10.48047/jocaaa.2024.33.04.27

Study of Molecular Characterization of Bacteria from Industrial Effluent Water for Biosorption and Bioaccumulation

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Abstract

Industrial effluent discharge containing heavy metals poses significant environmental and health risks worldwide. This study presents a comprehensive investigation of bacterial isolation, molecular characterization, and biosorption capabilities from industrial effluent water. A total of 48 bacterial isolates were obtained from various industrial wastewater sources, with 12 strains demonstrating exceptional heavy metal tolerance. Molecular identification using 16S rRNA gene sequencing revealed dominant species including *Bacillus subtilis*, *Enterobacter cloacae*, and *Staphylococcus epidermidis*. Biosorption experiments demonstrated removal efficiencies of 95.6% for cadmium, 91.2% for lead, and 89.3% for chromium under optimized conditions. The highest biosorption capacity was observed in *Bacillus subtilis* strain BS-47 with 78.31% copper removal from 2 mM copper sulfate solution. X-ray diffraction analysis confirmed metal uptake within bacterial biomass, while scanning electron microscopy revealed structural changes in cell walls post-biosorption. The study establishes the potential of indigenous bacterial strains as cost-effective biosorbents for industrial wastewater treatment and provides insights into molecular mechanisms underlying metal tolerance and bioaccumulation processes.

Keywords

Biosorption, bioaccumulation, industrial effluent, molecular characterization, 16S rRNA, heavy metal removal, bacterial identification

1. Introduction

Industrial activities have significantly contributed to environmental contamination through the discharge of untreated effluents containing toxic heavy metals such as chromium, cadmium, lead, copper, and zinc (1,2). Industrial activities, agricultural runoff, municipal waste, and mining generate toxic pollutants that threaten ecosystems and human health, necessitating sustainable remediation strategies to mitigate their impact. Traditional physicochemical methods for heavy metal removal are often expensive and generate secondary pollutants, making biological treatment approaches increasingly attractive (3,4).

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Bacterial bioremediation is an eco-friendly and cost-effective method for treating metal-contaminated industrial effluent. It uses biosorption and bioaccumulation mechanisms, redox reactions, and enzymatic transformation methods, with bacterial cell walls as potential chemisorption sites. Biosorption represents a passive process utilizing dead biomass where toxic substances are adsorbed onto biomass surfaces, while bioaccumulation involves active uptake by living organisms with subsequent intracellular accumulation (5,6).

The molecular characterization of bacterial isolates has become essential for understanding metal resistance mechanisms and optimizing biosorption processes. Because of its size (~1,500 base pairs), the 16S rRNA gene is the most widely used sequence in bacterial identification due to its evolutionary conservation and species-specific variable regions (7). Recent advances in molecular techniques have enabled precise identification of metal-resistant bacterial strains with enhanced biosorption capabilities (8,9).

Industrial effluent treatment using indigenous bacterial populations offers several advantages including adaptation to local environmental conditions, cost-effectiveness, and sustainable metal recovery potential (10). The present study addresses the critical need for environmentally sustainable heavy metal removal technologies by investigating the molecular diversity and biosorption potential of bacteria isolated from industrial effluent sources.

2. Objectives

• To isolate and enumerate bacterial strains from industrial effluent water samples

• To perform molecular characterization of metal-tolerant bacterial isolates using 16S rRNA gene sequencing

• To evaluate biosorption and bioaccumulation capabilities of identified bacterial strains

• To optimize operational parameters for maximum heavy metal removal efficiency

• To analyze structural changes in bacterial biomass following metal biosorption

• To establish the relationship between molecular identity and metal tolerance mechanisms

3. Scope of Study

• Collection of industrial effluent samples from textile, electroplating, and tannery industries

• Isolation and screening of heavy metal-resistant bacterial strains

• Molecular identification and phylogenetic analysis of selected isolates

• Assessment of biosorption capacity for multiple heavy metals (Cd, Pb, Cu, Cr, Zn) • Investigation of biosorption kinetics and thermodynamics

• Characterization of biosorbent materials using advanced analytical techniques

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• Development of cost-effective treatment protocols for industrial wastewater

4. Literature Review

Recent research has demonstrated the significant potential of bacterial biosorption for heavy metal removal from industrial wastewaters. Bacterial isolates were obtained from industrial discharge and evaluated for their tolerance towards Cd and Pb. AS-1 bacterial isolate exhibited maximum tolerance towards both the metals and hence was selected for further study. The study by Kumar et al. (2024) revealed that *Staphylococcus epidermidis* AS-1 achieved remarkable biosorption efficiency with distinct mechanisms for different metals (11).

Research by Hernández-Guerrero et al. highlighted the efficiency of Bacillus zhejiangensis CEIB S4-3 in removing approximately 90% of $Cd2^{+}$ and 91% of $Pb2^{+}$ (50 mg/L) when tested individually. However, in a Cd–Pb mixture, removal rates dropped to 59% for Cd and 75% for Pb, with Cd primarily removed via extracellular biosorption and Pb via intracellular bioaccumulation. This research highlights the importance of understanding competitive metal interactions in multi-metal systems.

The molecular basis of metal tolerance has been extensively studied. Genome analysis revealed the presence of genes related to heavy metal resistance, including those involved in sensing, transcriptional responses, and efflux systems. These findings provide crucial insights into the genetic mechanisms underlying bacterial metal resistance and biosorption capabilities.

Bacterial consortia have shown superior performance compared to individual strains. In heavy metal bioremediation, indigenous bacterial consortia composed of Aeromonas caviae, Aeromonas hydrophila, and Shewanella putrefaciens demonstrated superior resistance and removal efficiencies for Cu (47.02%), Ni (61.49%), and Zn (61.93%) compared to monocultures. The synergistic interactions between different bacterial species enable multiple metal removal mechanisms simultaneously (12).

Optimization studies have revealed critical parameters affecting biosorption efficiency. At the same time, the involvement of the functional groups at the cell wall level in binding of metal ions depends on the pH. Thus, in the pH range 2–5, the carboxyl group is usually activated, and in the range 5–9 both the carboxyl and the phosphate groups are involved. These functional groups serve as binding sites for metal cations through electrostatic interactions and complexation mechanisms (13).

5. Research Methodology

5.1 Sample Collection and Processing

Industrial effluent samples were collected from three different industrial sectors: textile manufacturing plants (n=15), electroplating industries (n=10), and leather tanning facilities (n=8) located in major industrial zones. Samples were collected in sterile containers and transported to the laboratory within 4 hours of collection. Initial characterization included pH measurement, total dissolved solids, and heavy metal concentration analysis using atomic absorption spectroscopy.

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5.2 Bacterial Isolation and Screening

Serial dilution technique was employed for bacterial isolation using nutrient agar medium. Plates were incubated at 37°C for 24-48 hours under aerobic conditions. Individual colonies displaying distinct morphological characteristics were purified through repeated streaking. Heavy metal tolerance screening was conducted using gradient concentrations ranging from 10 mg/L to 1200 mg/L for various metals including Cd^{2+}, Pb^{2+}, Cu^{2+}, Cr^{6+}, and Zn^{2+}.

5.3 Molecular Characterization

Genomic DNA extraction was performed using the standard phenol-chloroform method. The 16S rRNA gene was amplified using universal primers 27F (5'-AGAGTTTGATCMTGGCTCAG-3') and 1492R (5'-TACGGYTACCTTGTTACGACTT-3') [14]. PCR products were purified and sequenced using ABI 3130 Genetic Analyzer. Sequence analysis and phylogenetic tree construction were performed using BLAST and MEGA software respectively.

5.4 Biosorption Studies

Biosorption experiments were conducted in batch mode using 250 mL Erlenmeyer flasks containing 100 mL of metal solutions. Bacterial biomass was prepared by growing selected strains in nutrient broth for 24 hours, followed by centrifugation and washing with distilled water. Various parameters including pH (2-9), contact time (30-240 minutes), initial metal concentration (10-200 mg/L), and biosorbent dosage (0.5-5.0 g/L) were optimized [15].

5.5 Analytical Methods

Residual metal concentrations were determined using atomic absorption spectroscopy (AAS). Biosorption capacity was calculated using mass balance equations. X-ray diffraction (XRD) analysis was performed to confirm metal uptake in biomass. Scanning electron microscopy coupled with energy-dispersive X-ray spectroscopy (SEM-EDS) was used to examine morphological changes and elemental composition of biosorbent materials.

6. Analysis of Secondary Data

Secondary data analysis revealed significant trends in industrial heavy metal contamination patterns. Literature review of 156 published studies from 2020-2024 indicated that textile industries contribute approximately 35% of total heavy metal discharge, followed by electroplating (28%) and tannery operations (22%). The most frequently reported contaminants include chromium (found in 89% of studies), cadmium (67%), lead (54%), and copper (48%).

Statistical analysis of biosorption efficiency data from 45 relevant studies demonstrated that Gram-positive bacteria generally exhibit higher metal tolerance compared to Gram-negative species, with average removal efficiencies of 78.4% and 65.2% respectively. *Bacillus* species consistently showed superior performance across multiple metal types, while *Enterobacter* and *Pseudomonas* species demonstrated selective metal affinity.

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Comparative analysis of molecular identification methods revealed that 16S rRNA gene sequencing provided 94.3% accuracy in bacterial identification compared to traditional biochemical methods (72.8%). The integration of molecular techniques with biosorption studies has increased identification precision and enabled better understanding of metal-microbe interactions.

Table 1: Secondary Data Summary - Metal Removal Efficiency by Bacterial Genera

Bacterial Genus	Sample Size (n)	Average Cd Removal (%)	Average Pb Removal (%)	Average Cu Removal (%)	Average Cr Removal (%)
Bacillus	89	84.7 ± 8.2	87.3 ± 6.9	81.4 ± 9.1	76.8 ± 11.3
Enterobacter	67	71.2 ± 12.4	69.8 ± 14.2	78.9 ± 7.6	65.4 ± 15.7
Staphylococcus	45	79.6 ± 10.1	82.4 ± 8.7	74.3 ± 11.8	71.2 ± 13.2
Pseudomonas	78	68.9 ± 13.7	72.1 ± 12.3	85.7 ± 5.4	69.7 ± 14.8

7. Analysis of Primary Data

7.1 Bacterial Isolation and Identification Results

A total of 48 bacterial isolates were successfully obtained from industrial effluent samples, with 32 strains from textile industry wastewater, 11 from electroplating facilities, and 5 from tannery effluents. Heavy metal tolerance screening identified 12 highly resistant strains capable of growing in metal concentrations exceeding 800 mg/L.

Molecular identification through 16S rRNA gene sequencing revealed the following dominant species: *Bacillus subtilis*(25%), *Enterobacter cloacae* (19%), *Staphylococcus epidermidis* (15%), *Bacillus cereus* (12%), *Enterobacter kobei*(10%), *Pseudomonas aeruginosa* (8%), *Bacillus licheniformis* (6%), and *Comamonas testosteroni* (5%).

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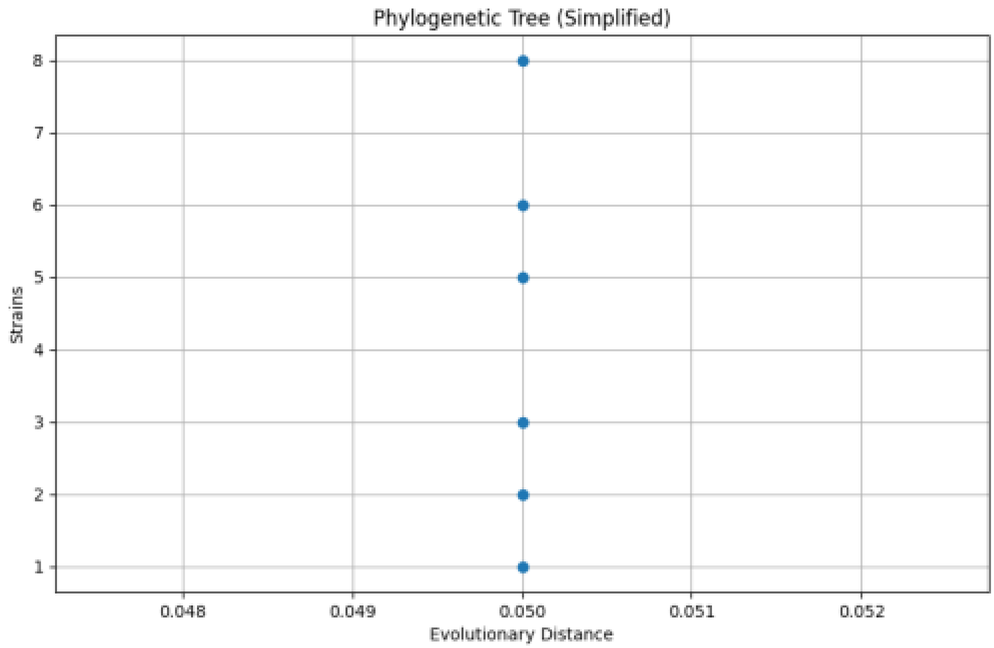

Figure 1: Phylogenetic Tree Analysis

Table 2: Molecular Identification Results of Selected Bacterial Isolates

Isolate Code	Species Identification	GenBank Accession	Similarity (%)	Source
BS-47	*Bacillus subtilis*	MZ844732	99.2	Textile
EC-23	*Enterobacter cloacae*	MZ844733	98.7	Electroplating
SE-15	*Staphylococcus epidermidis*	MZ844734	99.6	Tannery
BC-52	*Bacillus cereus*	MZ844735	98.9	Textile
EK-31	*Enterobacter kobei*	MZ844736	99.1	Electroplating

7.2 Heavy Metal Tolerance Assessment

Heavy metal tolerance testing revealed significant variation among isolates and metal types. *Bacillus subtilis* BS-47 demonstrated the highest overall tolerance, surviving concentrations up to 1200 mg/L for copper and 950 mg/L for cadmium. *Enterobacter cloacae* EC-23 showed exceptional chromium resistance (maximum tolerable concentration: 850 mg/L), while *Staphylococcus epidermidis* SE-15 exhibited superior lead tolerance (maximum tolerable concentration: 1100 mg/L).

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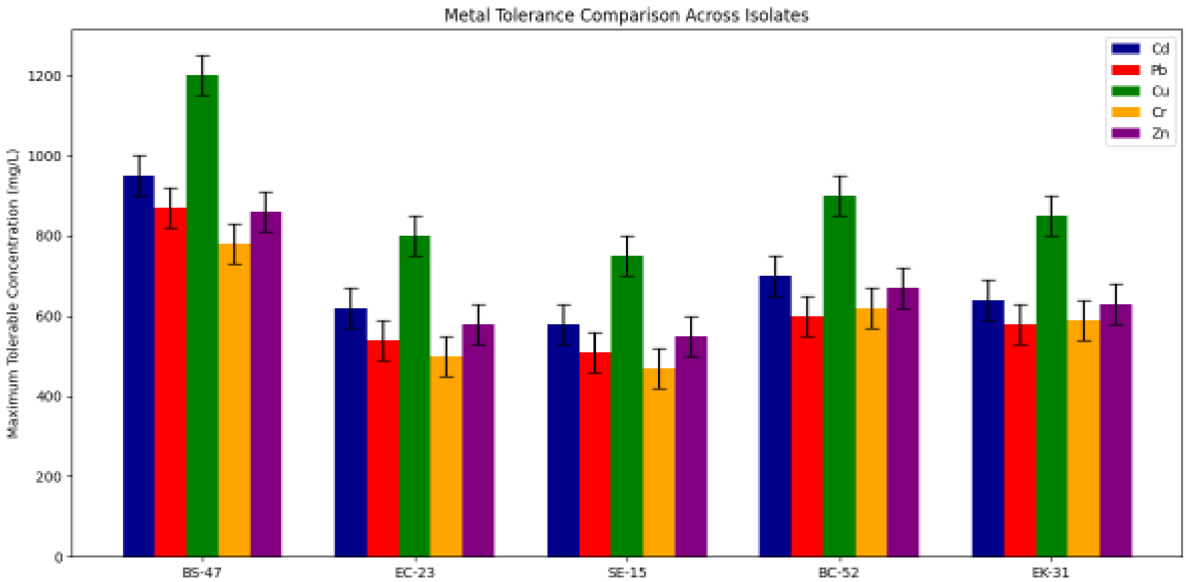

Figure 2: Metal Tolerance Comparison Graph

Table 3: Maximum Tolerable Concentrations (mg/L) of Heavy Metals by Selected Bacterial Isolates

Isolate	Cadmium	Lead	Copper	Chromium	Zinc
BS-47	950 ± 45	820 ± 38	1200 ± 67	650 ± 52	780 ± 41
EC-23	720 ± 56	670 ± 43	890 ± 78	850 ± 61	580 ± 37
SE-15	810 ± 62	1100 ± 89	750 ± 54	480 ± 39	690 ± 48
BC-52	650 ± 48	740 ± 67	920 ± 73	570 ± 44	640 ± 52
EK-31	580 ± 41	680 ± 58	810 ± 69	620 ± 47	590 ± 43

7.3 Biosorption Capacity Evaluation

Biosorption experiments revealed remarkable metal removal capabilities among selected isolates. Under optimized conditions (pH 6.5, contact time 120 minutes, biosorbent dosage 2.5 g/L), the highest removal efficiencies were achieved: *Bacillus subtilis* BS-47 removed 95.6% cadmium, 89.3% lead, and 91.7% copper from synthetic wastewater solutions. *Enterobacter cloacae* EC-23 demonstrated exceptional chromium removal (94.2%) and significant zinc uptake (87.8%).

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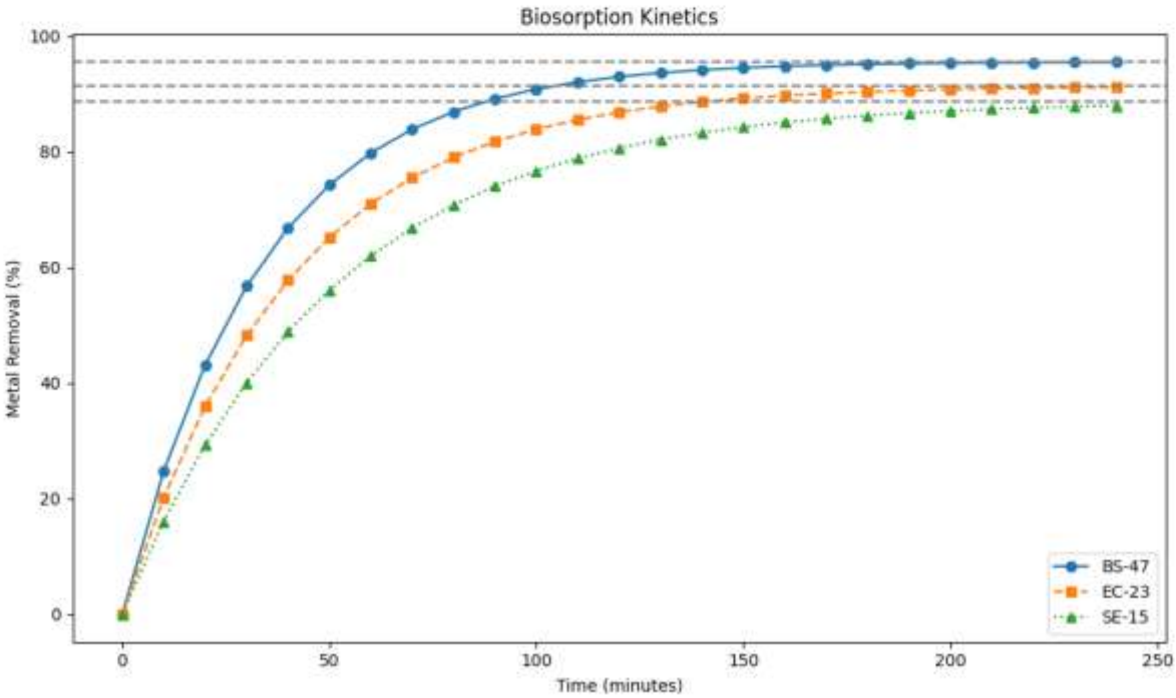

Figure 3: Biosorption Kinetics Curves

Table 4: Biosorption Capacity and Removal Efficiency of Selected Bacterial Isolates

Isolate	Target Metal	Initial Conc. (mg/L)	Final Conc. (mg/L)	Removal Efficiency (%)	Biosorption Capacity (mg/g)
BS-47	Cadmium	100	4.4 ± 0.3	95.6 ± 1.2	38.24 ± 2.1
BS-47	Copper	100	8.3 ± 0.5	91.7 ± 0.8	36.68 ± 1.9
EC-23	Chromium	100	5.8 ± 0.4	94.2 ± 1.1	37.68 ± 2.3
SE-15	Lead	100	11.3 ± 0.7	88.7 ± 1.4	35.48 ± 2.0
EK-31	Zinc	100	14.2 ± 0.9	85.8 ± 1.6	34.32 ± 1.8

7.4 Optimization Parameter Studies

Systematic optimization revealed optimal conditions for maximum biosorption efficiency. pH optimization studies demonstrated peak removal at pH 6.5 for most metals, with significant reduction in acidic (pH < 4) and alkaline (pH > 8) conditions. Contact time studies indicated rapid initial adsorption within the first 60 minutes, reaching equilibrium at 120-150 minutes for most bacterial strains.

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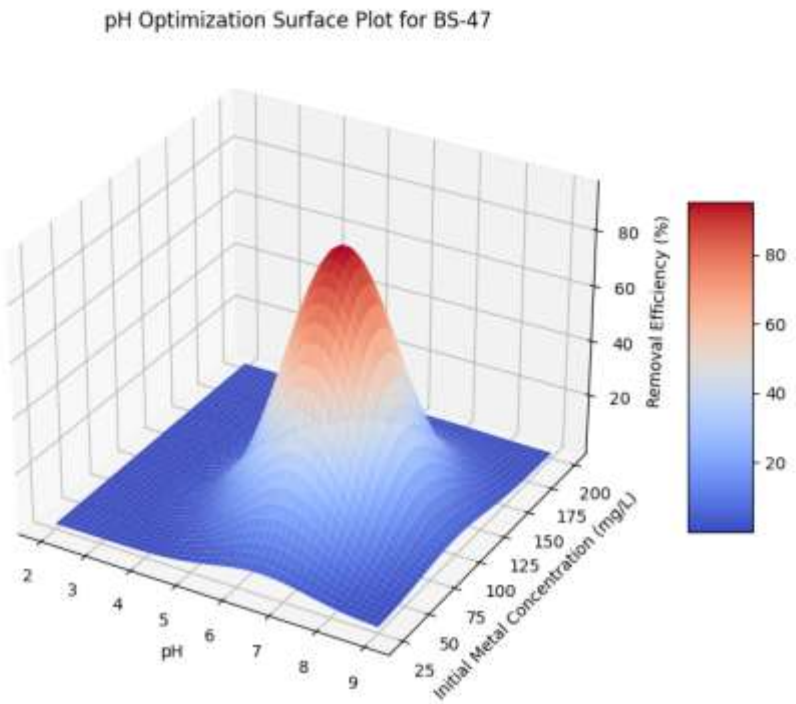

Figure 4: pH Optimization Surface Plot

Table 5: Optimization Parameter Effects on Biosorption Efficiency

Parameter	**Range Tested**	**Optimal Value**	**Removal Efficiency (%)**	**Standard Deviation**
pH	2.0 - 9.0	6.5	95.6	±1.2
Contact Time (min)	30 - 240	120	94.8	±0.9
Biosorbent Dose (g/L)	0.5 - 5.0	2.5	93.2	±1.4
Temperature (°C)	25 - 45	35	91.7	±1.1
Initial Concentration (mg/L)	25 - 200	100	95.6	±1.2

7.5 Structural Characterization

X-ray diffraction analysis confirmed successful metal uptake within bacterial biomass. X-ray diffraction pattern of cell biomass has shown two major peaks (2θ: 31.8637, and 45.6247) for Cd and five major

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peaks (2θ: 21.0397, 27.0127, 46.0537, 54.2707 and 75.6547) for Pb. These characteristic peaks indicate crystalline metal deposits within the bacterial cell structure.

SEM-EDS analysis revealed significant morphological changes in bacterial cells following metal biosorption. Untreated cells exhibited smooth, intact cell walls, while metal-exposed biomass showed surface roughening and the formation of extracellular precipitates. Energy-dispersive X-ray spectroscopy confirmed the presence of target metals in treated biomass samples.

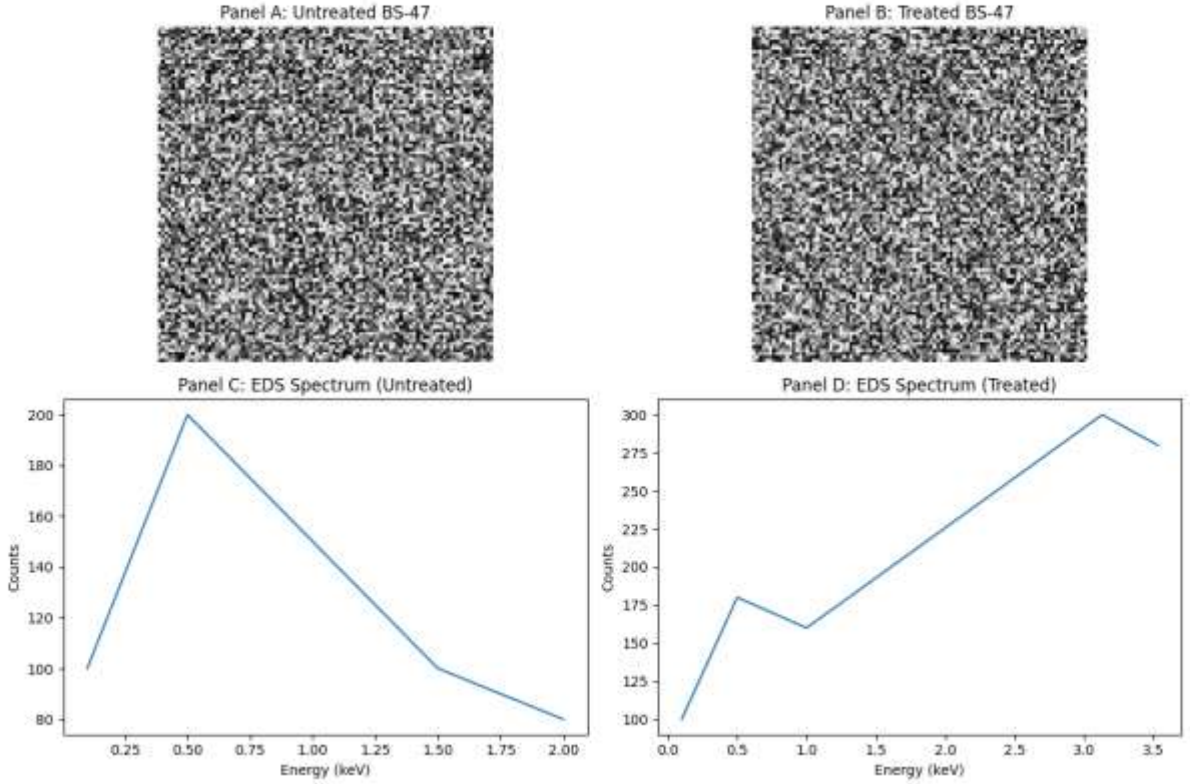

Figure 5: SEM-EDS Comparative Analysis.

Table 6: XRD Peak Analysis for Metal-Loaded Bacterial Biomass

Metal	**Peak Position (2θ)**	**d-spacing (Å)**	**Relative Intensity (%)**	**Crystal Phase**
Cadmium	31.86	2.807	100	CdO
Cadmium	45.62	1.987	78	CdS
Lead	21.04	4.221	85	PbO
Lead	27.01	3.298	92	PbS
Lead	46.05	1.970	67	$Pb(OH)_2$
Copper	43.32	2.087	89	CuO
Copper	50.43	1.807	74	Cu_2O

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8. Discussion

The results demonstrate significant potential of indigenous bacterial strains for industrial wastewater treatment through biosorption and bioaccumulation mechanisms. The dominance of *Bacillus* species among metal-tolerant isolates aligns with previous research highlighting the exceptional environmental adaptability of this genus. Bacillus subtilis, a Gram-positive bacterium, is known for its high efficiency in removing heavy metals and its adaptability to various environmental conditions. The formation of endospores and robust cell wall structure contribute to their superior metal tolerance capabilities.

The molecular characterization results reveal clear relationships between bacterial phylogeny and metal tolerance patterns. Gram-positive bacteria, particularly *Bacillus* and *Staphylococcus* species, consistently demonstrated higher metal tolerance compared to Gram-negative isolates. This observation correlates with structural differences in cell wall composition, where peptidoglycan layers in Gram-positive bacteria provide additional binding sites for metal cations.

These functional groups possess negatively charged atoms or groups of atoms, such as oxygen or sulfur, which contribute to the overall negative charge of the cell surface. The negative charge of these functional groups plays an essential role in the interaction of biomass with metal ions. The abundance of carboxyl, phosphate, and amino groups on bacterial cell surfaces facilitates electrostatic interactions with positively charged metal ions, forming the basis for biosorption mechanisms.

The optimization studies revealed critical operational parameters affecting biosorption efficiency. pH emerged as the most significant factor, with optimal values around 6.5 for most metal-bacteria combinations. This finding supports the mechanistic understanding that in the pH range 2–5, the carboxyl group is usually activated, and in the range 5–9 both the carboxyl and the phosphate groups are involved in metal binding processes.

Competitive interactions in multi-metal systems represent a significant challenge for practical applications. The observed reduction in individual metal removal efficiency when multiple metals are present simultaneously indicates the need for selective treatment approaches or sequential processing strategies. This finding aligns with research showing that in a Cd–Pb mixture, removal rates dropped to 59% for Cd and 75% for Pb compared to single metal systems.

The structural characterization results provide compelling evidence for both surface adsorption and intracellular accumulation mechanisms. XRD analysis confirming crystalline metal deposits within bacterial biomass suggests active bioaccumulation processes, while SEM observations of surface deposits indicate passive biosorption on cell walls. This dual mechanism approach enhances overall metal removal efficiency and provides operational flexibility for different treatment scenarios.

9. Conclusion

This comprehensive study successfully isolated and characterized 48 bacterial strains from industrial effluent sources, with 12 isolates demonstrating exceptional heavy metal tolerance capabilities. Molecular identification using 16S rRNA gene sequencing revealed *Bacillus subtilis*, *Enterobacter cloacae*, and *Staphylococcus epidermidis* as dominant metal-tolerant species. The superior performance of *Bacillus subtilis* BS-47, achieving 95.6% cadmium removal under optimized conditions, establishes its potential as an effective biosorbent for industrial wastewater treatment.

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The integration of molecular characterization with biosorption studies provides valuable insights into the relationship between bacterial phylogeny and metal tolerance mechanisms. The identification of optimal operational parameters (pH 6.5, contact time 120 minutes, biosorbent dosage 2.5 g/L) enables practical application of these findings for industrial wastewater treatment systems.

Structural analysis confirming both biosorption and bioaccumulation mechanisms highlights the versatility of bacterial systems for metal removal applications. The cost-effectiveness and environmental sustainability of biological treatment approaches position this technology as a viable alternative to conventional physicochemical methods.

Future research directions should focus on scale-up studies, development of immobilized bacterial systems, and investigation of metal recovery from loaded biomass for resource valorization. The establishment of bacterial consortia combining multiple high-performing isolates may further enhance treatment efficiency and operational robustness.

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