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Isolation and Screening of Heavy Metal-Resistant Bacteria from Hasdeo River Sediments

Sadhana Gupta[1], Bhagyashree Deshpande[2*] and Bhawana Pandey[3]

[1,2*]School of Sciences, MATS University, Raipur, Chhattisgarh
[3]Dept. of Biotechnology and Microbiology, Bhilai Mahila Mahavidyalaya, Bhilai, Chhattisgarh

***Corresponding Author**: Bhagyashree Deshpande
*Email Id - bhagyashree.deshpande851@gmail.com

Abstract
The increasing contamination of aquatic ecosystems with heavy metals poses significant environmental and health risks. Microorganisms, particularly bacteria, possess the remarkable ability to resist and detoxify heavy metals, making them potential candidates for bioremediation strategies. This study aimed to isolate and screen heavy metal-resistant bacteria from the sediments of the Hasdeo River, a site impacted by industrial activities and pollution. Sediment samples were collected from various locations along the river, and bacterial isolates were cultured and screened for resistance to a range of heavy metals. The isolates were characterized based on their growth patterns in the presence of different concentrations of heavy metals, with their resistance profiles assessed through optical density measurements. The study successfully identified bacterial strains exhibiting significant resistance to heavy metals, highlighting their potential for use in bioremediation applications. The findings provide valuable insights into the microbial diversity present in river sediments and underscore the role of bacteria as nature's defenders against environmental pollution.

Keywords: Bioremediation, bacterial isolates, resistance profiles, environmental pollution.

1.0 Introduction

The rapid industrialization and urbanization across the globe have significantly impacted the quality of environmental resources, especially water and soil, leading to increased contamination with heavy metals (Sarma et al., 2019; Gadd, 2010;Dewangan et al., 2023). Heavy metal pollution, originating from various anthropogenic activities such as mining, industrial discharges, agricultural runoff, and wastewater disposal, poses a severe threat to ecosystems and human health. Among the most toxic heavy metals are lead (Pb), mercury (Hg), cadmium (Cd), chromium (Cr), and arsenic (As), which can accumulate in the environment and in living organisms, resulting in long-term ecological damage, bioaccumulation, and adverse health effects. These metals are often persistent in the environment due to their low biodegradability and mobility, making them difficult to remove through conventional treatment methods (Liu et al., 2016).

In this context, microorganisms, particularly bacteria, have emerged as a promising solution for mitigating heavy metal contamination. Certain bacteria have developed the ability to resist, tolerate, and even detoxify heavy metals through various biochemical mechanisms such as biosorption, bioaccumulation, and biotransformation. These bacteria, often referred to as *heavy metal-resistant bacteria* (HM-RB), play a crucial role in maintaining ecosystem balance by reducing the toxicity of heavy metals in contaminated environments. Furthermore, these microorganisms possess unique potential for bioremediation—an eco-friendly and cost-effective technique for cleaning up polluted environments (Sarma et al., 2018; Rauf et al., 2017; Rajwade and Deshpande, 2023).
The Hasdeo River, located in the heart of Chhattisgarh, India, is an important water resource for the local population and supports a variety of aquatic life. However, the river has been exposed to increasing levels of heavy metal pollution due to industrial activities, mining operations, and agricultural practices in its surrounding areas. Consequently, the river sediments, which serve as a repository for pollutants, are highly contaminated, raising concerns about the long-term ecological health of the river and the safety of its water resources (Chauhan et al., 2020; Rajwade and Deshpande, 2024).
This study aims to isolate and screen *heavy metal-resistant bacteria* from the soil sediments of the Hasdeo River, particularly from regions with high contamination levels. By identifying bacteria with resistance to multiple heavy metals, this research seeks to contribute to a better understanding of the natural microbial communities that thrive in polluted environments. Furthermore, it aims to explore the potential of these bacteria for use in bioremediation applications to mitigate the harmful effects of heavy metal pollution in the river ecosystem (Dewangan et al., 2024).
By identifying and understanding the properties of *heavy metal-resistant bacteria* in the Hasdeo River sediments.

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2.0 Material and Method

2.1 Selection of sampling sites

Samples were collected from (SS-1) Swarmangla and (SS-2) Deori. Collected samples was brought in laboratory and was processed under aseptic condition and stored in refrigerator at 4ºC for the further investigation (Figure 1).

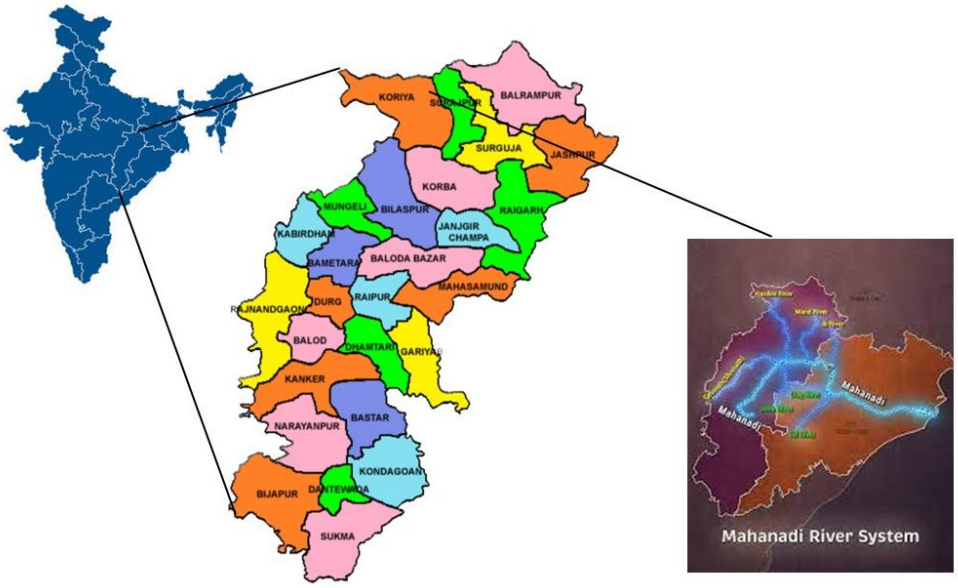

Figure 1: Sample collection site

2.2 Isolation and Identification of Metallo Tolerant Bacteria

The isolation of metal-tolerant bacteria involves preparing Luria Bertani (LB) agar medium supplemented with heavy metal salts Fe, $Pb(NO_3)_2$, Mn at concentrations of 50–200 mg/L. Soil samples are serially diluted, and 0.1 mL from each dilution is spread onto metal-supplemented LB agar plates using the pour plate technique, followed by incubation at 30–37°C for 24–48 hours to select distinct metal-tolerant colonies. Identification of these isolates is carried out through colony morphology analysis, Gram staining, and biochemical tests such as catalase, oxidase, IMViC, carbohydrate fermentation, and hydrolytic enzyme activity (Bergey's Manual of Systematic Bacteriology, 2005). Heavy metal tolerance is assessed by growing isolates in LB broth with increasing metal concentrations, measuring optical density (OD_{600} nm) to determine the minimum inhibitory concentration (MIC) (Kavamura & Esposito, 2010). Additionally, the effect of physiological parameters, including pH, temperature, salt concentration, and carbon and nitrogen sources, is studied by measuring bacterial growth under varying conditions using a spectrophotometer, ensuring optimal conditions for their survival and resistance (Roane & Pepper, 2000).

2.3 Optimization by different Parameter

Metal-tolerant bacteria were isolated by plating 0.1 mL of serially diluted soil samples onto Luria Bertani (LB) agar supplemented with Fe, $Pb(NO_3)_2$, and Mn salts (50–200 mg/L), followed by incubation at 30–37°C for 24–48 hours. Identification of isolates was performed using colony morphology, Gram staining, and biochemical tests (catalase, oxidase, IMViC, carbohydrate fermentation, and hydrolytic enzymes) (Bergey's Manual of Systematic Bacteriology, 2005). Metal tolerance was determined by growing isolates in LB broth with increasing metal concentrations, measuring optical density (OD_{600} nm) to assess the minimum inhibitory concentration (MIC) (Kavamura & Esposito, 2010). The effect of physiological parameters (pH, temperature, salt concentration, and carbon/nitrogen sources) on growth was analyzed using a spectrophotometer (Roane & Pepper, 2000).

2.4 Determination of Minimum Inhibitory Concentration (MIC) of Heavy Metal-Resistant Bacteria

The minimum inhibitory concentration (MIC) of heavy metal-resistant bacteria was determined by preparing filter-sterilized stock solutions of $Pb(NO_3)_2$, $MnSO_4$, and $(NH_4)_2Fe(SO_4)_2$, and adding them to LB broth at concentrations ranging from 10 to 500 mg/L. Bacterial cultures were inoculated and incubated at 30–37°C for 24–48 hours with shaking. Growth was measured by OD_{600} nm, and the MIC was recorded as the lowest concentration that completely inhibited growth (Gadd, 2010). Growth patterns under metal stress were analyzed by measuring OD_{600} nm at regular intervals, identifying different phases of growth (Schmidt et al., 2005). LB broth was prepared with peptone, yeast extract, and NaCl (pH 7.2 ± 0.2) and autoclaved at 121°C for 15 minutes (Malik, 2004). Stock solutions of metals were

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prepared at 1000 mg/L, filter-sterilized, and added to LB broth to assess bacterial tolerance (Roane & Pepper, 2000). Growth curves were plotted to determine the MIC (Nies, 1999).

3.0 Result and Discussion

The presence of bacteria in environments contaminated with heavy metals indicates their potential to tolerate, adapt to, or even metabolize these metals, which has significant implications for bioremediation and microbial ecology. Table 1 presents the bacterial counts observed in industrial samples (S-1 and S-2) under the influence of three heavy metals: iron (Fe), lead (Pb), and manganese (Mn). The highest bacterial count associated with iron was observed in S-1 . Since iron is an essential micronutrient for bacterial metabolism, its availability influences microbial growth (Andrews et al., 2003). However, excessive iron concentrations can induce oxidative stress, thereby limiting bacterial survival (Touati, 2000). The higher bacterial presence in S-1 suggests that this site may 4113avoura iron-tolerant or iron-utilizing species, possibly due to prolonged exposure leading to microbial adaptation (Braud et al., 2010).

The bacterial count for lead was highest in S-1 (2 bacteria), followed by S-2 (1 bacteria). Lead is a well-known toxic heavy metal that inhibits bacterial enzymatic functions and disrupts cellular homeostasis (Bruins et al., 2000). Studies have shown that lead-resistant bacteria, including strains from genera like *Pseudomonas* and *Bacillus*, can adapt to such environments and contribute to bioremediation efforts by bioaccumulating or transforming lead into less toxic forms (Kumar et al., 2016).

The highest bacterial count for manganese was recorded in S-2 (4 bacteria). Manganese is a crucial cofactor for bacterial enzymes and is generally less toxic than other heavy metals (Archibald & Duong, 1986). However, excessive manganese exposure can still be inhibitory, affecting microbial growth depending on environmental factors such as pH, metal speciation, and competing microbial populations (Kotrba et al., 2011). The relatively higher bacterial presence in S-2 suggests that the site provides 4113avourable conditions for manganese-tolerant bacterial species, which could be involved in manganese cycling and biotransformation (Su et al., 2015).

The variation in bacterial counts across samples highlights the complex interactions between microbial communities and heavy metal contamination. S-1 exhibited the highest bacterial count for iron, with moderate levels for lead and manganese, suggesting the presence of iron-tolerant bacteria. S-2 had the highest bacterial presence for manganese, likely due to site-specific environmental factors 4113avourabl manganese-resistant species.

These findings support previous research indicating that bacteria inhabiting heavy metal-contaminated environments develop resistance through multiple adaptive mechanisms, including biofilm formation, enzymatic detoxification, and metal efflux systems (Silver & Phung, 2005). The observed variations in bacterial counts suggest that microbial communities can serve as bioindicators of heavy metal pollution, with potential applications in bioremediation strategies aimed at mitigating industrial contamination (Giller et al., 2009). Further studies, including molecular and biochemical analyses, would be beneficial to identify the specific bacterial species involved and their potential for biotechnological applications in heavy metal detoxification.

Table 1: Number of bacteria in presence of Heavy Metals

S.N.	Samples	Types of Bacteria		
		Iron	Lead	Manganese
1.	S-1	5	2	2
2.	S-2	4	1	4

Based on frequency, three bacterial strains were selected from industrial samples contaminated with heavy metals. One iron-resistant bacteria were isolated from Sample 1 (S-1), one lead-resistant bacteria were identified in Sample 1 and one manganese-resistant bacteria were found in S-2. Table 2 provides detailed information on the screening of these bacterial strains based on their frequency of occurrence and their association with specific heavy metals.

3.2 Screening of Bacteria on the basis of Frequency

one bacterial strain, B-1 were isolated from S-1, indicating their adaptation to high iron concentrations. Iron is an essential micronutrient for bacterial growth and metabolism, playing a key role in electron transport and enzymatic reactions (Andrews et al., 2003). However, excessive iron can lead to oxidative stress due to the generation of reactive oxygen species (Touati, 2000). The ability of B-1 to survive in high-iron environments suggests that they may possess iron detoxification mechanisms such as siderophore production, iron efflux systems, or enzymatic pathways to neutralize oxidative damage (Braud et al., 2010). The presence of these bacteria in S-1 highlights their ecological role in iron cycling and their potential application in bioremediation efforts to mitigate iron contamination.

Bacterial strains B-2 demonstrating their resistance to lead contamination. Lead is a highly toxic heavy metal that disrupts bacterial cellular processes by interfering with enzyme activity, protein structure, and membrane integrity (Bruins et al., 2000). The presence of B-2 suggests adaptation to moderate lead concentrations, potentially through mechanisms such as metal efflux pumps, biosorption, or enzymatic detoxification (Nies, 1999). Previous studies have

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reported that lead-resistant bacteria, including species from genera like *Pseudomonas*, *Bacillus*, and *Acinetobacter*, can bioaccumulate lead and contribute to bioremediation strategies (Kumar et al., 2016).
One manganese-resistant bacterial strain, B-3 was identified. Manganese is an essential trace element required by bacteria for enzymatic functions, oxidative stress protection, and cellular metabolism (Archibald & Duong, 1986). However, excessive manganese levels can be toxic, affecting bacterial growth and metabolic pathways (Su et al., 2015). The presence of B-3 suggests that this site provides 4114favourable conditions for manganese-tolerant bacteria, which may play a role in manganese cycling and mineralization (Kotrba et al., 2011).

Table 2: Screening of Bacteria on the basis of Frequency

Heavy Metals	Bacteria	Isolated sites
Iron	B-1	(S-1)
Lead	B-2	(S-1)
Manganese	B-3	(S-2)

3.3 Biochemical analysis of Isolated Selected Bacteria
Table 3 presents a detailed biochemical analysis of three bacterial strains (B-1, B-2 and B-3) isolated from industrial samples contaminated with heavy metals. The biochemical tests provide insights into their metabolic and enzymatic activities, which are crucial for understanding their functional properties, environmental adaptability, and potential roles in heavy metal tolerance or bioremediation applications. The Indole Production Test revealed that B-1 and B-3 produced indole by breaking down tryptophan, indicating their ability to metabolize amino acids and survive in nutrient-limited environments (Kumar et al., 2011). The Citrate Utilization Test showed positive results in B-1 and B-2, demonstrating their ability to use citrate as a sole carbon source, an adaptation often linked to survival in carbon-limited conditions (Ghosh et al., 2003). The Catalase Test detected catalase activity in B-1 and B-3, highlighting their potential to counter oxidative stress caused by reactive oxygen species (ROS), which is particularly relevant in metal-contaminated environments (Imlay, 2013). In the Amylase Production Test, positive results in B-1 suggest that these strains can hydrolyze starch, making them useful in industrial applications such as bioremediation and biotechnology (Gupta et al., 2003). The Cellulase Production Test showed that all strains were capable of degrading cellulose, which is significant for organic matter decomposition and nutrient cycling (Singh et al., 20). The Gelatin Hydrolysis Test was positive for B-1 , indicating proteolytic activity, which is crucial for nutrient acquisition in protein-rich environments (Gupta & Ramnani, 2006).The Casein Hydrolysis Test demonstrated that all strains could hydrolyze casein, a property often linked to extracellular enzyme production in soil and water bacteria (Sharma et al., 2018). The Urease Test was positive in B-1, indicating their role in nitrogen metabolism, which is important for microbial adaptation in polluted environments. The Hydrogen Sulfide Production Test was positive in B-1 and B-3 suggesting sulfur metabolism, an essential adaptation for survival in metal-contaminated areas (Rathore et al., 2016).
The Carbohydrate Fermentation Test revealed metabolic differences among the strains. All strains fermented mannitol, indicating its widespread utilization as an energy source. Dextrose fermentation was observed in B-1 suggesting their ability to use simple sugars, while sucrose was not fermented by any strain, reflecting selective metabolic preferences (Madigan et al., 2014). The Litmus Test showed variations in acid and alkaline pH production, with B-1 producing acidic byproducts and all strains showing alkaline metabolism, indicating their adaptability to different pH conditions.
The Curd Formation Test showed that B-2 and B-3 formed acid curd, while only B-3 produced rennet curd, suggesting its potential for dairy waste degradation. Gas production was observed in B-1 and B-2 indicating fermentative metabolism, which is advantageous for survival in anaerobic or microaerophilic environments (Green and Sambrook, 2019).).

Table 3: Biochemical analysis of Isolated Selected Bacteria

S.No.	Biochemical Test	B-1	B-2	B-3
1.	Indole production test	+ve	-ve	+ve
2.	Citrate utilization test	+ve	+ve	-ve
3.	Catalase test	+ve	-ve	+ve
4.	Amylase production test	+ve	-ve	-ve
5.	Cellulase production test	+ve	+ve	+ve
6.	Hydrolysis of gelatine test	+ve	-ve	-ve
7.	Casein hydrolysis test	+ve	+ve	+ve
8.	Urease test	+ve	-ve	-ve
9.	Hydrogen sulfide test	+ve	-ve	+ve
10.	Carbohydrate fermentation test			
a.	Dextrose	+ve	-ve	-ve

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b.	Mannitol	+ve	+ve	+ve
c.	Sucrose	-ve	-ve	-ve
11.	Litmus test			
a.	Acid pH	+ve	-ve	-ve
b.	Alkaline pH	+ve	+ve	+ve
c.	Reduction	-ve	-ve	-ve
12.	Acid curd	-ve	+ve	+ve
13.	Rennet curd	-ve	-ve	+ve
14.	Gas production	+ve	+ve	-ve

3.4 Antibiotics Test of Isolated Bacteria

Table 4 presents the antibiotic sensitivity test results for three bacterial strains using various antibiotics. The zone of inhibition, measured in centimeters, reflects the degree of bacterial sensitivity or resistance to each antibiotic. A larger inhibition zone indicates greater susceptibility, while a smaller or absent inhibition zone signifies resistance. Bacitracin, an antibiotic primarily effective against Gram-positive bacteria, showed limited effectiveness, with only B-1 exhibiting a minimal inhibition zone (0.16 ± 0.04 cm), indicating partial sensitivity. The complete resistance of B-2 and B-3 suggests that these strains possess mechanisms to inactivate bacitracin or prevent its uptake (Sahl & Bierbaum, 1998). Ampicillin, a broad-spectrum antibiotic, was more effective against B-3, which exhibited the highest sensitivity (2.11 ± 0.008 cm). B-1, B-2, B-3 demonstrated moderate sensitivity (0.7 ± 0.004 cm), suggesting partial resistance mechanisms in these strains. Resistance to ampicillin is often associated with β-lactamase enzyme production, which hydrolyzes the β-lactam ring, rendering the antibiotic ineffective (Bush & Bradford, 2016). Amoxicillin, another broad-spectrum β-lactam antibiotic, showed moderate sensitivity in B-1, B-2 and B-3 (1.3 ± 0.004 cm to 1.33 ± 0.04 cm). Resistance to amoxicillin is often due to efflux pumps or altered penicillin-binding proteins (PBPs) that reduce antibiotic binding (Livermore, 2000). B-2 and B-3 exhibited intermediate sensitivity (0.5 ± 0.004 cm and 0.5 ± 0.009 cm, respectively) against Penicillin. Resistance to penicillin often arises from β-lactamase activity or modifications in PBPs, reducing the antibiotic's ability to inhibit bacterial cell wall synthesis (Ghuysen, 1991). Rifampicillin, an antibiotic used against both Gram-positive and some Gram-negative bacteria, exhibited high sensitivity in B-3 (2.11 ± 0.008 cm). Resistance to rifampicillin is often linked to mutations in the rpoB gene encoding the RNA polymerase β-subunit, leading to reduced drug binding (Campbell et al., 2001) (Figure 2).

Table 3 Antibiotics test of selected bacteria

Antibiotics	**Zone of inhibition (in cm)**		
	B-1	**B-2**	**B-3**
Bacitracin	0.16±0.04	0±0	0±0
Ampicillin	0.70±0.004	0.7 1±0.008	2.1±0
Amoxicillin	1.33±0.04	1.11±0.08	1.3±0.004
Penicillin	0.31±0.01	0.5±0.004	0.1±0.004
Rifampicillin	0.3±0.004	2.11±0.008	0.1±0.004

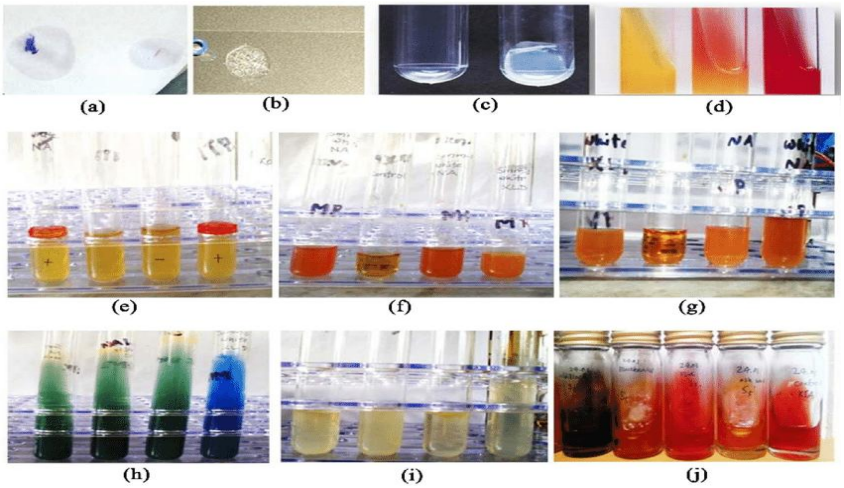

Figure 2: Biochemical Tests

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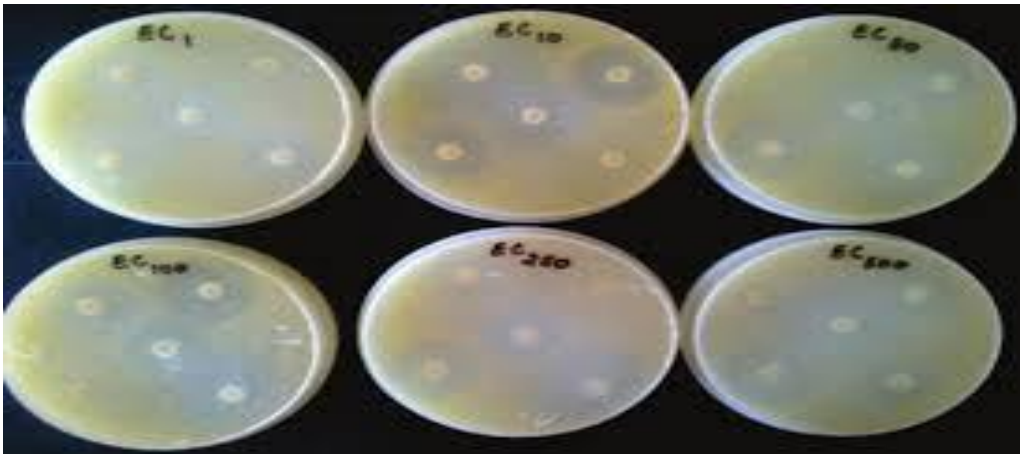

Figure 3: Antibiotic Sensitivity Test

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