Journal of Chemical Health Risks

www.jchr.org

JCHR (2026) 16(1), 2459-2473 | ISSN:2251-6727

Bioaccumulation of Chromium by Indigenous Bacteria isolated from Industrial Area, Belur, Dharwad in Karnataka, India

Ambarish Sindagi[1] and Manjula Shantaram[2,3*]

[1]Department of Studies and Research in Microbiology, [2]Department of Studies and Research in Biochemistry, Mangalore University, Jnana Kaveri Post Graduate Centre, Chikka Aluvara, Kodagu, Karnataka, India

[3]AJ Research Centre, AJ Institute of Medical Sciences and Research Centre, Mangalore, Karnataka, India

(Received: 05 December 2025 Revised: 15 January 2026 Accepted: 10 February 2026)

KEYWORDS

Bioaccumulation, bioremediation, Chromium, detoxification, eco-friendly.

ABSTRACT:

Chromium (Cr) contamination is a major environmental concern due to its toxicity and persistence. This study aimed to isolate and evaluate indigenous bacterial strains from Cr-polluted soils of the Belur Industrial area, Dharwad, Karnataka, India, for their bioremediation potential. Four Cr-resistant isolates- *Enterobacter mori* (BIDS I), *Pseudomonas alcaliphila* (BIDS II), *Pseudomonas aestus* (BIDS IV), and *Aeromonas hydrophila* (BIDS VI) were characterized morphologically, biochemically, and molecularly. Growth curve studies indicated their tolerance and adaptability to Cr stress. Bio-removal assays demonstrated that BIDS I removed up to 83% of Cr at 500 mg/L after 72 hours, while BIDS II, IV, and VI removed 75%, 69%, and 65%, respectively. Statistical analysis confirmed significant effects of initial Cr concentration on removal efficiency. These findings highlight the potential application of these indigenous strains for eco-friendly and efficient Cr bioremediation.

1. Introduction

Heavy metal pollution has emerged as one of the most critical environmental challenges of our time. Rapid industrialization, urban growth, and unsustainable practices- ranging from mining and smelting to the mismanagement of agricultural waste, effluent discharge, and electronic waste- have all intensified this problem [1]. The situation becomes even more complex when contaminated sites contain not just heavy metals but also organic pollutants, as these combinations are particularly difficult to treat. For bioremediation to succeed in such environments, it is crucial to understand how heavy metals interfere with the normal functioning of microorganisms [2].

Microorganisms are the foundation of wastewater treatment systems, breaking down organic matter and maintaining process efficiency. Yet, when heavy metals are present, they disrupt microbial metabolism and weaken treatment performance. This makes microbes capable of tolerating and thriving in metal-rich environments highly valuable for biological treatment strategies [3]. Among various heavy metals, Cr stands out due to its widespread industrial use and persistent toxicity. Electroplating, leather tanning, textile production, paint manufacturing, pulp and paper processing, fertilizers, and mining are all major contributors to Cr pollution [4]. Once released into soil and water, Cr can accumulate in the food chain, causing severe health risks such as genetic damage, neurological disorders, and organ failure [5]. Cr typically occurs in two oxidation states: trivalent [Cr (III)] and hexavalent [Cr(VI)]. While Cr (III) is an essential trace element that supports carbohydrate and lipid metabolism, Cr (VI) is highly toxic, mobile, and easily absorbed. Its persistence and harmful effects make the removal of Cr (VI) a global environmental priority [6].

Journal of Chemical Health Risks
www.jchr.org
JCHR (2026) 16(1), 2459-2473 | ISSN:2251-6727

In recent years, biological solutions such as microbial biosorption and bioaccumulation have gained attention as sustainable alternatives to chemical treatment. Microbes interact with Cr in multiple ways: by binding it to their cell surface, sequestering it inside their cells, or transforming it into less toxic forms. Some mechanisms are passive, while others are active and energy-dependent. Identifying Cr-resistant strains and optimizing their use offers promising potential for effective bioremediation [7]. Microbial remediation is not only cost-effective and environmentally friendly but also aligns with the broader vision of green technology. It holds immense relevance for wastewater management, safe drinking water supply, and large-scale environmental restoration [8]. Against this background, the present study focuses on isolating Cr resistant bacteria from contaminated sites and evaluating their potential to remediate Cr rich effluents.

2. Material and Methods

Study Area and Sample Collection

The contaminated soil samples were collected as per APHA method [9] from Belur Industrial area, Dharwad in Karnataka, India. For microbiological analysis, polluted soil samples were obtained from a depth of approximately 4–5 inches (45 cm) using sterile polyethylene bags. Samples were collected from four distinct locations, including grass-root soil from outside the effluent disposal site. Samples were stored at 4°C until further processing. For composite sample preparation, 50g of soil from each location was pooled, homogenized, and oven-dried at 60°C for 24 h. Representative samples were subsequently used for microbiological enrichment and analysis.

Enrichment Studies

Enrichment was initiated by adding 10g of contaminated soil to 250mL Erlenmeyer flask containing 100mL sterile distilled water (SDW). The mixture was incubated at 30 ± 1 °C for 24h on an orbital shaker at 150rpm. Following enrichment, 1 mL of the suspension was inoculated into 50mL of Luria Bertani (LB) broth supplemented with Cr (Cr) at 100 mg/L and incubated at 30°C for 72 h [10].

Isolation and Screening of Cr-resistant bacterial isolates

After incubation, 1mL of culture was serially diluted up to 10^{-6} in SDW, and 100µL aliquots were spread-plated onto Soyabean Casien Digest Agar (SCDA) plates. Plates were incubated at 30 ± 1°C for 24h. Ten heavy-metal-resistant isolates were recovered, of which four (BIDS I, BIDS II, BIDS IV, and BIDS VI) demonstrating robust growth at 100mg/L Cr was selected for further experimentation. These isolates were maintained in nutrient broth and preserved at -20°C for future use [11].

Growth Curves of Bacterial Isolates with Metal Induction

Growth kinetics of the isolates were evaluated in LB medium supplemented with Cr (100 mgL^{-1}). Heavy metal stock solutions were autoclaved separately prior to use. Each flask was inoculated with 500µL of overnight-grown heavy metal–resistant bacterial culture and incubated at 30°C with shaking (150 rpm) for 96 h. LB broth without Cr served as the control. Growth was monitored at 6, 12, 24, 48, 60, 72, 84, and 96 h by measuring optical density (OD) at 610 nm, and growth curves were plotted for each isolate. Optimal growth conditions with respect to pH (4, 5, 6, and 7) and temperature (30, 35, and 37°C) were also determined by culturing isolates in LB broth medium. All four bacterial strains exhibited the highest growth rate up to 72 h at pH 7.0 and 30°C, consistent with previous studies [12-14].

Metal Tolerance Assay

Stock solutions of $K_2Cr_2O_7$ for Crwas prepared in Milli-Q water, filter-sterilized, and stored at 4°C.

Journal of Chemical Health Risks
www.jchr.org
JCHR (2026) 16(1), 2459-2473 | ISSN:2251-6727

Maximum tolerance levels were determined on SCDA media supplemented with increasing metal concentrations (100–200mg/L). Tolerance in liquid cultures was evaluated by inoculating 500µL of overnight culture (1×10^6 CFU/mL) into 50 mL Bushnell Hass (BH) broth containing Cr (100–200mg/L). Flasks were incubated at 30±1°C, 180rpm for 4 days. Isolates exhibiting growth at 200 mg/L were selected for subsequent studies and maintained on SCDA slants at −20 °C [15,16].

Morphological and biochemical characterization

Colony morphology and Gram staining were performed for preliminary characterization. Biochemical assays included oxidase, catalase, Voges–Proskauer, methyl red, casein hydrolysis, potassium cyanide (KCN) tolerance, urease, starch hydrolysis, motility, indole production, and citrate utilization tests [17]. Identification was carried out following Bergey's Manual of Systematic Bacteriology.

Molecular identification and characterization

Molecular identification of the isolates was sent to Eurofins Genomics India Pvt Ltd, Bangalore opting universal primer of 16SrRNA. Fragment of 16S rDNA gene was amplified by 27F and 1492R primers. Forward and reverse DNA sequencing reaction of PCR amplicon was carried out with forward primer and reverse primers using BDT v3.1 Cycle sequencing kit on ABI 3730xl Genetic Analyzer. Consensus sequence of 16S rDNA gene was generated from forward and reverse sequence data using aligner software Clustal W. Distance matrix was generated and the phylogenetic tree was constructed using MEGA 12 [18].

Fourier Transform Infrared Spectroscopy (FTIR) analysis bacterial biomass response to Cr metal stress

Fourier Transform Infrared Spectroscopy (FTIR) was employed to identify the functional groups and determine the chemical nature of the extracted strain. The lyophilized sample was finely ground with potassium bromide (KBr) and compressed into a thin, transparent pellet under a pressure of 5–6 tons cm^{-2} using a hydraulic press. The prepared pellet was analyzed using a Shimadzu UV-FTIR spectrometer within the wavenumber range of 4000–500 cm^{-1}.

Bioaccumulation Cr by bacterial isolates

Bio-accumulation potential assays were performed by inoculating 500µL of overnight-grown metal-resistant isolates into 50mL Bushnell Hass (BH) broth containing Cr (100–200mg/L). Flasks were incubated at 30 ±1°C, 180 rpm for 72h. Samples (10mL) were withdrawn every 12h, centrifuged at 10,000rpm, 4°C for 10 min, and the supernatant analyzed for residual metal concentration using Atomic Absorption Spectrophotometry (AAS) [19, 20].

SEM analysis

The morphological characteristics of Cr-resistant *Enterobacter mori* (BIDS I) and *Pseudomonas alcaliphila* (BIDS II) cells were examined as control and treated using scanning electron microscopy (SEM) following standard protocols [21, 22]. Actively growing bacterial cultures were harvested by centrifugation at 5,000 × g for 10min and washed twice with 0.1M phosphate buffer (pH 7.2). The cell pellets were fixed in 2.5% glutaraldehyde prepared in the same buffer for 2h at room temperature, followed by three rinses in phosphate buffer to remove excess fixative. Post-fixation was carried out using 1% osmium tetroxide for 1h to enhance cell wall contrast [23]. The fixed samples were dehydrated through a graded ethanol series (30%, 50%, 70%, 90%, 95%, and 100%) for 10 min each and finally treated with hexamethyldisilazane (HMDS) for drying [24]. The dried specimens were mounted on aluminum stubs using conductive carbon adhesive and sputter-coated with a thin layer of gold for 60s. The coated samples were examined under a

Journal of Chemical Health Risks
www.jchr.org
JCHR (2026) 16(1), 2459-2473 | ISSN:2251-6727

scanning electron microscope (ZEISS model) at an accelerating voltage of 5–10 kV using a secondary electron detector [25, 26].

Statistical Analysis

All experiments were performed in triplicates, and data were analyzed using SPSS v21.0 and Microsoft Excel. Two-way ANOVA was applied to determine statistically significant differences between bacterial strains and metal concentrations for Cr bio-removal efficiency.

3. Results and Discussion

Isolation and Screening of Cr Resistant Bacterial Strains

Ten Cr-resistant bacterial strains were isolated from the contaminated soil. Among these, four isolates — BIDS I, BIDS II, BIDS IV, and BIDS VI — demonstrated luxuriant growth at high Cr concentrations (100–200mg/L) and were selected for further analysis. The selected strains were sub-cultured on nutrient agar slants and stored at –20°C for subsequent studies.

Growth Curve Studies of Bacterial Strains

Growth behaviour of four bacterial isolates was investigated, and all isolates showed maximum growth up to 72h under optimal conditions of pH 7.0 and temperature 30°C. The growth kinetics was further evaluated in the presence of Cr. The results indicated that Cr exerted no significant inhibitory effect, suggesting that the isolates possess resistance to Cr. Among them, isolates BIDS I, II, IV, and VI attained peak growth after 96 hours when exposed to Cr. Based on these findings, the growth period for all isolates was standardized to 72 hours for subsequent experiments.

Growth Curve Analysis of Bacterial Strains

The growth dynamics of the four Cr-resistant bacterial isolates—*Enterobacter mori* (BIDS I), *Pseudomonas alcaliphila* (BIDS II), *Pseudomonas aestus* (BIDS IV), and *Aeromonas hydrophila* (BIDS VI)—were assessed through OD_{600} monitoring across 96h. Among these, *E. mori* (BIDS I) demonstrated strong but fluctuating growth, with an early peak at 12h (0.0473) followed by additional rises at 36 and 60h, reaching its maximum OD at 84 hours (0.0553). A similar trend was observed in *P. aestus* (BIDS IV), which maintained steady growth in the early phase and achieved its highest OD (0.0550) at 84 hours, reflecting both adaptability and prolonged metabolic activity. *A. hydrophila* (BIDS VI) showed moderate, relatively stable growth, with peaks at 48h (0.0310) and 84h (0.0340), whereas *P. alcaliphila* (BIDS II) exhibited weaker, inconsistent growth, with its highest OD at 12h (0.0423) but declining thereafter (Figure 1).

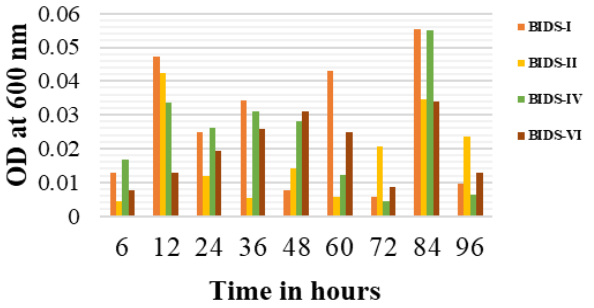

Figure 1. Growth curve studies of bacterial strains with Cr (100mg/L)

Taken together, the isolates displayed early-phase growth peaks at around 12h, followed by fluctuating patterns throughout the 96h incubation. Notably, both *E. mori* (BIDS I) and *P. aestus* (BIDS IV) exhibited a biphasic growth response, characterized by an initial increase and a secondary late-phase rebound at 84h. This biphasic behavior is consistent with earlier reports of bacteria adapting to heavy metal stress by activating secondary metabolic pathways that support extended growth under adverse conditions [27]. The late-phase rebound likely reflects activation of chromate-reduction mechanisms,

Journal of Chemical Health Risks
www.jchr.org
JCHR (2026) 16(1), 2459-2473 | ISSN:2251-6727

which transform toxic Cr (VI) into the less harmful Cr (III), thereby alleviating oxidative stress and allowing continued survival [28].

In contrast, the moderate growth pattern of *A. hydrophila* (BIDS VI) suggests reliance on more general stress-response mechanisms, such as antioxidant defenses or extracellular polymeric substance (EPS) production, rather than highly efficient chromate-reductase activity. Meanwhile, the weak and inconsistent performance of *P. alcaliphila* (BIDS II) indicates limited chromate-reduction capability or insufficient metabolic resilience under prolonged Cr exposure, which aligns with reports that Cr (VI) reduction efficiency can vary considerably across bacterial taxa depending on enzyme activity and electron donor availability [29]. Overall, these findings identify *E. mori* (BIDS I) and *P. aestus* (BIDS IV) as the most promising isolates for Cr bioremediation. Their biphasic growth and late-phase adaptability suggest a higher potential for sustained Cr (VI) detoxification. Similar observations have been reported in recent studies, where efficient Cr (VI) reducing bacteria achieved maximum reduction between 48 and 84 hours, primarily through the conversion of Cr (VI) to Cr (III) [30].

Growth Curve Studies under Control Conditions

The growth response of the four bacterial isolates under control conditions (without Cr supplementation) was monitored over 96h using OD_{600} measurements. *Enterobacter mori* (BIDS I) showed negligible growth, with OD values remaining close to baseline throughout the incubation period, indicating poor adaptability in the absence of Cr stress. In contrast, *Pseudomonas alcaliphila* (BIDS II) exhibited the highest growth potential among the isolates, with OD steadily increasing from 0.151 at 6h to a peak of 0.206 at 72h, followed by a slight decline thereafter. *Pseudomonas aestus* (BIDS IV) displayed gradual but consistent growth, with OD values rising from 0.010 at 6 hours to 0.036 at 72 hours, before decreasing slightly in later phases. Similarly, *Aeromonas hydrophila* (BIDS VI) demonstrated moderate and steady growth, increasing from 0.045 at 6h to a maximum of 0.083 at 72h, followed by a decline at 84–96h (Table 1).

Table 1. Growth curve of bacterial isolates under control conditions

Time in	O.D of Bacterial Strains			
(hours)	BIDS-I	BIDS-II	BIDS-IV	BIDS-VI
06	0.000	0.151	0.010	0.045
12	0.001	0.158	0.014	0.049
24	0.002	0.175	0.019	0.059
36	0.002	0.189	0.023	0.065
48	0.005	0.190	0.028	0.072
60	0.011	0.198	0.032	0.079
72	0.013	0.206	0.036	0.083
84	0.010	0.202	0.031	0.075
96	0.009	0.200	0.024	0.068

Overall, the isolates exhibited slow growth under control conditions, with *P. alcaliphila* (BIDS II) showing the most pronounced and sustained increase, while *E. mori* (BIDS I) remained largely inactive. Both *P. aestus* (BIDS IV) and *A. hydrophila* (BIDS VI) displayed moderate adaptability with gradual increases up to 72h before entering a stationary or decline phase. The growth curve analysis under control conditions revealed clear differences in adaptability among the isolates. *Pseudomonas alcaliphila* exhibited the highest and most sustained growth, consistent with the known metabolic versatility of *Pseudomonas* spp. [31]. *P. aestus* and *A. hydrophila* showed moderate, steady growth up to 72h before entering a decline phase, aligning with earlier findings that these genera often display slower baseline proliferation in

Journal of Chemical Health Risks
www.jchr.org
JCHR (2026) 16(1), 2459-2473 | ISSN:2251-6727

nutrient-limited media but maintain persistence under stress [32]. In contrast, *Enterobacter mori* remained largely inactive, suggesting its metabolism may be more responsive to metal-rich conditions, as previously reported for certain *Enterobacter* strains with metal-dependent enzymatic activity [33]. These observations highlight species-specific physiological traits that could influence their potential in Cr bioremediation.

Metal Tolerance Studies

Metal tolerance assays indicated that isolates BIDS I and BIDS II exhibited superior resistance to Cr induced stress, demonstrating the ability to acclimate at relatively higher metal concentrations compared to BIDS IV and BIDS VI. Under optimal experimental conditions (pH 7.0; 30°C), all four isolates displayed pronounced tolerance to Cr, suggesting their potential applicability in metal-stressed environments.

This finding is consistent with earlier reports highlighting that *Enterobacter* and *Pseudomonas* species often possess strong metal resistance mechanisms, including efflux pumps, enzymatic reduction, and biofilm-mediated protection [34]. Furthermore, the observation that all isolates demonstrated pronounced tolerance under optimal conditions (pH 7.0; 30°C) underscores the role of environmental parameters in enhancing microbial survival and activity in metal-stressed habitats. Similar studies have shown that physiological optimization of pH and temperature can significantly improve bacterial tolerance and Cr reduction efficiency, reinforcing the potential of these strains for bioremediation applications [35].

Molecular identification and characterization

Using a combination of morphology, biochemical tests, and molecular tools, the four Cr resistant isolates were identified. BIDS I was confirmed as *Enterobacter mori*, BIDS II as *Pseudomonas alcaliphila*, BIDS IV as *Pseudomonas aestus*, and BIDS VI as *Aeromonas hydrophila*. Each isolate showed its own unique features. For example, the colonies differed in color BIDS I was white, BIDS II pink, BIDS IV cream, and BIDS VI red. All were Gram-negative, but their cell shapes varied: BIDS I and II were mostly short rods or coccobacilli, BIDS IV was coccobacilli, and BIDS VI appeared as rods. Among them, only BIDS II showed motility.

The biochemical tests painted an even clearer picture. BIDS I, IV, and VI were positive for the Methyl Red test, but none showed activity for Voges-Proskauer or indole. Citrate use was exclusive to BIDS VI. Urease and casein hydrolysis were positive in BIDS II and IV, while BIDS VI showed only casein hydrolysis. All four tolerated potassium cyanide, while oxidase activity was found only in BIDS II and VI. Interestingly, none of the isolates showed catalase activity, but all were able to hydrolyze starch. When it came to sugar utilization, glucose and fructose were universally used. Lactose was metabolized by BIDS I and IV, while sucrose was used by BIDS I, IV, and VI. Mannitol stood out as it was fermented only by BIDS VI (Table 2). All isolates thrived at 30°C, 35°C, and 37°C, showing they are well-suited to mesophilic conditions. In short, each strain carried its own distinct traits, and together they represent a diverse group of Cr resistant bacteria. Their unique abilities make them interesting candidates for applications such as bioremediation in environments contaminated with heavy metals.

The morphological and biochemical profiling revealed distinct traits that underline the diversity and adaptability of the isolates. *Pseudomonas alcaliphila* showed the greatest metabolic versatility with motility, oxidase activity, and multiple hydrolytic enzymes, consistent with the adaptability of *Pseudomonas* spp. [36]. *Aeromonas hydrophila* was distinguished by citrate utilization and mannitol fermentation, features previously

Journal of Chemical Health Risks
www.jchr.org
JCHR (2026) 16(1), 2459-2473 | ISSN:2251-6727

noted as survival strategies in stressed environments [37]. The common ability of all isolates to hydrolyze starch and tolerate potassium cyanide indicates baseline resilience, supporting persistence in metal-contaminated sites. Such phenotypic variability enhances microbial survival and supports their potential in Cr bioremediation [38].

The 16SrRNA sequences of the all isolated bacterial strains BLAST searched in the NCBI server and sequences were identified as was confirmed as *Enterobacter mori*, *Pseudomonas alcaliphila*, *Pseudomonas aestus*, and *Aeromonas hydrophila* for BIDS I, II, IV and VI respectively (Figure 2 and 3). Further, the phylogenies were constructed using neighbor joining method by utilizing MEGA 12 software.

Table 2. Morphological and biochemical characteristics of Cr resistant bacterial isolates

Bacterial Strains	BIDS-I	BIDS-II	BIDS-IV	BIDS-VI
Morphological Characters				
Colony colour	White	Pink	Cream	Red
Gram Nature	Negative	Negative	Negative	Negative
Cell Morphology	Cocco bacilli	short rods	Coccobacilli	Bacilli
Motility	-	+	-	-
Biochemical Tests				
MR	+	-	+	+
VP	-	-	-	-
Indole	-	-	-	-
Citrate	-	-	-	+
Urease	-	+	+	-
Casien	-	+	+	+
KCN growth	+	+	+	+
Oxidase	-	+	-	+
Starch Hydrolysis	+	+	+	+
Catalase	-	-	-	-
Utilization of Sugars				
Glucose	+	+	+	+
Lactose	+	-	+	-
Sucrose	+	-	+	+
Mannitol	-	-	-	+
Fructose	+	+	+	+
Growth at				
30°C	+	+	+	+
35°C	+	+	+	+
37°C	+	+	+	+

FTIR spectroscopic analysis of bacterial strain cellular responses to Cr stress.

The intra molecular functional groups were identified by the FTIR spectrum of the cell free culture filtrate extracts, these exhibited distinct absorption peaks confirming the presence of major molecular functional groups (Figure 4). A broad absorption peaks at 3456.28 cm^{-1} corresponding to O–H stretching vibrations of hydroxyl was accumulated, a feature commonly observed in glycolipid-type cell free bacterial culture filtrate [39, 40]. The peaks at 2928.54 cm^{-1} and 2857.89 cm^{-1} were attributed to C–H stretching of aliphatic $–CH_2$ and $–CH_3$ groups, indicating the presence of

Journal of Chemical Health Risks
www.jchr.org
JCHR (2026) 16(1), 2459-2473 | ISSN:2251-6727

long hydrocarbon chains associated with lipid moieties [41].

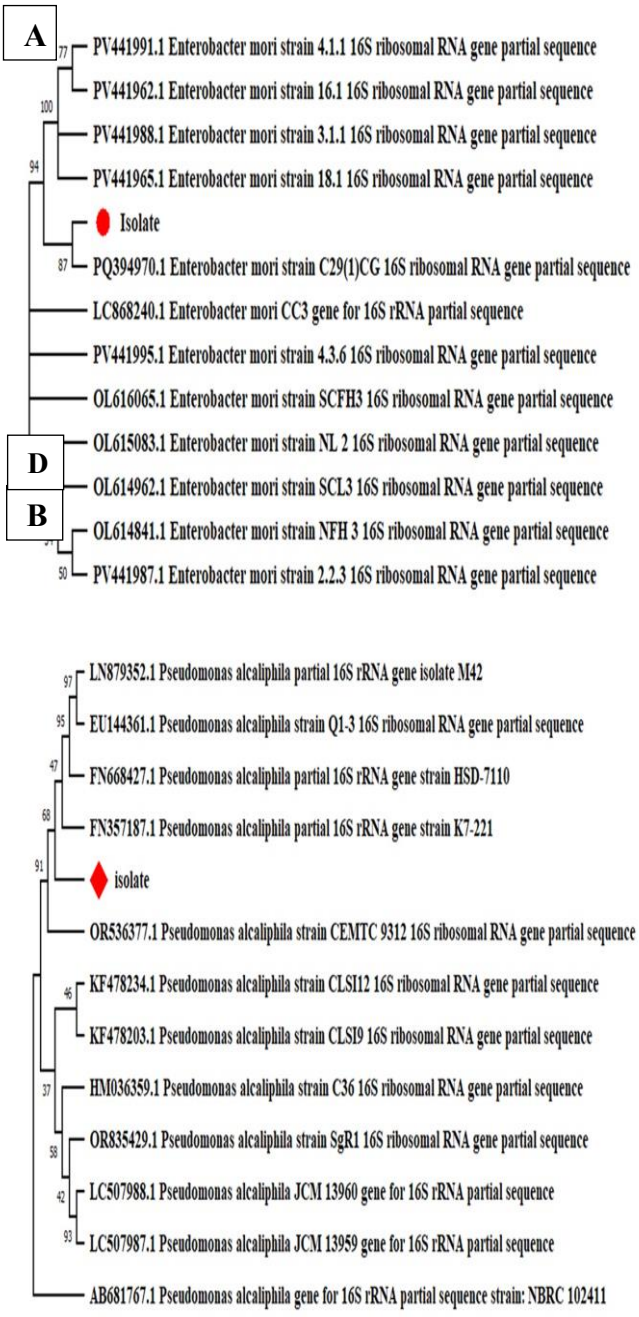

Figure 2. Molecular identification of bacterial isolates BIDS I (A) and II (B)

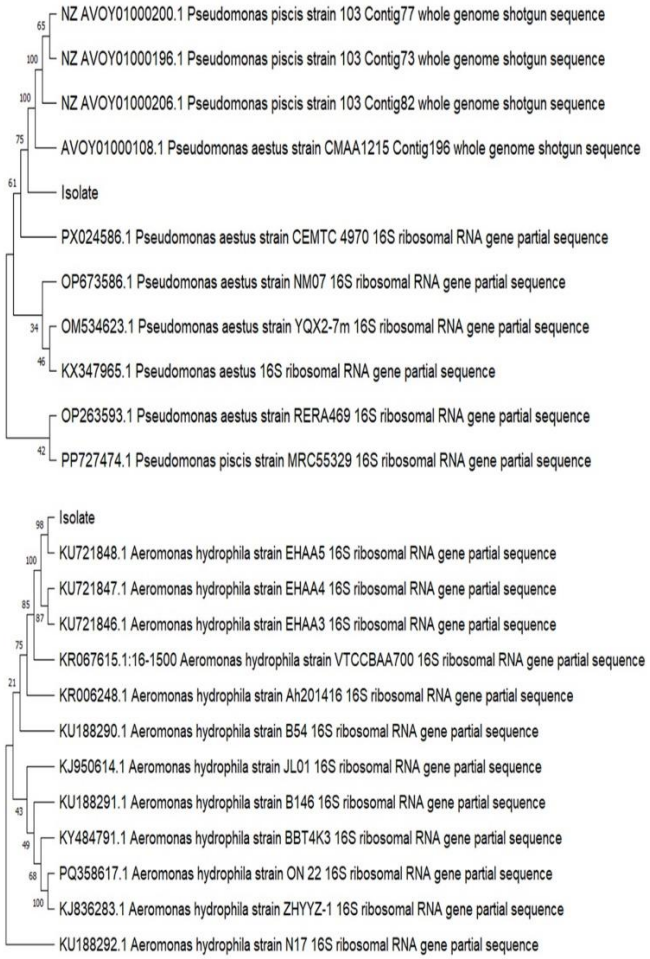

Figure 3. Molecular identification of bacterial isolates BIDS IV (C) and VI (D)

Distinct bands at 1768.20 cm^{-1} and 1635.90 cm^{-1} represented C=O stretching of ester and amide linkages, respectively, confirming the coexistence of lipid and peptide components [42]. Additional peaks at 1384.03 cm^{-1} and 1271.83 cm^{-1} were assigned to C–H bending and C–O stretching, which are characteristic of ester and ether bonds present in the carbohydrate portion of glycolipids [43]. Minor absorptions observed at 880.88 cm^{-1}, 832.60 cm^{-1}, and 439.32 cm^{-1} corresponded to C–H out-of-plane bending and C–Br stretching, respectively.

Journal of Chemical Health Risks
www.jchr.org
JCHR (2026) 16(1), 2459-2473 | ISSN:2251-6727

Overall, the FTIR data confirmed the presence of hydroxyl, aliphatic, carbonyl, and ester groups, indicating that the extracted compound possesses both lipid and carbohydrate moieties, consistent with the structural features of glycolipid-type bacterial based intra cellular molecular functional groups [43].

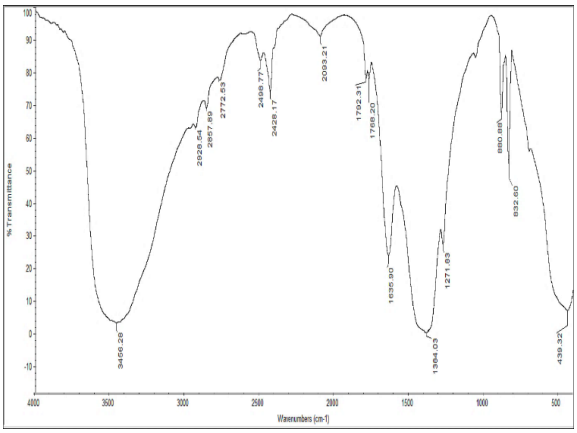

Figure 4. FT-IR chromatogram of the *Enterobacter mori* BIDS I strain treated with Cr exhibiting distinct molecular functional groups

Bioaccumulation of Cr by bacterial strains

This study was undertaken to isolate Cr-resistant bacterial strains with multiple heavy metal resistances, with the potential for simultaneous removal of several metals from contaminated environments. The four bacterial isolates were evaluated for their ability to remove Cr from aqueous solutions. *E. mori* strain BIDS I exhibited the highest removal efficiency, eliminating up to 83% of Cr from a medium containing 500 mg after 72 hours of incubation. BIDS II also demonstrated substantial Cr removal, achieving 75% under the same conditions. BIDS IV and BIDS VI effectively removed 69% and 65% of Cr, respectively, after 72 hours (Figure 5). All four isolates showed resistance to a range of heavy metals, indicating that environmental bacteria can adapt to their ecological niches and may possess specialized mechanisms for metal tolerance. These characteristics highlight their potential application in bioremediation of heavy-metal-contaminated sites.

The ability of these isolates to tolerate and reduce high Cr concentrations indicates specialized resistance mechanisms such as enzymatic reduction, efflux activity, and biosorption, which are well-documented in Cr resistant bacteria [44]. Similar studies have shown that Enterobacter and Pseudomonas spp. efficiently perform Cr (VI) bio-reduction and removal, reinforcing their value as bioremediation candidates [45]. Moreover, the observed multi-metal resistance suggests ecological adaptation to polluted environments, consistent with reports that environmental isolates can simultaneously withstand and transform multiple toxic metals [46].

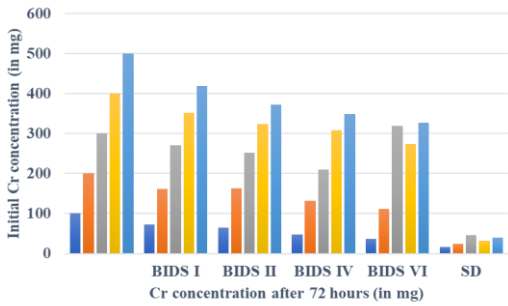

Figure 5. Accumulation of Cr by different bacterial isolates

SEM analysis

Scanning electron micrographs of the Cr-resistant isolates, *E. mori* (BIDS I) and *P. alcaliphila* (BIDS II), revealed clear morphological differences between control and Cr treated cells (Figure 6A–D). The untreated control cells of *E. mori* (Figure 6A) and *P. alcaliphila* (Figure 6C) exhibited typical smooth, intact, rod-shaped morphology with well-defined cell boundaries, indicating normal cellular architecture under non-stress conditions. In contrast, Cr-exposed cells (Figure 6B and D) showed pronounced surface irregularities, roughened texture, and partial

Journal of Chemical Health Risks
www.jchr.org
JCHR (2026) 16(1), 2459-2473 | ISSN:2251-6727

deformation, along with occasional clumping and aggregation of cells. These alterations suggest that Cr stress induces distinct structural modifications in the bacterial cell envelope, possibly due to metal binding, membrane damage, or extracellular polymeric secretion as a defence response. Such morphological adaptations are consistent with earlier findings in Cr tolerant bacterial strains [47, 48], reflecting the ability of these isolates to withstand and adapt to heavy-metal toxicity through cell surface remodelling mechanisms.

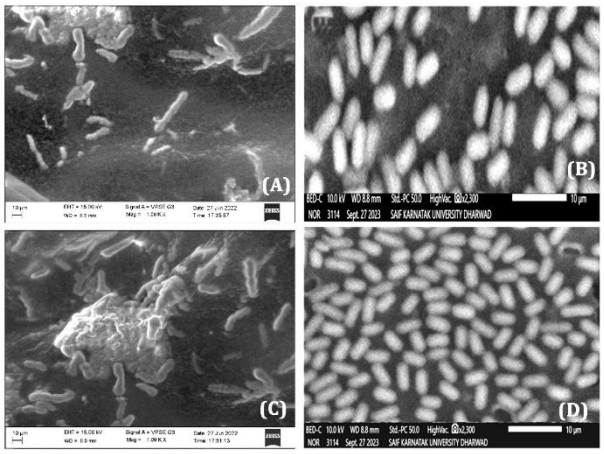

Figure 6. Scanning electron microphotographs of the Cr-resistant isolates, A. and B. *E. mori* (BIDS I) control and treated; C. and D. *P. alcaliphila* (BIDS II) control and treated.

Statistical Analysis

The table shows the Cr concentrations remaining after 72 hours of incubation with four bacterial isolates (BIDS I, II, IV, and VI) at different initial Cr levels, along with the standard deviation (SD) to indicate variability among the isolates. At the lowest Cr concentration (100 mg), BIDS I removed the most Cr (73 mg), followed by BIDS II (64.67 mg), BIDS IV (48 mg), and BIDS VI (37.33 mg), with a relatively low SD of 16.09, indicating that the isolates behaved similarly at this concentration. As the initial Cr level increased, all isolates removed more Cr, but the variation between them also grew, reflected in higher SD values. For example, at 300 mg, the SD reached 45.66, showing greater differences in Cr removal efficiency among the isolates.

Overall, BIDS I consistently achieved the highest Cr removal across all tested concentrations, while BIDS VI showed more variability. The increasing SD at higher Cr levels highlights how these bacterial strains differ in their tolerance and ability to remove metals under more stressful conditions. This analysis demonstrates that each isolate has a distinct capacity for Cr remediation, and SD provides a clear measure of this variability.

The Cr removal data after 72 hours revealed distinct differences in the efficiency of the four isolates across varying initial Cr concentrations. *Enterobacter mori* (BIDS I) consistently showed the highest removal, while *Aeromonas hydrophila* (BIDS VI) displayed greater variability, particularly at higher Cr levels. The increasing standard deviation at elevated concentrations indicates that stress conditions amplify differences in tolerance and detoxification capacity among the isolates. These findings suggest that while all four strains are capable of Cr remediation, their effectiveness is strain-specific and influenced by metal concentration, highlighting the importance of selecting robust, high-performing bacteria for bioremediation applications. Similar observations have been reported for Cr-resistant *Enterobacter* and *Pseudomonas* strains, where removal efficiency and tolerance vary with metal load and environmental stress [49, 50].

Statistical Hypothesis

The ANOVA results indicate that the initial Cr concentration had a significant effect on Cr removal by the bacterial isolates (Table 3). The variation across different concentrations (rows) was highly significant, with an F-value of 77.87 and a p-value of 1.77×10^{-8}, showing that the amount of Cr in the medium strongly influenced removal efficiency. In contrast, the differences in

Journal of Chemical Health Risks
www.jchr.org
JCHR (2026) 16(1), 2459-2473 | ISSN:2251-6727

removal among the four bacterial isolates (columns) were not statistically significant at the 5% level ($F = 2.69$; $p = 0.093$), suggesting that all isolates performed relatively similarly under the tested conditions. The error term accounted for a small portion of the variability, indicating that the observed differences were largely explained by the initial Cr concentrations. Overall, these results highlight that while Cr concentration plays a major role in removal efficiency, the four bacterial strains show comparable performance in Cr remediation. These findings support the rejection of H2, confirming that initial metal levels significantly impact removal, while H1 cannot be rejected, showing that all four bacterial strains performed similarly under the tested conditions. Similar patterns have been reported in studies on *Enterobacter* and *Pseudomonas* species, where removal efficiency is largely dependent on substrate concentration rather than strain differences [51].

H1: There is no significant difference in the bio-removal of Cr after 72h for different bacterial strains (Columns).

H2: There is no significant difference in the bio-removal of Crafter 72h for different initial concentration of Cr (Rows).

Table 3. Effect on Cr removal by the bacterial isolates

ANOVA Test						
Source *of Variation*	*SS*	*Df*	*MSS*	*F*	*P-value*	*F crit*
Rows	260464.6	4	65116.14	77.86908	1.77E-08	3.259167
Columns	6754.594	3	2251.531	2.692492	0.093112	3.490295
Error	10034.71	12	836.2259			
Total	277253.9	19				

Conclusions

The present study successfully isolated and characterized four indigenous bacterial strains; *E. mori* (BIDS I), *P. alcaliphila* (BIDS II), *P. aestus* (BIDS IV), and *A. hydrophila* (BIDS VI) from Cr-contaminated soils of the Belur Industrial area, Dharwad in Karnataka, India. These isolates demonstrated remarkable resistance to Cr stress and exhibited distinct morphological, biochemical, and molecular traits. Growth curve analysis confirmed their ability to thrive in the presence of Cr, with BIDS I and BIDS IV showing the highest adaptability and potential for sustained bioremediation. Cr removal assays revealed that all four isolates could effectively reduce Cr concentrations from aqueous solutions, with BIDS I, achieving the highest removal efficiency (83% at 500 mg/L Cr), followed by BIDS II, BIDS IV, and BIDS VI. Statistical analysis (ANOVA) indicated that the initial Cr concentration significantly influenced bio-removal efficiency, while differences among the bacterial strains were not statistically significant, suggesting comparable performance among the isolates. Overall, these findings highlight the potential of indigenous bacterial strains as eco-friendly and efficient agents for bioremediation of Cr contaminated environments**.** Their inherent metal tolerance and bioaccumulation capabilities make them promising candidates for sustainable wastewater treatment and environmental detoxification strategies. Future studies could focus on scaling up these bioremediation processes and exploring their effectiveness in multi-metal contaminated sites.

Acknowledgments

The authors gratefully acknowledge the Chairperson, Department of Studies and Research in Microbiology, Mangalore University, Jnana Kaveri Post Graduate Centre, Chikka Aluvara, Kodagu, Karnataka, India, for her constant support throughout the research work. We gratefully acknowledge Nichrome Testing Laboratory and Research Pvt Ltd., Dharwad, for providing the facilities and essential support for conducting this research.

Journal of Chemical Health Risks

www.jchr.org

JCHR (2026) 16(1), 2459-2473 | ISSN:2251-6727

Authors' Contributions

The first author conceived and designed the study, analysed the results, and prepared the initial draft of the manuscript. All the authors contributed to the critical review and revision of the manuscript, participated in drafting the final version, and approved the final manuscript for publication. All the authors agree to be accountable for all aspects of the work.

Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

Source of Funding

This research received no specific grant from any funding agency in the public, commercial, or not for profit sectors.

References

1] Briffa J., Sinagra E., Blundell R., 2020. Heavy metal pollution in the environment and their toxicological effects on humans. Heliyon. 6(9), e04691. https://doi.org/10.1016/j.heliyon.2020.e04691

2] Kondakindi V.R., Pabbati R., Erukulla P., Maddela N.R., Prasad R., 2024. Bioremediation of heavy metals-contaminated sites by microbial extracellular polymeric substances. Environ Chem Ecotoxicol. 6, 408–421. https://doi.org/10.1016/j.enceco.2024.05.002

3] Pande V., Pandey S.C., Sati D., Bhatt P., Samant M., 2022. Microbial interventions in bioremediation of heavy metal contaminants in agroecosystem. Front Microbiol. 13, 824084.

4] Ayele A., Godeto Y.G., 2021. Bioremediation of chromium by microorganisms and its mechanisms related to functional groups. J Chem. 2021, 7694157. https://doi.org/10.1155/2021/7694157

5] Sharma A.K., Sharma M., Sharma S., Malik D.S., Sharma M., Sharma M., Sharma A.K., 2024. A systematic review on assessment of heavy metals toxicity in freshwater fish species: Current scenario and remedial approaches. J Geochem Explor. 262, 107472. https://doi.org/10.1016/j.gexplo.2024.107472

6] Sharma P., Singh S.P., Parakh S.K., Tong Y.W., 2022. Health hazards of hexavalent chromium (Cr(VI)) and its microbial reduction. Bioengineered. 13(3), 4923–4938. https://doi.org/10.1080/21655979.2022.2037273

7] Ma Y., Bantec T.N., Oliveira R.S., Coutinho A.X.P., Zhang C., Freitas H., 2022. The role of bacteria in metal bioaccumulation and biosorption. In: Bauddh K., Ma Y., Eds., Advances in Microbe-Assisted Phytoremediation of Polluted Sites. Elsevier: Amsterdam. pp. 103–112. https://doi.org/10.1016/B978-0-12-823443-3.00012-0

8] Chatterji T., Singh T., Khanna N., Bhagat T., Tyagi D., Totlani R., 2025. Microbial bioremediation strategies for sustainable wastewater treatment. Sustain Process Connect. 1, Article ID 0005. https://doi.org/10.69709/SusProc.2025.122304

9] American Public Health Association, 2017. Standard Methods for the Examination of Water and Wastewater, 23rd ed., American Public Health Association: Washington, DC.

10] Jeyasingh J., Philip L., 2005. Bioremediation of chromium-contaminated soil: Optimization of operating parameters under laboratory conditions. J Hazard Mater. 118, 113–120. https://doi.org/10.1016/j.jhazmat.2004.10.005

11] Nwaehiri U.L., Akwukwaegbu P.I., Nwoke B.E.B., 2020. Bacterial remediation of heavy metal polluted soil and effluent from paper mill industry. Environ Anal Health Toxicol. 35(2), e2020009. https://doi.org/10.5620/eht.e2020009

12] Jayanthi M., Kanchana D., Saranraj P., Sujitha D., 2013. Bioremediation of toxic heavy metal chromium in tannery effluent using bacteria. Appl J Hyg. 2(2), 8–14.

13] Sudhanshu S., Sundaramanickam K., Vijayansiva G., 2014. Detoxification of hexavalent chromium by chromate-reducing bacteria. Am J Res Commun. 2(2), 205–216.

14] Satapute P., Kaliwal B., 2016a. Biodegradation of propiconazole by Burkholderia sp. BBK_9. 3 Biotech. 6(1), 110. https://doi.org/10.1007/s13205-016-0429-3

15] Sundar K., Vidya R., Amitava M., Chandrasekaran N., 2010. High chromium-tolerant bacterial strains from Palar River Basin. Res J Environ Earth Sci. 2(2), 112–117.

16] Satapute P., Paidi M.K., Kurjogi M., Jogaiah S., 2019. Physiological adaptation of cadmium-resistant Pseudomonas taiwanensis. Environ Pollut. 251, 555–563. https://doi.org/10.1016/j.envpol.2019.05.048

17] Smrithi A., Usha K., 2012. Isolation and characterization of chromium removing bacteria from tannery effluent disposal site. Int J Adv Biotechnol Res. 3(3), 644–652.

18] Satapute P., Kaliwal B., 2016b. Biodegradation of propiconazole by Pseudomonas aeruginosa PS-4. Ann Microbiol. 66, 1355–1365. https://doi.org/10.1007/s13213-016-1222-6

19] Ramadass K., Megharaj M., Venkateswarlu K., Naidu R., 2016. Soil bacterial strains with heavy metal resistance and hydrocarbon degradation potential. Int J Environ Sci Technol. 13, 2863–2874. https://doi.org/10.1007/s13762-016-1113-1

20] Khansa R., Menon U., Sivasubramanian V., Ranjitha, 2021. Removal of cadmium by heavy metal–resistant bacteria isolated from Hussain Sagar Lake—Hyderabad. Biomass Convers Biorefin. 12, 1703–1713. https://doi.org/10.1007/s13399-021-01840-1

21] Bozzola J.J., Russell L.D., 1999. Electron Microscopy: Principles and Techniques for Biologists, 2nd ed., Jones and Bartlett Publishers: Boston.

22] Beveridge T.J., 2001. Use of the Gram stain in microbiology. Biotech Histochem. 76(3), 111–118.

23] Hayat M.A., 2000. Principles and Techniques of Electron Microscopy: Biological Applications, 4th ed., Cambridge University Press: Cambridge.

24] Nation J.L., 1983. A new method using hexamethyldisilazane for preparation of soft insect tissues for scanning electron microscopy. Biotech Histochem. 58, 347–351.

25] Kim J., Lee S., Choi Y., 2020. Morphological analysis of metal-resistant bacteria by scanning electron microscopy. J Environ Sci Health A. 55, 347–355.

26] Satapute P., Nagaraja G., Jogaiah S., 2024. Microbial metabolites associated with degradation of imidacloprid. Environ Geochem Health. 46, 114. https://doi.org/10.1007/s10653-024-01892-w

27] Gonzalez J.M., Aranda B., 2023. Microbial growth under limiting conditions—Future perspectives. Microorganisms. 11(7), 1641. https://doi.org/10.3390/microorganisms11071641

28] Adhikary S., Saha B.K., Roy V., Saha J., Pal A., 2025. Deciphering chromate tolerance and reduction ability of an indigenous Bacillus strain isolated from polluted pond sludge for chromium bioremediation. Sci

Journal of Chemical Health Risks
www.jchr.org
JCHR (2026) 16(1), 2459-2473 | ISSN:2251-6727

Rep. 15(1), 23323. https://doi.org/10.1038/s41598-025-07031-4

29] Ramli N.N., Othman A.R., Kurniawan S.B., Abdullah S.R.S., Hasan H.A., 2023. Metabolic pathway of Cr(VI) reduction by bacteria: A review. Microbiol Res. 268, 127288. https://doi.org/10.1016/j.micres.2022.127288

30] Kholisa B., Matsena M., Chirwa E.M.N., 2021. Evaluation of Cr(VI) reduction using indigenous bacterial consortium isolated from municipal wastewater sludge. Catalysts. 11(9), 1100. https://doi.org/10.3390/catal11091100

31] Wackett L.P., 2003. Pseudomonas putida—A versatile biocatalyst. Nat Biotechnol. 21(2), 136–138.

32] Janda J.M., Abbott S.L., 2010. The genus Aeromonas: Taxonomy, pathogenicity, and infection. Clin Microbiol Rev. 23(1), 35–73. https://doi.org/10.1128/CMR.00029-09

33] Sun Y., Lan J., Du Y., Guo L., Du D., Chen S., Ye H., Zhang T.C., 2020. Chromium(VI) bioreduction and removal by Enterobacter sp. SL. Bioresour Technol. 302, 121974. https://doi.org/10.1016/j.biortech.2019.121974

34] Elfadadny A., Ragab R.F., AlHarbi M., Badshah F., Ibáñez-Arancibia E., Farag A., et al., 2024. Antimicrobial resistance of Pseudomonas aeruginosa: Navigating clinical impacts, current trends, and innovations. Front Microbiol. 15, 1374466. https://doi.org/10.3389/fmicb.2024.1374466

35] Ahemad M., 2015. Enhancing phytoremediation of chromium-stressed soils through plant-growth-promoting bacteria. J Genet Eng Biotechnol. 13(1), 51–58. https://doi.org/10.1016/j.jgeb.2015.02.001

36] Karaman M.Z., Yetiman A.E., Zhan J., Fidan O., 2025. Biochemical characterization and genome analysis of Pseudomonas loganensis sp. nov., a novel endophytic bacterium. MicrobiologyOpen. 14(4), e70051. https://doi.org/10.1002/mbo3.70051

37] Semwal A., Kumar A., Kumar N., 2023. Pathogenicity of Aeromonas hydrophila and mitigation through medicinal herbs. Heliyon. 9(3), e14088. https://doi.org/10.1016/j.heliyon.2023.e14088

38] Syed A., Zeyad M.T., Shahid M., Elgorban A.M., Alkhulaifi M.M., Ansari I.A., 2021. Chromium-resistant bacteria and hexavalent chromium remediation. ACS Omega. 6(38), 25076–25088. https://doi.org/10.1021/acsomega.1c04396

39] Singh P., Patil Y., Rale V., 2016. Biosurfactant production: Emerging trends and promising strategies. J Microbiol Biotechnol Res. 6(1), 31–37.

40] Ambaye T.G., Vaccari M., van Hullebusch E.D., Rtimi S., 2021. A critical review on progress, challenges and perspectives of biosurfactants. J Clean Prod. 295, 126333. https://doi.org/10.1016/j.jclepro.2021.126333

41] Essghaier B., Mallat N., Khwaldia K., Mottola F., Rocco L., Hannachi H., 2023. Production and characterization of new biosurfactants from bacterial isolates. Molecules. 28(4), 1912. https://doi.org/10.3390/molecules28041912

42] Gharaie S., Ohadi M., Hassanshahian M., Shakibaie M., Shahriary P., Forootanfar H., 2023. Glycolipopeptide biosurfactant from Bacillus pumilus SG: Structural and functional characterization. Sci Rep. 13, 11589. https://doi.org/10.1038/s41598-023-38809-2

43] Dias M.A.M., Rossini E.L., de Britto D., Nitschke M., 2025. Production and characterization of a novel glycolipid biosurfactant from microbial origin. Fermentation. 11(8), 471. https://doi.org/10.3390/fermentation11080471

44] Pushkar B., Sevak P., Parab S., Nilkanth N., 2021. Chromium pollution and its bioremediation mechanisms in bacteria: A review. J Environ Manage. 287, 112279. https://doi.org/10.1016/j.jenvman.2021.112279

45] Ruparelia J.R., Patel H.K., 2024. Harnessing the chromium reduction potential of Pseudomonas aeruginosa JRHM33. Heliyon. 10(15), e35650. https://doi.org/10.1016/j.heliyon.2024.e35650

46] Balta I., Lemon J., Gadaj A., Cretescu I., Stef D., Pet I., et al., 2025. The interplay between antimicrobial resistance, heavy metal pollution, and the role of microplastics. Front Microbiol. 16, 1550587. https://doi.org/10.3389/fmicb.2025.1550587

47] Karthik C., Barathi S., Arulselvi P.I., 2016. Isolation and characterization of chromium-resistant bacteria from tannery effluent contaminated soil and their potential in bioremediation. Appl Microbiol Biotechnol. 100, 341–352.

48] Khusro A., Aarti C., Agastian P., 2021. SEM analysis of chromium-tolerant Bacillus sp. isolated from tannery effluents. Environ Technol Innov. 22, 101408.

49] More A.G., Gupta S.K., 2018. Evaluation of chromium removal efficiency of a novel bioelectrochemical system. Bioprocess Biosyst Eng. 41, 1547–1554. https://doi.org/10.1007/s00449-018-1982-4

50] Plestenjak E., Kraigher B., Leskovec S., Mandic Mulec I., Marković S., Ščančar J., Milačič R., 2022. Reduction of hexavalent chromium using bacterial isolates from tannery effluent. Sci Rep. 12, 20197. https://doi.org/10.1038/s41598-022-24797-z

51] Mahmoud A.S., Mohamed N.Y., Mostafa M.K., Mahmoud M.S., 2021. Effective chromium adsorption using bimetallic Fe/Cu nanoparticles. Air Soil Water Res. 14, 1–14.