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Original Article

Bioremediation Efficacy of Chromium-Resistant Bacterial Isolates from Industrial Effluents at Krishna Ghat and Patliputra Industrial Area, Patna

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Abstract

Chromium (Cr), released from different industries poses risks to living organisms, especially when anthropogenic activities raise its concentration in the environment. The present study, therefore, aims to find chromium-resistant bacteria for potential use in bioremediation. Water samples from two sites were collected, and six chromium-tolerant bacterial strains were isolated. Minimum inhibitory concentration (MIC) of all the six isolates were estimated against chromium. The results demonstrated that these isolates possess strong resistance to chromium, suggesting their potential effectiveness in detoxifying contaminated environments. This study reports the isolation of chromium resistant bacteria from Krishna Ghat water sample and Patliputra industrial area of Patna. The novelty of this research lies in the isolation and characterization of chromium-resistant bacteria from industrial effluents at Krishna Ghat and Patliputra industrial area water samples, highlighting their potential for bioremediation of chromium-contaminated environments. The present study demonstrates that isolates K5 (*Streptococcus spp.),* and K6 (*Enterococcus spp.*) achieve a 75% reduction in chromium concentration, underscoring their high competence in hexavalent chromium bioremediation. Therefore, the study concludes that these chromium-resistant bacteria could serve as promising agents for bioremediation strategies aimed at reducing chromium pollution.

Keywords: *Bacteria; Bioremediation; Chromium; MIC (Minimum Inhibitory concentration)*

Introduction

Chromium (Cr), a heavy metals vastly used in industries, plays a vital role in several manufacturing processes. Chromium is a major constituent of industries involved in leather processing, printing, dyeing, pigment production, electroplating, and automobile fabrication. While chromium's unique properties provide significant benefits to these industries, the waste and by-products generated during these processes pose serious environmental challenges. Improperly managed industrial waste, often containing chromium compounds, is frequently released into water bodies and soil, causing considerable pollution and ecological disruption (Shekhawat *et al*., 2015; Adhikary *et al*., 2025).

Being one of the most perilous metals, chromium enters the atmosphere through both natural and anthropogenic sources, threatening the health of all living organisms, including humans. Consumption of chromium-contaminated water can adversely affect the cardiovascular and urinary systems and is associated with health issues such as bone deformities, emphysema, infertility, and high blood pressure (Irshad *et al.,* 2023). Chromium primarily exists in two forms: trivalent chromium [Cr(III)] and hexavalent

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chromium [Cr(VI)]. While Cr(III) is an essential element involved in metabolism of glucose, proteins, and fats, an imbalance in its levels can disrupt normal metabolic functions (McLean *et al.,* 2001, Sharma *et al.,* 2022). On the other hand, Cr(VI) is highly soluble, mobile, and toxic, posing significantly greater risks than Cr(III) (Monga *et al.,* 2022).

Inhalation of Cr(VI) is associated with severe adverse health effects under acute as well as chronic exposure conditions. It mainly affects the respiratory system, with many studies confirming its carcinogenic properties and its ability to increase the risk of lung cancer (Irshad *et al.,* 2023). In contrast, Cr(III) is generally considered less harmful due to its low solubility and tendency to bind with organic matter, which limits its mobility in the environment (Zulfiqar *et al.,* 2023). Excessive levels of Cr(III) can disrupt the activity of metalloenzymes, which may result in reproductive problems and increased oxidative stress (Farina *et al.,* 2013).

Addressing chromium contamination through bioremediation offers a promising solution. This process utilizes biological organisms like microorganisms, plants, and fungi to detoxify or immobilize chromium in contaminated soils and water. Microorganisms, particularly, assist the reduction of Cr(VI) to less harmful Cr(III), reducing chromium's mobility and toxicity (Basu *et al.,* 1997;Camargo *et al.,* 2003; Ayele *et al.,* 2021; Padma *et al.,* 2023; Saxena et al., 2025; Aththanayake *et al*., 2025; Khanam *et al*., 2026).These microorganisms are effective Cr^{+6} biosorbers and hence assist in bioremediation (Acharya *et al.,* 2024). Given the high levels of chromium discharge from industries such as leather tanning, textile dyeing, and electroplating, bioremediation represents a cost-effective and eco-friendly alternative to traditional methods (Ganguli *et al.,* 2002; Abo Elazm *et al.,* 2020; Tuli *et al*., 2024; Liza *et al.,* 2025).

The current study therefore seeks to screen and characterize chromium resistant bacteria from effluent impacted water bodies. The isolates were screened for chromium resistant property and further Minimum Inhibitory Concentration (MIC) was calculated for all the isolates. The bioremediation property to degrade chromium was also checked for the resistant isolates. Hence, this study will assist in identification of the bioremediation potential of microorganisms to degrade heavy metals.

Materials and Methods

Collection of water samples

Water samples were obtained from one industrial location in Patna (Patliputra Industrial Area) and one Ghat area (Krishna Ghat) known for significant heavy metal contamination zone, particularly chromium. The collected samples were stored in sterilized, labelled containers, and essential physico-chemical parameters, including temperature, pH, colour and turbidity, were measured.

Detection of chromium in different samples

The collected water samples were first filtered using Whatman filter paper. After filtration, 1 mL of solution containing sulfuric acid, phosphoric acid, and diphenyl carbazide solution was added to each sample. The mixture was kept at room temperature for approximately 10 minutes. A purple colour development indicates the presence of chromium, confirming its detection through qualitative analysis.

Isolation of bacteria

Bacteria resistant to chromium were isolated using enrichment culture method. Serial dilution of the water samples were prepared and plated on nutrient agar and bacterial growth was observed. Colony enumeration was performed in colony-forming units per ml (CFU/mL). Each distinct colony was characterized for further analysis.

Primary screening of chromium-resistant bacteria

To determine chromium resistance, single bacterial isolate was streaked onto nutrient agar plates supplemented with potassium dichromate ($K_2Cr_2O_7$) at concentrations of 100 ppm, 200 ppm, and 300 ppm. The plates were then incubated at 37°C for 24 hours, after which bacterial growth was monitored (Figure 1).

Figure 1: *Primary screening of chromium resistant bacteria after 24 hours of incubation at 37°C.*

Characterization of chromium-resistant Bacteria

Morphological characterization of the isolated strains was conducted using Gram's staining, following the guidelines in Bergey's Manual of Determinative Bacteriology (Vos *et al.,* 2009) (Figure. 2). Morphological parameters such as colony shape, colour, texture, elevation, margin, Gram's nature, and cell shape were recorded. Additionally, several biochemical tests were performed to identify the isolates, including the Voges-Proskauer (VP) test, catalase test, indole test, methyl red test and catalase test.

Determination of Minimum Inhibitory Concentration (MIC)

The MIC for chromium was estimated through the plate dilution method. Bacterial isolates were cultured in nutrient broth for 24 hours. Standardized bacterial suspensions were swabbed onto nutrient agar plates using sterile cotton swabs. Whatman filter paper discs, each containing potassium dichromate ($K_2Cr_2O_7$) at concentrations of 5000 ppm, 10000 ppm, and 15000 ppm, were placed on the agar surface. The plates were incubated at 37°C for 48 hours, and the zones of inhibition were measured after 24 and 48 hours. The resulting data were recorded and analyzed (Kalsoom *et al.,* 2021).

Bioremediation of chromium by bacteria

The study aimed to explore the potential of bacterial isolates to reduce chromium toxicity and mobility in the environment. Selected bacterial strains were cultured in nutrient broth supplemented with chromium to assess their growth and capacity for chromium reduction. Bacterial growth and the reduction of chromium were monitored for 48 hours by estimating the optical density (OD) at 540 nm wavelength.

The percentage of reduction was calculated using equations (Kumar & Dwivedi 2019)

$Y = C_i – C_f / C_i * 100$

where Y is the reduction percentage, Ci is the initial Cr [VI] concentration, Cf is the final Cr [VI] concentration.

Statistical Analysis

All the statistical analysis and data were plotted using Graphpad prism 5.0

Results

Physico-chemical analysis of water samples

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This study involved the analysis of two water samples collected from industrial regions in Patna, specifically Krishna Ghat and the Patliputra Industrial Area. Table 1 provides significant insights into the physico-chemical characteristics of these sample. This information provides a better understanding of the environmental conditions of the sampling sites and their influence on chromium-resistant bacterial populations.

Table 1. Physico-chemical properties of water samples used in this study

Samples	pH	Temperature	Colour
Krishna Ghat	6.5	23°C	Slightly yellow
Pataliputra industrial area	7	27°C	Greenish

Isolation of chromium resistant bacteria from samples

Six bacterial isolates with distinct morphologies were obtained from two chromium contaminated water samples using nutrient agar with chromium concentrations of 100 ppm, 200 ppm and 300 ppm. Five isolates (K1, K2, K3, K4, K5) were derived from the Krishna Ghat sample, while one isolate (K6) was from the Patliputra Industrial Area.

The bacterial colonies in the water samples were estimated to range between 100 and 300 colonies per milliliter. For the sample from Krishna Ghat, the CFU values at different concentration of chromium i.e. 100ppm, 200ppm and 300ppm were calculated as 7×10^3 CFU/mL, 14×10^3 CFU/mL, and 6×10^3 CFU/mL respectively. Similarly, for the sample from the Patliputra Industrial Area, the CFU values at different concentration of chromium i.e. 100ppm, 200ppm and 300ppm were calculated as 6×10^4 CFU/mL, 27×10^3 CFU/mL, and 14×10^4 CFU/mL respectively (Table 2).

Table 2. CFU/mL of different water samples at different chromium concentrations

Samples	100ppm	200ppm	300ppm
Krishna Ghat	7×10^3	14×10^3	6×10^3
Pataliputra Industrial area	6×10^4	27×10^3	14×10^4

The isolates displayed various cultural characteristics: K1, K2, K3, K4, K5, and K6 were described as off-white, creamy white, textured, pale white, and greyish, respectively. Based on Bergey's Manual of Determinative Bacteriology (Vos *et al.,* 2009), these isolates showed similarities to *coccus* and *bacillus* morphologies.

Biochemical characterization of chromium degrading bacterial isolates

The six bacterial isolates were subjected to a series of biochemical tests, including the Methyl Red, Indole, Voges-Proskauer (VP), and Catalase tests. The results of the Indole test indicated that all isolates were negative, except for K5. In the Methyl Red test, isolates K3, K5, and K6 showed positive results, while the others tested negative. The VP test yielded positive results for isolates K1, K2, and K5. For the Catalase test, all isolates tested positive except K6. Based on Gram staining and the biochemical tests, the isolates K1, K2 and K3 showed similarity to *Staphylococcus spp.,* K4 showed similarity to *Bacillus spp.*, K5, and K6 showed similarity to *Streptococcus spp.,* and *Enterococcus spp.* respectively (Table 3, Figure.2).

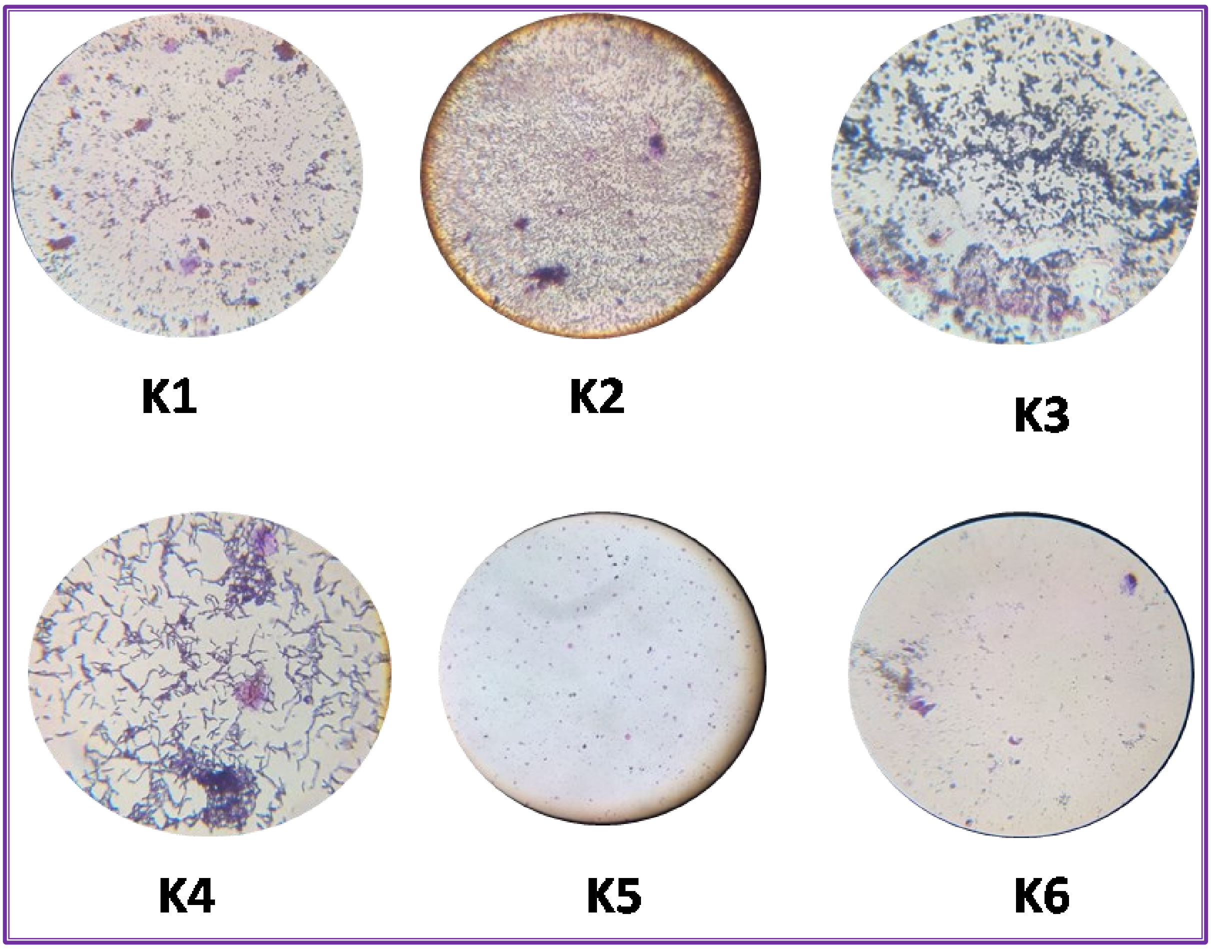

Figure 2: *Gram staining of all the six chromium resistant isolates designated as K1, K2, K3, K4, K5 and K6*

Table 3. Cultural and morphological characteristics of chromium resistant isolates

Isolates	Colour	Shape	Margin	Elevation	Texture	Gram Stain
K1	Off white	Coccus	Regular	Flat	Creamy	+ve
K2	white	Coccus	Irregular	Flat	Creamy	-ve
K3	Creamy white	Coccus	Entire	Flat	Rough	+ve
K4	Off white	Bacilli	Entire	Flat	Creamy	+ve
K5	Greyish	Coccus	Regular	Opaque	Creamy	+ve
K6	Pale white	*Coccus*	Entire	Raised	Mucoid	-ve

Detection of Minimum Inhibitory Concentration (MIC Test)

The study evaluated the chromium tolerance of six bacterial isolates (K1, K2, K3, K4, K5, and K6) by assessing their growth responses under varying chromium concentrations. The minimum inhibitory concentration (MIC) was tested on nutrient agar medium at three chromium levels: 5000 ppm, 10000 ppm, and 15000 ppm (Table 4). At 1000ppm isolate K3, K4 and K5 displayed the small zone of inhibition. K6 showed inhibition zone at 5000 ppm itself (Figure. 3).

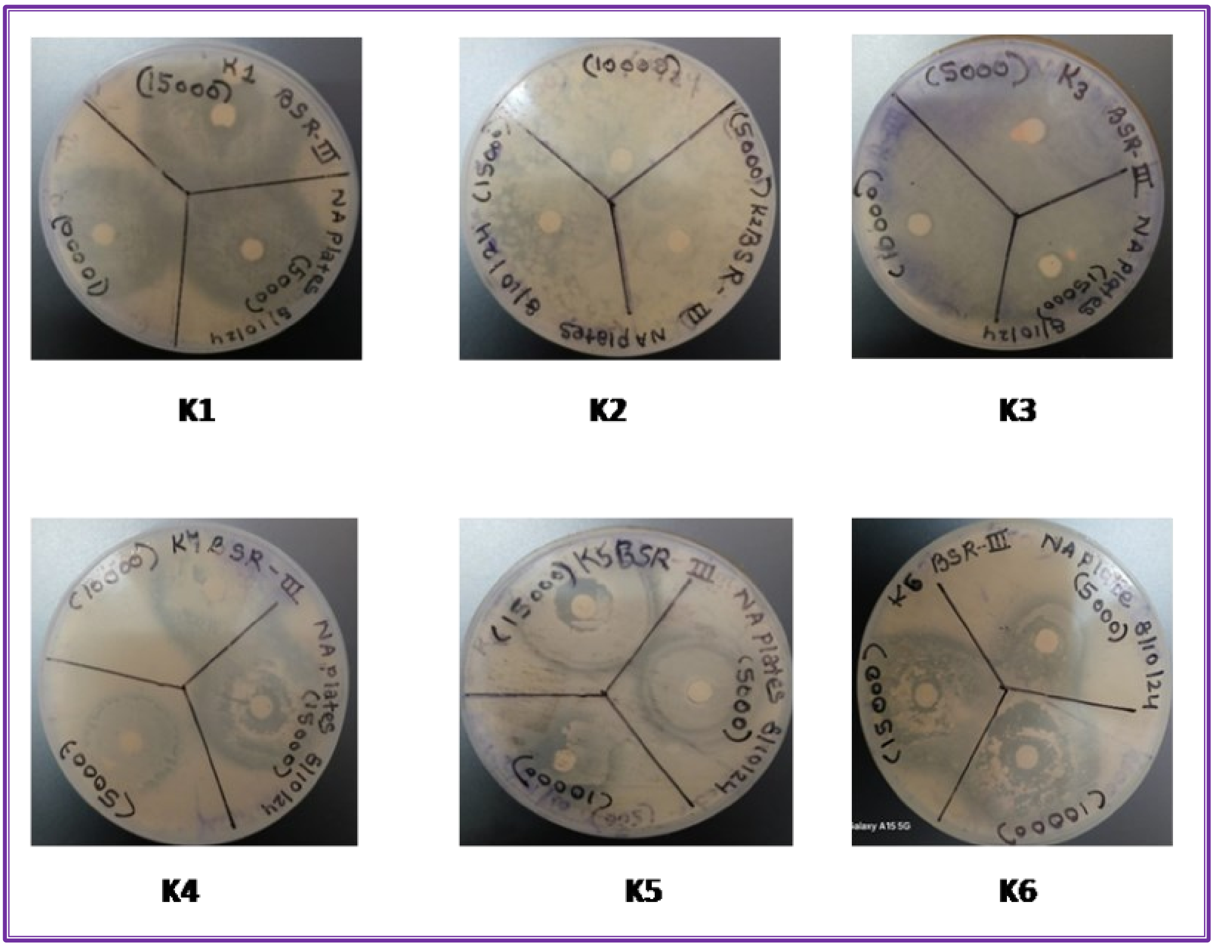

Figure 3. *Zone of inhibition of different isolates (K1-K6) at different chromium concentrations*.

Table 4. Zone of inhibition of all six chromium resistant isolates at different concentrations

Isolates	5000ppm	10000ppm	15000ppm
K1	+	+	-
K2	+	+	-
K3	+	-	-
K4	+	-	-
K5	+	-	-
K6	-	-	-

Degradation of chromium

After 48 hours of incubation, the control sample *Lactobacillus spp.* procured from Dept. of Microbiology, Patna Women's College, Patna, Bihar (India), showed no chromium reduction. The observed chromium reduction by different bacterial isolates is as follows: K1 reduced chromium from 0.8 mg/100 mL to 0.6 mg/100 mL, K2 reduced it from 0.8 mg/100 mL to 0.75 mg/100 mL, K3 reduced it from 0.8 mg/100 mL to 0.7 mg/100 mL, K4 reduced it from 0.8 mg/100 mL to 0.3 mg/100 mL, K5 reduced it from 0.8 mg/100 mL to 0.25 mg/100 mL, and K6 reduced it from 0.8 mg/100 mL to 0.2 mg/100 mL (Figure.4).

The scatter plot (Figure 4) shows a strong negative linear relationship between final concentration and reduction value. A total of six data points were analyzed (Number of XY pairs = 6). Pearson's correlation coefficient was $r = -1.000$, indicating a perfect negative correlation. The 95% confidence interval ranged from +1.000 to −1.000. The correlation was statistically significant ($P < 0.0001$, two-tailed; ***), with an R^2 value of 1.000, confirming complete linear dependence between the variables.

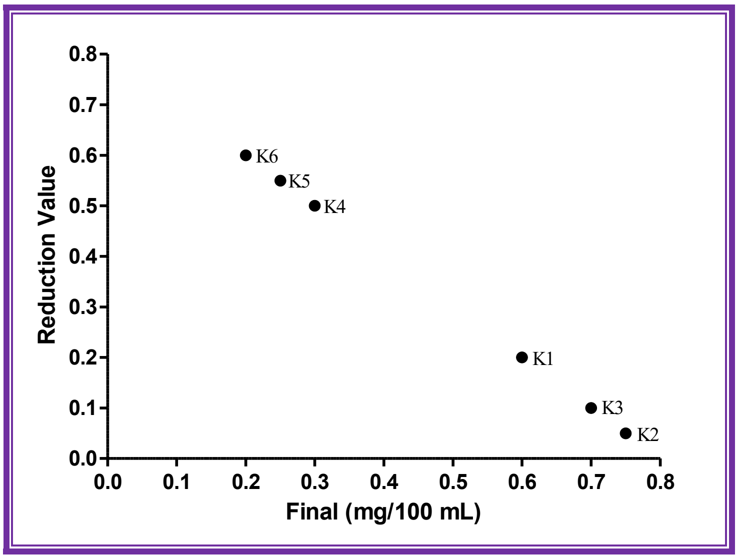

Figure 4. *Chromium degradation by different isolates after 48 hours of incubation in chromium containing media. The graph shows correlation between final concentration (mg/100 mL) and reduction value in samples K1–K6.*

Discussion

Microbes have evolved several mechanisms to handle a variety of harmful metals for their better survival on this Earth accumulated with such contaminants. Presence of heavy metal can influence the diversity and also change the community structure and function (Shakoori *et al.,* 2010). In this study, the study found that there was presence of chromium degrading bacteria on chromium contaminated water through the process known as bioremediation. This process utilizes biological organisms like microorganisms to detoxify or immobilize chromium in contaminated samples (Congeevaram *et al.,* 2007; Ilias *et al.,* 2011; Garg *et al*., 2025). Different strains of bacteria present in the industrial areas have default capability of degrading heavy metals. Hence, the present study deals with the isolation of chromium resistant bacteria from industrial waste for bioremediation purposes. In this study, two different water samples were procured from industrial sites areas in Patna. On the basis of colony characteristics, six different bacterial isolates were selected and named as K1, K2, K3, K4, K5, K6. All the isolates were found to reduce chromium, but it is significant in the case of isolate K5 which reduced chromium from 0.8 mg/100 mL to 0.25 mg/100 mL, and K6 which reduced it from 0.8 mg/100 mL to 0.2 mg/100 mL. The present study shows that isolates K5 and K6 resulted in upto 75% reduction in hexavalent chromium. Isolation of these isolates from Ganga, shows that the Ganges water is polluted with heavy metals like chromium (Banerjee *et al*., 2016; Paul, 2017). The presence of heavy metal in the Ganges water has compelled the microbial community in water to be resistant to these heavy metals. Khanam et al. (2024) reported bacterial isolates capable of achieving complete (100%) chromium reduction. However, Khanam *et al*. (2024) reported the isolates from tannery waste whereas this study first time reports isolation of bacteria from Krishna Ghat and industrial area of Patna. Some research reveals that the isolated bacterium from tannery effluent can tolerate upto 10 mg/100ml of chromium (Sanjay *et al.,* 2020). A study by Plestenjak *et al*., (2022) shows that bacteria belonging to the genera *Pseudomonas* and *Mammaliicoccus* isolated from tannery effluent were enriched with increasing concentration of chromate, ultimately enhanced their ability to grow and biodegrade chromium. Nowadays the process of biosorption by microbial biomass is mainly used to remediate hexavalent chromium (Cr(VI)). Zhu *et al*., (2021) in his study, mentioned that a Gram positive bacteria *Lysinibacillus* sp., a common inhabitant of soil, when immobilized in magnetite was found to remove Cr(VI) very efficiently. One of the similar studies by Upadhyay *et al*., (2017) showed that a bacterium isolated from contaminated coal mines soil was able to reduce hexavalent chromium by 75%. 16SrRNA typing revealed its maximum similarity to *Bacillus* sp. The enzyme involved for this efficient chromium removal is Chromium reductase which can be found both extracellulary as well as intracellulary (Ramli *et al*., 2023; Kalsoom *et al*., 2025; Singh *et al*., 2025). These bacteria, which thrive in heavy-metal-rich environments, offer potential applications in detecting and mitigating heavy metal pollution. The study reports the first isolation and characterization of indigenous chromium-resistant bacteria from Krishna Ghat and the Patliputra Industrial Area, Patna, demonstrating their high tolerance and efficient Cr(VI) reduction capability.

Limitations

The findings highlight the potential of these native strains for developing site-specific, eco-friendly bioremediation strategies for chromium-contaminated environments. These results contribute valuable insights into chromate bioremediation strategies. While this study demonstrates promising Cr(VI) reduction by indigenous isolates, lab-scale tests limit scalability to complex field conditions with variable pH, metals, and consortia.

Future Scope of Research

Further studies need to be conducted to identify bacterial strains and to enhance its biodegradation capabilities

Conclusion

Heavy metal pollution of soil and water bodies is a worldwide concern and needs novel approaches for bioremediation. The present study demonstrates that bacteria isolated from chromium polluted water bodies' exhibit chromium degrading properties and can be a suitable candidate for bioremediation. However, this is a preliminary study, and more experiments needs to be done to determine the potency of the isolates for bioremediation at larger scale. These chromium resistant isolates can be employed for bioremediation of industrial waste and for purification of Ganges water.

Conflict of interest

The authors declare that they have no conflicts of interest among the authors.

Acknowledgement

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