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Characterization of Multiple Heavy Metal Resistant *Bacillus cereus* IEI-01 Isolated from Industrial Effluent and its *In Vitro* Bioremediation Potential

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ABSTRACT

Heavy metal (HM) pollution has been a significant issue for the environment and public health. Unmonitored industrial effluents are a major source of HM pollution. However, metallotolerant bacteria thriving in such environments could be potentially useful for bioremediation purposes. In this study, *Bacillus cereus* IEI-01 was isolated from water samples of Badshahpur Lake, Gurugram, showcasing resilience to HM exposure and thriving under optimal conditions at 37°C and pH 7.0. Morphological and biochemical characterization showed its Gram-positive rod shape and metabolic versatility, including glucose fermentation and nitrate reduction capabilities. Molecular analysis further affirmed its close relation to the *Bacillus cereus* strain. Dynamic bacterial growth patterns were observed, with typical sigmoidal curves indicating significant growth over 72 h. When exposed to various HMs, the strain IEI-01 exhibited differential tolerance and promoting patterns, with cadmium (Cd) and lead (Pb) compared to other metals. Over 72 h, the strain exhibited substantial removal rates ranging from 60.64% to 87.43% for Cd and 41.87% to 52.62% for Pb. The concentration-dependent bio-removal efficiency of IEI-01 in Cd-spiked cultures displayed a declining trend with increasing concentrations, with removal rates ranging from 80.23% to 60.72% over the same period. These findings highlight the potential of *Bacillus cereus* IEI-01 for HM bioremediation, particularly at lower concentrations. Its efficacy in removing Cd and Pb from contaminated environments suggests promising applications in environmental cleanup efforts.

INTRODUCTION

Heavy metal pollution is a significant environmental issue arising from various industrial activities, including mining, metallurgy, chemical manufacturing, and waste disposal. These activities discharge substantial amounts of toxic metals such as lead (Pb), cadmium (Cd), chromium (Cr), and mercury (Hg) into natural ecosystems, posing severe risks to human health and the environment (Bhakta et al. 2014). Heavy metals are persistent contaminants that do not degrade naturally, leading to bioaccumulation in food chains and prolonged ecological and ecotoxicological impact (Ali & Rehman 2014, Das et al. 2016). Traditional methods for HM removal, such as chemical precipitation, ion exchange, and adsorption, are often expensive, inefficient for low-concentration contaminants, and can produce secondary pollution. In this context, bioremediation, using microorganisms to detoxify or immobilize HMs, has emerged as a promising,

eco-friendly alternative. Various bacteria, including those from the *Bacillus* genus, have shown considerable potential in bioremediation due to their metabolic versatility and ability to thrive in harsh conditions (Shakoori et al. 2010, Bestawy et al. 2013). *Bacillus cereus* has been widely studied for its resilience in environments contaminated with HMs. This resilience is largely attributed to its unique metabolic pathways and resistance mechanisms, which include biosorption, bioaccumulation, and biotransformation of HMs (Afzal 2023). *Bacillus cereus* strains have been isolated from various contaminated sites, including industrial effluents, where they exhibit capabilities to reduce, oxidize, or otherwise mitigate HM toxicity (Patra et al. 2010, Kelany et al. 2023). The ability of *Bacillus cereus* to form endospores further enhances its survival in extreme conditions, making it a suitable candidate for bioremediation applications (Monga et al. 2022). Shakoori et al. (2010b) isolated and characterized arsenic (As) -reducing bacteria from industrial effluents, demonstrating their potential in bioremediation. Similarly, Kour et al. (2019) explored zinc biosorption in *Bacillus* strains, providing insights into their metal tolerance mechanisms. The identification of specific genes and proteins involved in metal resistance, such as those encoding for metal efflux pumps and metal-binding proteins, is crucial for developing genetically engineered strains with enhanced bioremediation capabilities (Afzal et al. 2017, Kumari et al. 2021a). The ability of *Bacillus cereus* to tolerate and potentially remediate various HMs makes it a reliable microorganism for bioremediation (Kalsoom et al. 2021). Previous research by Al Azad et al. (2020) has shown that *Bacillus cereus* strains from different environments, including milk and soil, possess significant bioremediation properties. However, strains isolated from industrial effluents are particularly interesting due to their exposure to high concentrations of HMs, which could have induced unique adaptive mechanisms (Begum & Aundhati 2016).

Bioremediation efficiency *in vitro* is assessed by evaluating the bacterium's ability to reduce, sequester, or transform HMs in controlled conditions. Zahoor & Rehman (2009) isolated Cr (VI)-reducing bacteria capable of converting toxic Cr to less harmful forms. Similar studies have shown that *Bacillus cereus* can significantly reduce the concentrations of various metals, making them less bioavailable and toxic (Sharma et al. 2021, Ameen et al. 2020). Saduzzaman et al. (2023) also found that DAS1 exhibited optimal growth and 85% Cr(VI) remediation at pH 8, with immobilized bacteria increasing reduction efficiency to 90.4%. When combined with DAS2, the mixed culture achieved complete Cr(VI) removal using the X3Y2Z1 experiment design.

This study aims to screen and identify HM-tolerant *Bacillus cereus* and its potential for bioremediation of metals. The biochemical characterization of *Bacillus cereus* includes an array of tests to determine its metabolic capabilities and resistance to HMs. Furthermore, molecular techniques, 16s RNA gene sequencing were employed to identify specific strains of the microorganisms.

MATERIALS AND METHODS

Sample Collection and Premilinary Isolation

Samples were collected in March 2023 from Badshahpur Lake in Gurugram, India, located at latitude 28.39 and longitude 77.0466318. The samples were collected in sterilized 250 mL amber-color acid-washed polyethylene bottles from ten different locations within the lake to ensure a representative sample of the water quality. To isolate *Bacillus cereus*, the water samples underwent primary culture preparation on Mannitol-egg yolk-polymyxin agar (MYP) medium, which is selective for *B. cereus*. The cultures were incubated at 37°C for 24 to 72 h, starting from the 9^{th} step of serial dilution of the water sample to ensure optimal growth conditions and isolate the desired bacteria. The samples underwent seven consecutive contamination-free subcultures following the initial incubation to obtain pure isolates. This rigorous subculturing process was essential to eliminate non-target microorganisms and achieve a pure culture of *B. cereus*. Finally, eight single colonies, each displaying distinct morphological characteristics, were selected from the final pure culture plate. These colonies were selected for further biochemical and molecular characterization to assess their potential for HM bioremediation.

Screening of Heavy Metal Resistant Bacteria

Screening of HM-resistant bacteria was conducted following the method previously described by Kumari et al. (2021a). Briefly, several isolated bacterial strains were tested for their ability to resist HMs by observing their growth on LB agar plates enriched with a mixture of 10 $mg.L^{-1}$ concentrations of Cd, Cu, Pb, Cr, As, and Hg over two days. *Bacillus* isolate IEI-01, which exhibited significant growth, was selected as the most promising strain for further analysis (Desoky et al. 2020).

Morphological Characterization of Bacteria

The morphological characterization of *Bacillus* isolates involved examining their size, shape, motility, and staining properties. Bacterial smears were prepared, air-dried, heat-fixed, and stained with crystal violet or methylene blue to assess cell size and shape under a light microscope at 1000x

magnification. Motility was evaluated by inoculating a semi-solid agar medium with the isolates and observing the spread of growth from the stab line after incubating at 37°C for 24 to 48 h. Gram staining determined the bacteria's Gram-positive nature by retaining the crystal violet dye. Capsular staining identified the presence of a clear halo around cells, indicating a positive capsule, while spore staining revealed spore formers by retaining malachite green within the spores, contrasting with red-stained vegetative cells. Additionally, colony morphology on solid agar plates was observed for shape, texture, elevation, margin, size, and transparency, providing further insight into the isolates' characteristics. The colonies were typically rod-shaped, rough in texture, raised with irregular margins, measured 4-6 µm in size, opaque, and confirmed as spore-forming and Gram-positive (Chauhan & Jindal 2020)

Biochemical Characterization

To biochemically characterize *Bacillus* isolates, pink colonies were initially picked from MYP agar plates, streaked onto nutrient agar, and incubated at 37°C for 24 h to obtain pure cultures. Several biochemical tests were conducted on these cultures. Gram staining confirmed the isolates as Gram-positive rods. Glucose fermentation tests were performed to assess acid production. The Voges-Proskauer test was conducted to detect acetoin production from glucose fermentation. Methyl Red testing was carried out to determine stable acid production. The Citrate Utilization test was performed to assess citrate utilization. Indole production was examined using Kovac's reagent. The Oxidase test was conducted to detect cytochrome c oxidase activity. The catalase test was performed to determine the presence of the catalase enzyme. The nitrate reduction test was conducted to assess nitrate reduction to nitrite. Finally, the starch hydrolysis test was performed to determine starch degradation (Chauhan & Jindal 2020)

Molecular Characterization Using 16S rRNA Gene Sequencing

DNA isolation: Molecular characterization of our bacterial isolate began with the extraction of DNA. Samples were homogenized with extraction buffer and processed through phenol-chloroform extraction. Following centrifugation, the aqueous phase was collected, and DNA was precipitated using sodium acetate and isopropanol. The DNA was then washed with ethanol, air-dried, and dissolved in TE buffer, with RNA removal achieved using RNAse A. The extracted DNA was quantified using spectrophotometry, and purity was assessed by calculating the OD 260/280 ratio, aiming for values between 1.8 and 2.0 (Arora et al. 2024)

PCR amplification and sequencing: A fragment of the 16S rRNA gene was amplified using universal primers 27F and 1492R. The PCR amplicon was purified to remove contaminants and resolved on an agarose gel. Forward and reverse DNA sequencing was performed using an ABI 3730×l Genetic Analyzer with the BDT v3.1 Cycle sequencing kit. A consensus sequence was generated from the forward and reverse sequence data using alignment software.

Phylogenetic tree construction: The genetic sequences were identified using NCBI's BLAST software, comparing them to the closest relatives based on maximum identity scores. The top ten sequences were aligned using Clustal W, and a phylogenetic tree was constructed using the neighbor-joining algorithm in MEGA 10 software. The stability of the tree clades was determined through a 1000-replication bootstrap analysis, which involved creating pseudo-alignments by resampling the original alignment to generate a majority consensus tree. This tree displayed the percentage of times specific groups appeared on each side of a branch across 100 bootstrap replicates.

Optimizing Growth Conditions for *Bacillus* Sp. IEI-01: Temperature and pH Effects

To determine the optimal growth conditions for *Bacillus cereus* strain IEI-01, the methods described by Sheer et al. (2021) were employed, focusing on temperature and pH. For temperature optimization, LB-broth was prepared in sets and autoclaved, then inoculated with an overnight log phase culture of the isolate. These sets were incubated at 25°C, 37°C, and 45°C on a shaker for 24 h. The growth was measured by recording the optical density (OD) at 600 nm UV/vis spectrophotometer. For pH optimization, LB broth was adjusted to different pH levels (5.0, 7.0, 9.0) and similarly inoculated and incubated at 37°C on a shaker for 24 h. Post-incubation, the OD at 600 nm was measured to assess bacterial growth across the pH spectrum.

Impact of Heavy Metals on the Growth of *Bacillus cereus* Strain IEI-01

To investigate the effect of various HMs on the growth of *Bacillus cereus* strain IEI-01, OD at 600 nm was monitored over 72 days using LB broth. The metals examined included Cd, Cu, Pb, Cr, As, and Hg. Growth measurements were taken at specific intervals, 0, 12, 24, 48, and 72 days.

Evaluation of Bioremoval Efficiency of Heavy Metals by Bacterial Strains in LB Broth

LB broth (100 mL) in a 250 mL flask was individually spiked with 10 $mg.L^{-1}$ of HMs Cd, Cu, Pb, Cr, As, and Hg and then inoculated with 50 µL of an overnight bacterial culture of (2×10^7 $CFU.mL^{-1}$). Metal concentrations in these spiked

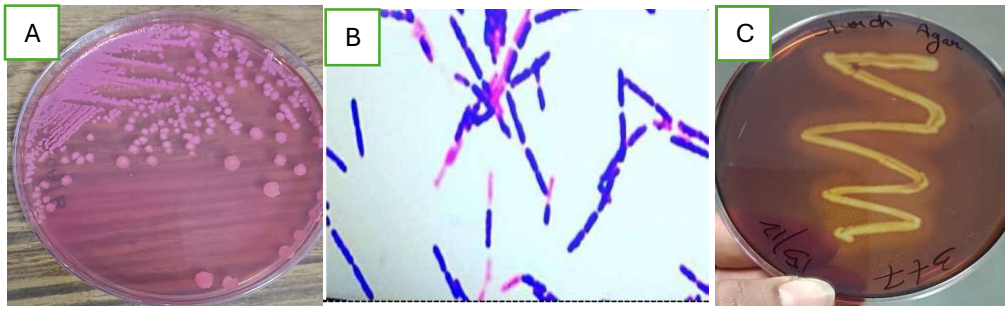

Fig. 1: Morphological and biochemical characteristics of *Bacillus* isolate IEI-01, showing (A) pink-colored colonies on MYP agar, (B) Gram-positive rod shape, and (C) starch hydrolysis on starch agar.

cultures were measured at specified intervals (0, 12, 24, 48, 72 h) using an ICP-OES. Samples were centrifuged at 3500 rpm for 30 min, and the supernatant was filtered through a 0.25 µm filter before ICP-OES analysis. The bioremoval of metal ions by the bacteria was calculated by determining the difference between the initial and final concentrations of HMs in the media (Radhika et al. 2006). The bio-removal percentage (BR%) and the metal ion removal rate (RR) by the strain were calculated using the following equations (Kumari et al. 2021b):

$$\text{BR}\ (\%) = \left\{\frac{C_0 - C_t}{C_0}\right\} \times 100$$

C_0 and C_t are metal ion concentrations at the time 0 min and at time interval t, respectively.

Methodology for Concentration-Dependent Bioremoval Efficiency of *Bacillus cereus* IEI-01 in Cadmium-Spiked Cultures

The concentration-dependent bioremoval efficiency of *Bacillus cereus* IEI-01 in Cd-spiked cultures was investigated over a 72-h incubation period. Initially, cultures of *Bacillus cereus* IEI-01 were prepared in LB broth and incubated overnight to reach the log phase. Cd solutions of varying concentrations ranging from 0.1 mg.L^{-1} to 5 mg.L^{-1} were then prepared by diluting a Cd stock solution in sterile water. Sampling was conducted at 12 h intervals over 72 h, and the Cd concentration in the culture supernatant was determined using ICP-OES. Bioremoval efficiency was calculated as described in the previous section.

RESULTS AND DISCUSSION

Bacillus isolate IEI-01 was successfully isolated from water samples collected from Badshahpur Lake, Gurugram, using selective MYP agar and rigorous subculturing. The strain demonstrated strong resistance to HMs, showing growth on LB agar plates containing 10 mg.L^{-1} of Cd, Cu, Pb, Cr, As, and Hg. Further testing revealed that *Bacillus cereus* IEI-01 thrives optimally at a temperature of 37°C and a pH of 7.0, indicating its potential for effective HM bioremediation in contaminated environments.

Morphological and Biochemical Characterization Results

The HM-tolerant *Bacillus* sp., IEI-01, was identified as Gram-positive rods with metabolic versatility, as it could ferment glucose and produce acetoin, demonstrated by positive Voges-Proskauer tests. Additionally, the strain reduced nitrate to nitrite, as evidenced by positive nitrate reduction tests. Morphologically, IEI-01 formed rough,

Table 1: Morphological and Biochemical Characteristics of *Bacillus cereus*.

Characteristic	*Bacillus* isolate IEI-01
Morphological Characteristics	
Shape	Rod
Texture	Rough
Motility	Positive
Elevation	Raised
Margin	Irregular
Size	4-6 µm
Transparency	Opaque
Gram's Staining	Gram-positive
Capsular Staining	Positive
Spore Staining	Spore former
Biochemical Characteristics	
Methyl Red Reaction	Negative
Citrate	Positive
Indole Production	Negative
Voges–Proskauer Reaction	Positive
Oxidase Test	Negative
Catalase Test	Positive
Nitrate Test	Positive
Starch Hydrolysis	Positive

opaque, rod-shaped colonies with irregular margins and raised elevations, measuring 4-6 µm. Pink-colored colonies on MYP agar suggested mannitol fermentation, while clear zones around colonies on starch agar indicated starch hydrolysis. IEI-01 exhibited positive reactions for citrate utilization, catalase activity, and starch hydrolysis but tested negative for methyl red and oxidase. These findings highlight the potential of strain IEI-01 in bioremediation applications, attributed to its metabolic capabilities and resilience in HM environments. Fig. 1 illustrates these traits, highlighting pink colonies on MYP agar, Gram-positive rod-shaped cells, and starch hydrolysis on starch agar. Table 1 provides the morphological and biochemical characteristics of *Bacillus cereus*.

Molecular Characterization Results

Molecular characterization was carried out by performing 16S rRNA gene sequencing. For the amplification of 16S rRNA sequences, universal primers, 27F (5'-AGAGTTTGATCMTGGCTCAG-3') and 1492R (5'-TACGGYTACCTTGTTACGACTT-3'), were used. From the IEI-01 strain, the DNA extraction process yielded 133 ng of genetic material. On performing the NCBI BlastN analysis of the strains, their respective closest relatives could be identified. The genetic makeup of IEI-01 exhibited a striking 100% similarity to that of *Bacillus cereus* strain VC6, followed by a 99.71% similarity with *Bacillus cereus* strain SP4. This high level of genetic similarity can be indicative of common ancestry or shared genetic traits among these bacterial strains. The 16S rRNA phylogenetic tree of IEI-01 showing its relation with nearest neighbors can be seen in Fig. 2.

Bacterial Growth of *Bacillus cereus* IEI-01

In an optimal environment free from HM stress, *Bacillus cereus* IEI-01 exhibited typical bacterial growth characterized by a sigmoidal growth curve over 72 h. The absorbance values measured at OD600 showed an initial lag phase with an absorbance of 0.05 at 0 h, followed by exponential growth reaching an absorbance of 0.32 at 12 h. Subsequently, the growth rate peaked at 24 h with an absorbance of 1.21, indicating the onset of the stationary phase. By 48 and 72 h, the absorbance values slightly decreased to 1.19 and 1.01, respectively, suggesting stabilization of the bacterial population. This growth pattern illustrates the significant and typical growth behavior of *Bacillus cereus* IEI-01 in favorable conditions (Fig. 3).

Growth Dynamics of *Bacillus cereus* IEI-01 in the Presence of Various Heavy Metals

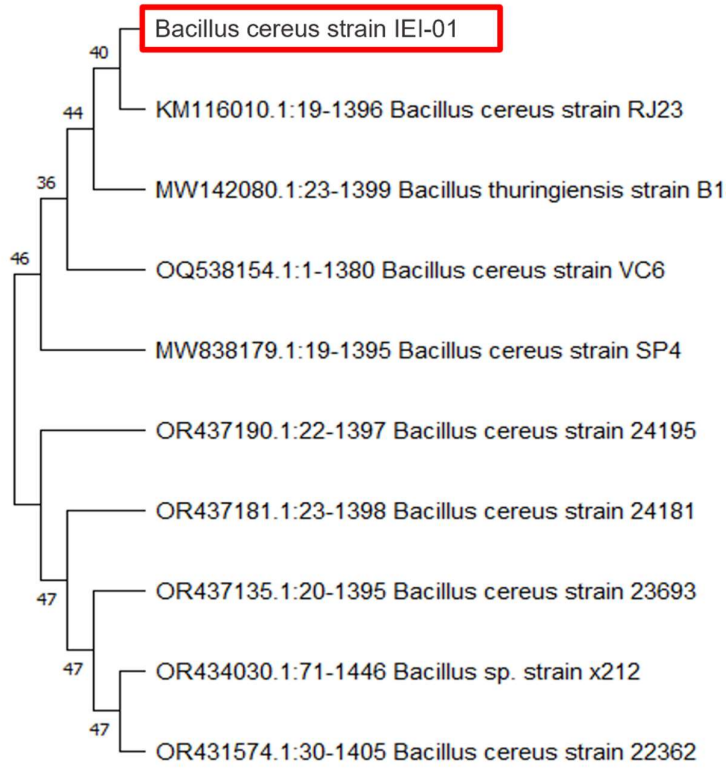

Fig. 2: A phylogenetic tree depicting the close relationship of *Bacillus cereus* IEI-01 with its nearest neighbors.

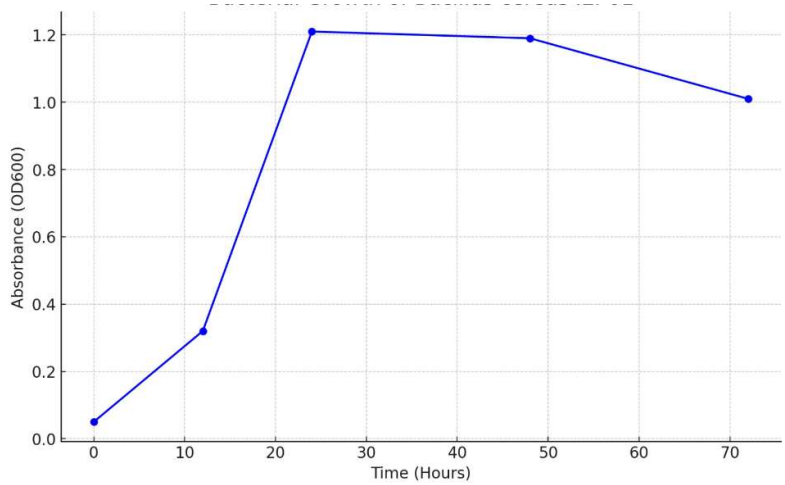

Fig. 3: Growth curve of *Bacillus cereus* IEI-01 under optimal environmental conditions. The graph shows absorbance (OD600) measurements taken at various time points (0, 12, 24, 48, and 72 h), illustrating the characteristic sigmoidal growth pattern of the bacterial culture.

The growth curves of *Bacillus cereus* strain IEI-01 in the presence of various HMs, measured by absorbance at OD600, reveal distinct patterns of bacterial growth over a 72-h incubation period. At the initial time point (0 h), all samples, including those with Cd, Cu, Pb, Cr, As, and Hg, exhibited low absorbance values between 0.04 and 0.06, indicating minimal initial growth. Over the first 12 h, significant growth was observed in the presence of Cd (0.37), Pb (0.34), and Cr (0.29), whereas Cu, As, and Hg showed lower growth rates with absorbance values below 0.25. By 24 h, the absorbance values peaked for most metals, with Cd (1.28), Pb (1.26), and Cr (1.20) supporting the highest bacterial growth. Cu, As, and Hg resulted in moderate growth with absorbance values ranging from 0.78 to 0.87. After 48 h, a slight decline in growth was noted for all metals except for a small increase in the presence of Cu (0.90). By 72 h, the overall absorbance decreased for most conditions, with Cd maintaining the highest absorbance (1.22), followed by Pb

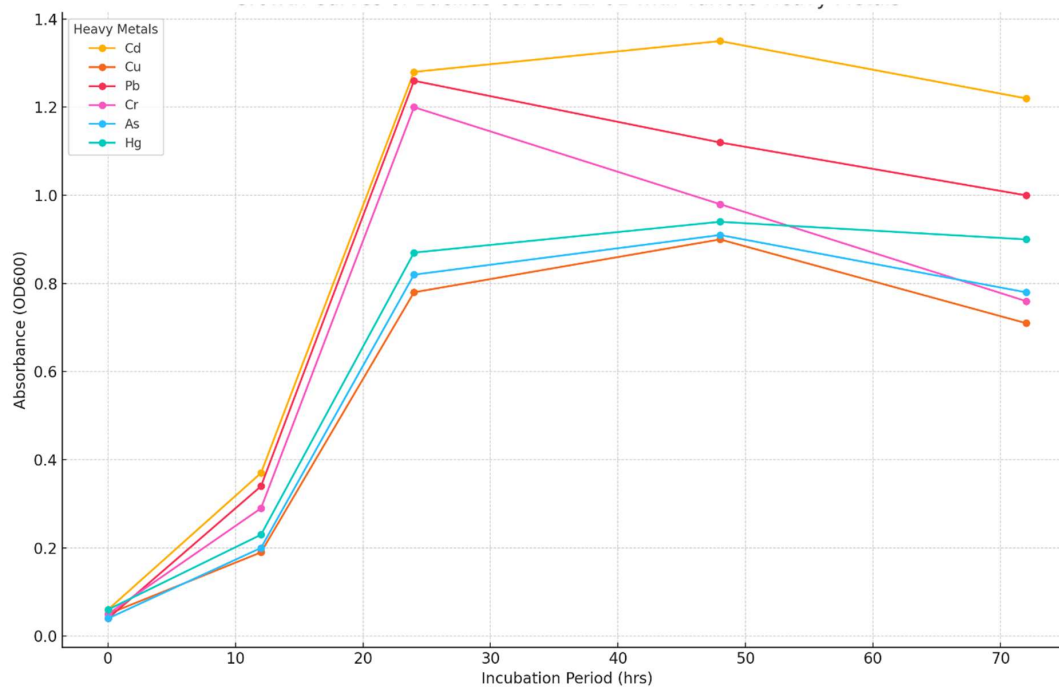

Fig. 4: Growth curves of *Bacillus cereus* IEI-01 with various HMs.

(1.00) and hg (0.90). The data indicate that *Bacillus cereus* IEI-01 exhibits differential tolerance and growth dynamics when exposed to various HMs, with Cd and Pb showing the most significant growth stimulation over time (Fig. 4).

Heavy Metal Bioremoval Efficiency of *Bacillus cereus* IEI-01 Over Time

The bio-removal capacity of *Bacillus cereus* IEI-01 for different HMs, expressed as Bioremoval (BR%) over a 72-hour incubation period, demonstrates varied efficiencies. At 12 h, Cd removal is the most effective at 58.41±2.3%, while Cu exhibits the lowest removal efficiency at 16.2±1.9%. Pb and Cr show moderate bioremoval capacities of 40.63±1.3% and 31.62±1.1%, respectively, whereas As and Hg are removed at 20.34±2.2% and 28.49±1.8%. By 24 h, the bio-removal of Cd significantly increases to 72.42±1.9%, making it the highest among all metals, while Hg also sees a notable rise to 37.52±2.1%. Pb maintains a steady removal rate (41.87±2.4%), whereas Cu, Cr, and As exhibit slight increases, reaching 19.52±0.6%, 38.83±1.4%, and 21.22±2.9%, respectively. After 48 h, the efficiency of Pb removal peaks at 52.62±2.1%, while Cd decreases slightly to 65.53±2.1%. Hg and As removal capacities improve to 32.82±1.4% and 24.52±2.4%, respectively, but Cu and Cr show minor declines to 17.43±1.1% and 34.34±1.8%. At 72 h, Cd's bioremoval decreases to 61.43±1.3%, but it remains the highest among the tested metals. The removal efficiencies for the other metals generally decline, with Cu dropping to 11.32±1.6% and mercury to 26.52±2.2%, indicating a gradual decrease in bioremoval capacity over time for most metals. This data highlights *Bacillus cereus* IEI-01's strong potential for removing Cd and Pb from contaminated environments, with varying efficiencies for other metals (Fig. 5).

Concentration-Dependent Bioremoval Efficiency of *Bacillus cereus* IEI-01 in Cadmium-Spiked Cultures

The bio-removal efficiency of *Bacillus cereus* IEI-01 in Cd spiked cultures shows a concentration-dependent variation over a 72-h incubation period. At 12 h, the highest bio-removal capacity is observed at the lowest Cd concentration of 0.1 mg.L^{-1}, with 80.23 ± 2.4% removal. As the concentration increases, the bio-removal efficiency decreases, with 71.62 ± 2.1% at 0.5 mg.L^{-1}, 65.62 ± 2.1% at 1 m.L^{-1}, 64.62 ± 2.3% at 2.5 mg.L^{-1}, and 60.64 ± 1.3% at 5 mg.L^{-1}. By 24 h, the bio-removal rates improve across all concentrations, reaching 87.43 ± 1.9% for 0.1 mg.L^{-1} and maintaining high removal rates for higher concentrations: 80.82 ± 1.9% at 0.5 mg.L^{-1}, 78.82 ± 1.7% at 1 mg.L^{-1}, 69.25 ± 1.2% at 2.5 mg.L^{-1}, and 70.12 ± 1.9% at 5 mg.L^{-1}. At 48 h, the removal efficiency slightly decreases for lower concentrations, with 86.62 ± 1.1% for 0.1 mg.L^{-1} and 72.83 ± 2.0% for 0.5 mg.L^{-1}, while showing a steady performance for higher concentrations: 70.45 ± 1.3% at 1 mg.L^{-1}, 70.86 ± 1.6% at 2.5 mg.L^{-1}, and an increase to 74.89 ± 2.1% at 5 mg.L^{-1}. By 72 h, the bio-removal efficiency generally

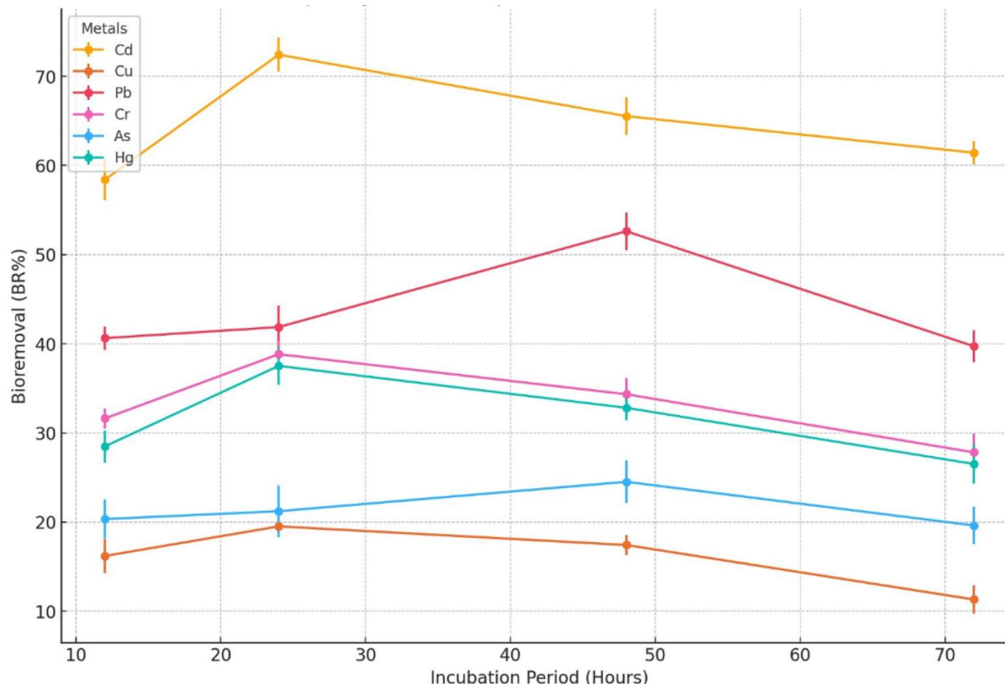

Fig. 5. Time-dependent heavy metal removal efficiency of *Bacillus cereus* IEI-01.

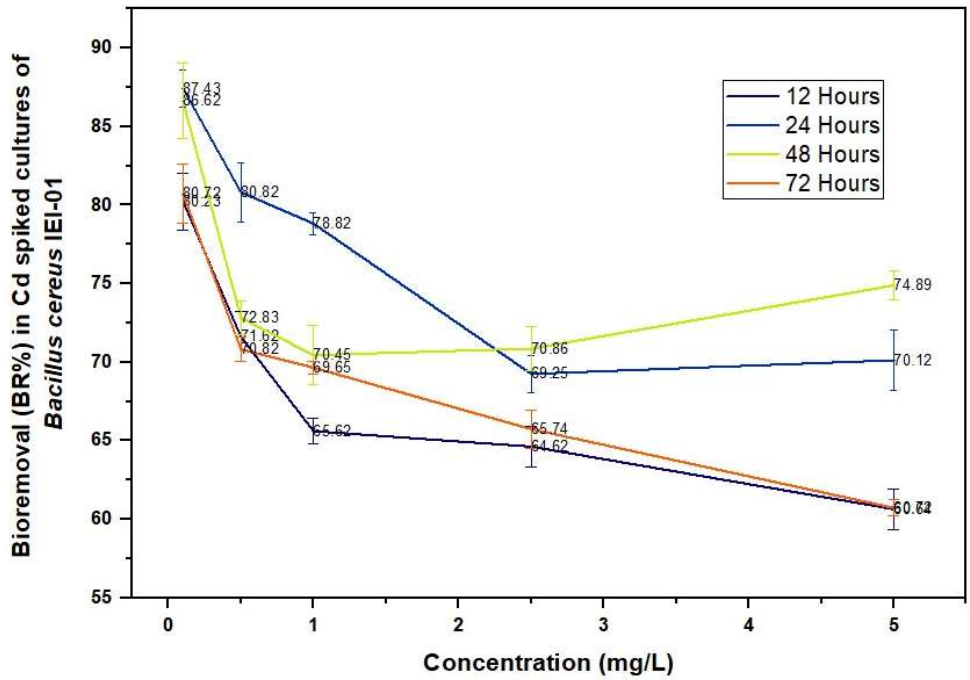

Fig. 6. Bioremoval capacity (BR%) of *Bacillus cereus* IEI-01 in cadmium (Cd) spiked cultures over 12, 24, 48, and 72 h across various Cd concentrations (0.1, 0.5, 1, 2.5, 5 mg.L^{-1}). Error bars represent the standard deviation.

declines but remains substantial, with 80.72 ± 2.0% at 0.1 mg.L^{-1} and decreasing as the concentration increases: 70.82 ± 2.1% at 0.5 mg.L^{-1}, 69.65 ± 1.4% at 1 mg.L^{-1}, 65.74 ± 3.2% at 2.5 mg.L^{-1}, and 60.72 ± 2.5% at 5 mg.L^{-1}. This data indicates that *Bacillus cereus* IEI-01 is highly effective at removing Cd from cultures, particularly at lower concentrations, with significant, albeit reduced, efficiency at higher Cd concentrations over time (Fig. 6).

DISCUSSION

Our study highlights *Bacillus cereus* IEI-01, a Gram-positive, rod-shaped bacterium isolated from Badshahpur Lake in Gurugram, which exhibits significant tolerance and bioremediation capabilities for various heavy metals (HMs). Identified through morphological and biochemical characterizations and confirmed via 16S rRNA sequencing, this strain is closely related to other *Bacillus cereus* strains, indicating shared resilient traits. Under optimal conditions, *Bacillus cereus* IEI-01 displayed a typical bacterial growth pattern, with heavy metals like Cd and Pb significantly stimulating its growth, while Hg and Cu resulted in moderate growth responses. Over 72 h, the strain demonstrated high bio-removal efficiencies, with cadmium showing the highest rate at 72.42% within 24 h, followed by notable but lesser rates for Pb and Hg and the lowest for Cu. The strain's ability to remove Cd was particularly impressive, achieving up to 87.43% removal at lower concentrations (0.1 mg.L^{-1}) within 24 h, though efficiency decreased with higher concentrations. Despite this decrease, significant removal capabilities were maintained even at higher concentrations (5 mg.L^{-1}). This finding suggests IEI-01's potential for application in HM bioremediation in contaminated environments.

Comparatively, Rengarajan et al. (2024) demonstrated the bioremediation potential of biochar and *Bacillus cereus* in treating HM-polluted mine surroundings. While our study focused on the bacterial strain's metal tolerance, Rengarajan et al. evaluated the combined effectiveness of biochar and bacteria. Both studies highlight the importance of integrated approaches for efficient metal removal. Furthermore, Maumela et al. (2024) investigated Pb bioremediation using a *Bacillus* sp. strain MHSD_36 isolated from *Solanum nigrum*. Our findings align with theirs in demonstrating the metal tolerance and bioremediation potential of Bacillus strains, albeit with variations in the targeted metals and specific bacterial strains. Harun et al. (2024) explored the bioremediation potential of *Paenebacillus* sp. and *Bacillus* sp. in lead-contaminated environments. While our study focused on *Bacillus cereus* IEI-01, their work underscores the broader applicability of bacterial remediation approaches across various HM contaminants. Moreover, Gupta et al. (2024) and Anusha et al. (2024) investigated Cr (VI) and multi-metal bioremediation, respectively,

showcasing diverse bacterial strains' versatility in metal removal. Although targeting different metals and bacterial strains, their findings complement ours by highlighting the collective contribution of bacterial remediation approaches to mitigating metal pollution.

In another study by Abo-Amer et al. (2014), *Azotobacter chroococcum's* role in HM bioremediation was examined, demonstrating the potential of diverse bacterial genera in environmental cleanup efforts. Similarly, Jahan et al. (2016) isolated and characterized *Bacillus thuringiensis* and *Bacillus pumilus* for Cr bioremediation, highlighting the importance of species-specific bacterial strains in metal detoxification processes. Raja et al. (2009) isolated HM-resistant bacteria from sewage water, illustrating the widespread distribution of metal-tolerant microorganisms in diverse environments. Their findings contribute to the understanding of microbial communities' potential role in mitigating metal pollution. Furthermore, Elahi et al. (2019) isolated and characterized *Microbacterium testaceum* B-HS2 from tannery effluent, emphasizing the importance of studying bacterial strains from specific industrial contexts for targeted bioremediation strategies. Additionally, Irawati et al. (2012) investigated copper-resistant bacteria from industrial wastewater, showcasing the relevance of studying microbial communities in anthropogenically impacted environments for pollution management. Nwagwu et al. (2017) isolated HM-tolerant bacteria from the Panteka stream in Nigeria, emphasizing the global relevance of studying bacterial bioremediation potentials in diverse ecosystems.

The study emphasizes *Bacillus cereus* IEI-01's metal resistance and bioremediation potential. The comparison with other studies underscores the broader spectrum of bacterial strains and metals targeted in HM remediation efforts. These findings collectively contribute to advancing our understanding of microbial-mediated approaches in environmental remediation.

CONCLUSIONS

In this study, *Bacillus cereus* IEI-01 was isolated from Badshahpur Lake in Gurugram and exhibited significant tolerance and bioremediation capabilities for various HMs. Morphological and biochemical characterizations identified it as a Gram-positive, rod-shaped bacterium with versatile metabolic abilities, such as glucose fermentation and nitrate reduction. Molecular analysis through 16S rRNA sequencing confirmed its close relationship to other *Bacillus cereus* strains, suggesting shared resilient traits. *Bacillus cereus* IEI-01 showed a typical bacterial growth pattern in optimal conditions and displayed distinct growth responses when exposed to HMs like Cd, Pb, and Cr). Cd and Pb significantly stimulated its growth, while Hg and Cu resulted in moderate growth. Over 72 h, the strain demonstrated high bio-removal efficiencies, particularly for Cd, which reached 72.42% at 24 h. Pb and Hg also exhibited considerable bio-removal rates, though less than Cd, while Cu removal was the lowest. When tested for Cd removal across different concentrations, *Bacillus cereus* IEI-01 achieved up to 87.43% removal at lower concentrations (0.1 $mg.L^{-1}$) within 24 h, though efficiency decreased as Cd concentrations increased. Despite this, the strain maintained significant removal capabilities even at higher concentrations (5 $mg.L^{-1}$), indicating its potential for diverse applications in contaminated environments. These findings suggest that *Bacillus cereus* IEI-01 is a promising candidate for bioremediation, particularly effective in environments contaminated with Cd and Pb. Future studies should explore its application in real-world scenarios, potentially combining it with other remediation techniques to enhance its effectiveness.

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