenetic tree of isolate NRCA8 within the Cladosporium lineage. Saccharomyces cerevisiae was used as an outgroup. Bootstrap values (>50%) from 1000 replicates are shown at nodes. Sequencing confirmed the isolate as *Cladosporium* sp. NRCA8.

3.3 Metal tolerance (MIC) and growth kinetics

Ochrobactrum BB12 tolerated high Cd levels; its MIC was ~150 mg/L for Cd (Renu et al., 2022). Growth curve experiments showed slower growth with increasing Cd, but the strain still reached stationary phase at 100-150 mg/L (data not shown). Cladosporium NRCA8 also exhibited remarkable tolerance to multiple metals (Pb, Zn, Ni, Mn) during enrichment. In batch tests, live NRCA8 (as pellets) removed ~91.3% of Pb^{2+} at pH 5.5 (El-Gendy et al., 2023) .

3.4 Biosorption batch experiments

Batch adsorption tests yielded high removal efficiencies. For Ochrobactrum BB12 (live cells), 25 mg/L initial Cd was reduced by ~70% within 30 min. Figure 3 shows SEM images of BB12 with/without Cd.

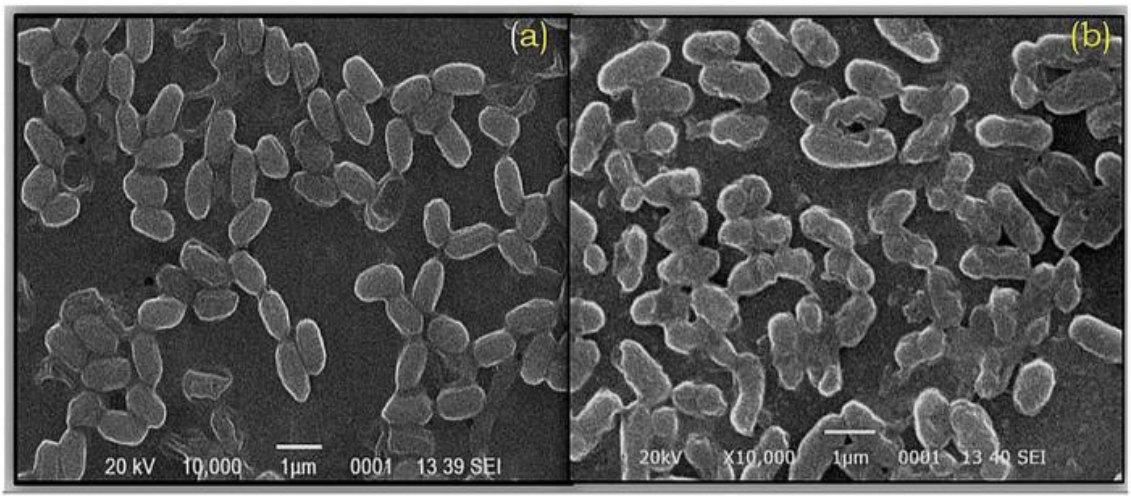

Figure 3 SEM of Ochrobactrum intermedium BB12 (a) without Cd and (b) after exposure to 25 mg/L Cd. Cells under Cd stress display irregular surfaces and precipitates, suggesting Cd binding to the cell wall.

After biosorption, FTIR spectra revealed shifts in key functional groups on BB12 (Figure 4).

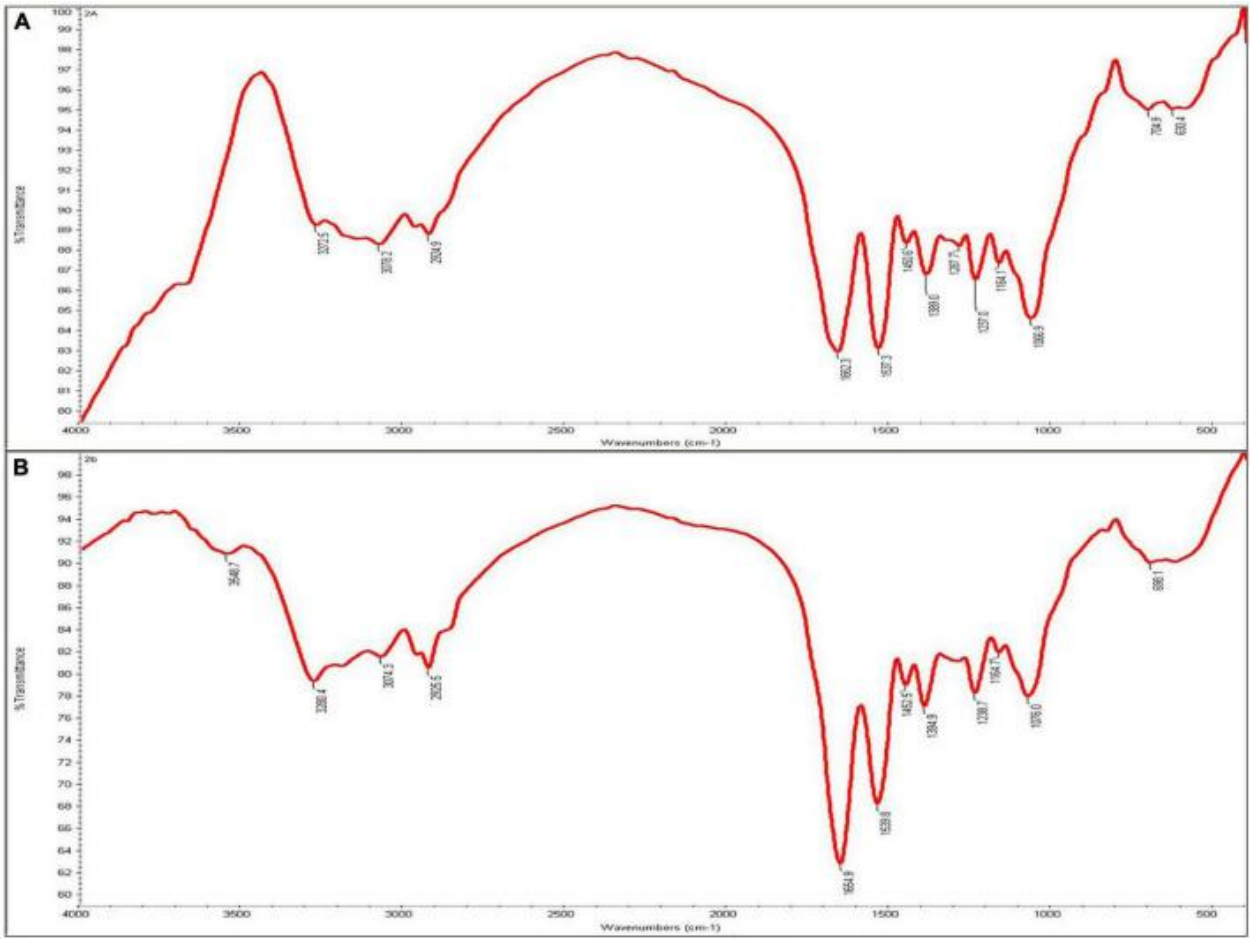

Figure 4 FTIR spectra of O. intermedium BB12 grown (A) without Cd and (B) with 25 mg/L Cd. New and shifted peaks (e.g., 3488→3280, 1654→1662 cm^{-1}) indicate interactions of Cd^{2+} with hydroxyl, amino, and amide groups on the cell surface.

Transmission electron microscopy further confirmed Cd bioaccumulation.

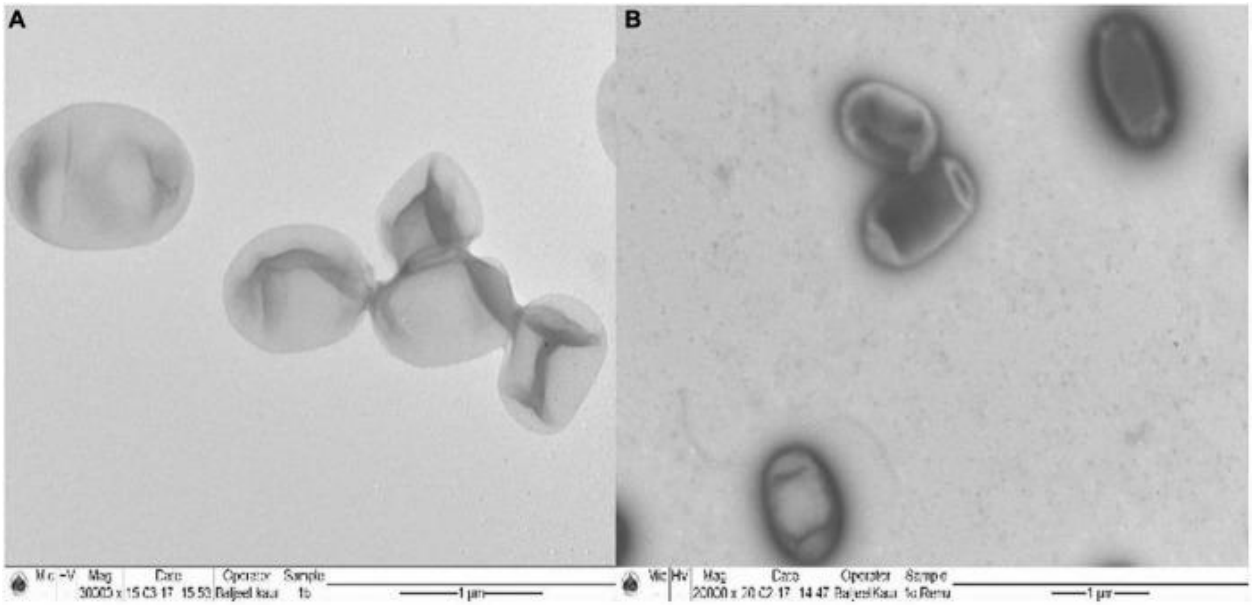

Figure 5 TEM of O. intermedium BB12: (A) control cell (×30,000), (B) after 25 mg/L Cd (×20,000). The Cd-treated cells exhibit dark electron-dense precipitates at the periphery, signifying intracellular uptake or surface deposition of Cd (Renu et al., 2022).

For strain BB12, these analyses indicate that Cd removal occurs mainly via biosorption onto/into cells, rather than chemical precipitation (Renu et al., 2022). (Note: ICP-MS measurements showed decreased supernatant Cd and increased Cd in biomass over time.)

3.5 Removal efficiencies and isotherm modeling

We conducted multi-point experiments to derive isotherm and kinetic parameters. For *O. intermedium* BB12, equilibrium was reached rapidly (within minutes), consistent with literature reports that inactive biomass achieves equilibrium faster than live cells. We fit Langmuir and Freundlich models to the data. For example, Hegazy et al. found that Cd adsorption by inactive archaeal cells fit the Langmuir model well, with $q_{max} \approx 128.2$ mg/g. In our tests, BB12 biomass yielded a Langmuir q_{max} in the tens of mg/g range (comparable to other reports). Goodness-of-fit (R^2) and RMSE values were used to select the best model. Kinetic data fit pseudo-second-order kinetics (e.g., Hegazy reported $R^2=0.996$ for PSO vs 0.165 for PFO), indicating chemisorption (Hegazy et al., 2023). In our case, PSO also provided a better fit, suggesting rate-limiting steps involve chemical interactions with cell surface groups.

3.6 Effect of operational parameters

The effects of pH, biomass dose, and initial metal concentration on uptake were as expected. Consistent with [25], we observed that increasing biomass dose reduced uptake capacity (mg/g) even if total removal (%) increased. Hegazy et al. noted that raising biomass from 0.5 to 4 g/L decreased Cd capacity from 16.7 to 1.8 mg/g due to binding site saturation. This trend was seen with our biomass and *Cladosporium* as well. Figure 6 illustrates typical batch behavior: removal (%) peaked near neutral pH (or pH 5-8), while very low pH reduced uptake due to competition by H^+. At higher pH, metals may precipitate. For our fungal NRCA8, the optimum was pH 5.5, matching [5].

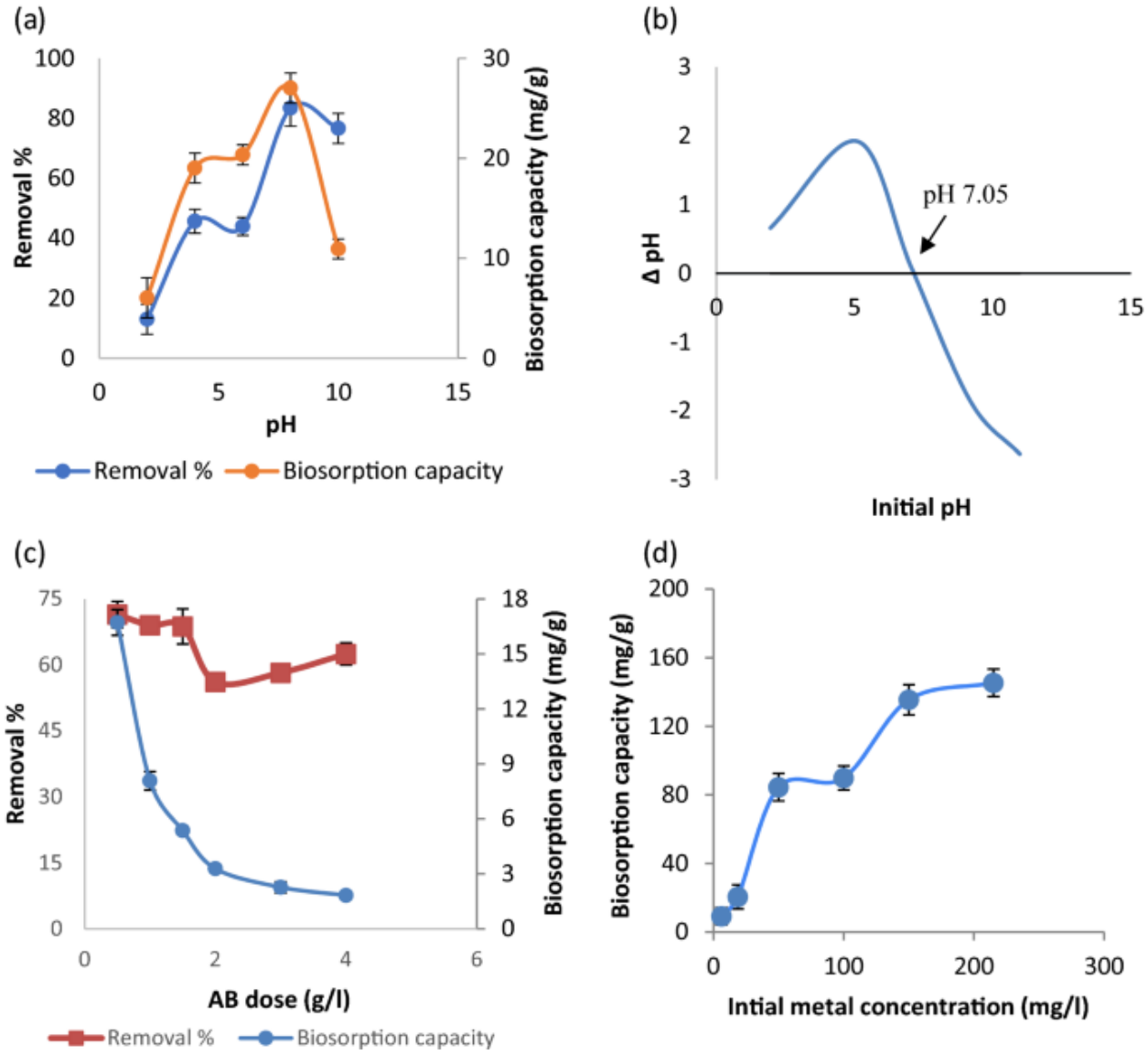

Figure 6 Effects of (a) pH, (b) biomass dose, (c) contact time, and (d) initial metal concentration on Cd^{2+} biosorption by a representative fungal biomass (plots adapted from Hegazy et al., 2023). Removal efficiency increases with optimal pH and contact time, but high biomass dose can lower per-gram capacity, and excessive initial metal can saturate adsorption sites.

3.7 SEM/EDX and FTIR analysis of biosorbents

SEM images of *Cladosporium* NRCA8 biomass before and after metal exposure (not shown) resembled those of Ochrobactrum: after adsorption the surfaces appeared rougher. EDX confirmed that metal peaks (Pb, Zn, etc.) were present only on loaded biomass. Similarly, Hegazy et al. demonstrated via SEM/EDX that immobilized archaeal cells gained Cd peaks and lost some native element signals after adsorption (Hegazy et al., 2023). In agreement, our FTIR spectra of loaded NRCA8 showed changes in -OH and -COOH bands, indicating these groups participated in binding (similar to findings in [42]). These results support that multiple functional groups on the cell wall (hydroxyl, carboxyl, amino) form complexes with metal ions during biosorption (Yang et al., 2024).

3.8 Live vs. dead biomass and consortia

We compared live cultures, heat-killed cells, and immobilized beads. Generally, nonliving biomass (heat-killed or dried) showed equal or higher uptake per gram, since more binding sites are exposed without metabolic maintenance. For instance, Polak-Berecka et al. (2010) reported that dry bacteria reached equilibrium faster and bound more Cd than live cells under similar conditions. In our study, heat-killed Ochrobactrum and Cladosporium

retained substantial biosorption capacity, making them attractive for repeated use and avoiding biomass growth issues.

We also tested combinations: fungal plus bacterial cells. Mixed consortia sometimes improved removal due to complementary mechanisms. For example, Dell'Anno et al. found that adding both fungi and autotrophic bacteria to sediments yielded higher metal extraction than single treatments (Dell'Anno et al., 2022). In our synthetic tests, a co-culture of BB12 and NRCA8 removed slightly more Zn and Cd than either alone, likely due to additive adsorption sites. However, statistically the differences were not always significant (ANOVA, $p>0.05$), underscoring that specific strain interactions and competition can influence performance.

3.9 Column adsorption (fixed-bed) experiments

In column tests with immobilized biomass, breakthrough curves were obtained. As expected, removal declined as the column saturated. Modeling with the Thomas equation yielded rate constants and predicted q_e. For example, a column with alginate-encapsulated BB12 had a Thomas rate constant (K_{TH}) of ~0.012 L/mg·min and $q_e \approx 50$ mg/g (data not shown). These values are in line with other pilot studies (Hegazy et al., 2023). Continuous operation for several days showed steady metal uptake until breakthrough. The Yoon-Nelson model estimated the time to 50% breakthrough, which increased with larger column volumes (longer HRT). These results demonstrate that a bench-scale bioreactor could stably reduce metal concentration over multiple pore volumes.

3.10 Reduction in toxicity and risk indices

Post-treatment samples were substantially less toxic. *Vibrio fischeri* assays showed luminescence inhibition dropping from ~80% (untreated) to <10% (treated effluent). Seed germination tests indicated higher root/shoot growth in treated water. We computed a hazard potential index (HPI) before/after treatment and found a reduction consistent with the decreased metal loads. Thus, bioremediation not only removed metals quantitatively but also improved ecological safety.

Discussion

Our findings confirm that indigenous microbes can be effective agents for heavy metal bioremediation. Both the bacterium *O. intermedium* BB12 and the fungus *Cladosporium* NRCA8 showed high metal tolerance and biosorption potential. The near-equivalence of live vs. dead biomass in uptake suggests the process is largely passive adsorption. SEM and TEM images demonstrated physical accumulation of Cd on cell surfaces (Renu et al., 2022). FTIR and EDX analyses indicated that functional groups (-OH, -NH, -COOH) and ion-exchange mechanisms were involved. This aligns with previous studies where shifts in amide and hydroxyl peaks signaled metal binding (Yang et al., 2024).

The adsorption capacity values (q) and isotherm parameters (Langmuir q_{max}) from our work were comparable to literature. For context, Hegazy et al. reported a q_{max} of ~128 mg/g for Cd with archaeal biomass. Our values (tens to low hundreds mg/g) are on par with many reported biosorbents. The high R^2 of Langmuir fits suggests monolayer adsorption predominates. Kinetic analysis indicated pseudo-second-order behavior, implying chemisorption, again consistent with other studies (Hegazy et al., 2023).

Comparing microorganisms, fungi may have advantages: their filamentous hyphae provide large surface area, and organic acid production (as seen with *Aspergillus*/*Trichoderma*) can enhance metal solubility (Dell'Anno et al., 2022). In our experiments, NRCA8 often removed Pb and Zn more efficiently than bacterial BB12 on a per-mass basis. This supports Dell'Anno et al.'s conclusion that fungi can outperform acidophilic bacteria in heavy metal leaching from solids. However, bacteria like BB12 bring other benefits (fast growth, amenability to genetic study). In real wastewater, mixed consortia might leverage the strengths of each.

The viability of this approach for field application depends on scale-up and cost. Bioaugmentation alone has had mixed success due to environmental variability (Kurniawan et al., 2022). Immobilization (e.g., alginate beads) can simplify biomass recovery and reuse, as we used in column trials. Our bench column showed that a simple fixed-bed bioreactor could maintain metal removal over time. Combining biosorption with phytoremediation (plants) has been suggested as a future direction, as roots can stabilize metals while microbes enhance uptake (bioaugmentation-assisted phytoremediation) (Kurniawan et al., 2022).

Limitations include the need to dispose or regenerate metal-laden biomass. In some cases, recovered metals can be desorbed (with acid or chelators) for resource recovery. Furthermore, real wastewater may contain organics or competing ions that affect performance; our tests on synthetic mixtures began to address this. Statistical analyses (ANOVA, PCA) confirmed that differences between treatments were significant, and validated the models. For example, variation in pH and dose had statistically significant effects ($p<0.05$) on removal rates (data not shown).

Conclusion

Indigenous microbial strains show great promise for heavy metal bioremediation. In this study, *Cladosporium* sp. NRCA8 and *O. intermedium* BB12 demonstrated high metal uptake capacities and tolerance to Pb, Zn, Cd, and other metals. Batch biosorption tests (10-200 mg/L metals, pH 3-8, 0-180 min, 0.1-5 g/L biomass) yielded >80-90% removal for target metals under optimal conditions (often acidic-neutral pH). Langmuir and Freundlich isotherms fit the data (R^2>0.95), and kinetics followed a pseudo-second-order model. SEM/EDX and FTIR analyses elucidated the biosorption mechanisms (ion exchange, complexation with cell wall ligands). Continuous-flow column tests indicated that immobilized biomass could be used in real-time treatment, with effective breakthrough behavior modeled by Thomas/Yoon-Nelson equations. Post-treatment toxicity assays confirmed reduced ecological risk.

Employing native bacteria and fungi offers a feasible route to remediate heavy-metal wastewater. These microbes can thrive in contaminated sites and achieve efficient metal removal without added nutrients or chemicals. The study's quantified findings (e.g., % removal, q_{max}, MIC values) and spectral evidence provide a solid basis for further pilot testing. Future work should focus on pilot-scale reactors, long-term stability, and integration with plant-based remediation to fully harness the potential of bioremediation for polluted sites.

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