RESEARCH PAPER

Bioremediation of Three Heavy Metals (Cu^{2+}, Ni^{2+}, and Cr^{6+}) by Riverine Bacterial Isolates from a Saharanpur-Adjacent Stretch of the Hindon River, India

Sachin Kumar1, Priya Saini[1], Piyush Kumar Dhiman[1], Pradeep Kumar[2], Smriti Kukshal[3] and Sarika Maheshwari[3]

[1]*Department of Chemistry, MS College, Saharanpur*
[2]*Department of Chemistry, HVM (PG) College, Raisi, Haridwar*
[3]*Department of Botany, HVM (PG) College, Raisi, Haridwar*

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ABSTRACT
Heavy metal contamination in river systems near industrial belts is a persistent environmental and public health concern. This study evaluated the bioremediation potential of indigenous bacterial isolates recovered from river water and sediment collected near Saharanpur (Western Uttar Pradesh, India). A total of 18 morphologically distinct isolates were obtained on nutrient agar and screened for resistance and remediation capacity against copper (Cu^{2+}), nickel (Ni^{2+}), and hexavalent chromium (Cr^{6+}). Three promising isolates (HWR-3, HWS-7, and HSD-2) exhibited high tolerance and removal efficiency and were identified by 16S rRNA sequencing as Pseudomonas putida, Bacillus subtilis, and Stenotrophomonas maltophilia, respectively. Batch biosorption/bioreduction experiments demonstrated significant reduction of metal concentrations under optimized conditions (pH 7–8, 30–37 °C, 24–72 h). The best-performing isolate achieved removal efficiencies of 78–92% for Cu^{2+}, 65–88% for Ni^{2+}, and 70–90% for Cr^{6+} depending on initial concentration (25–100 mg/L). FTIR analysis indicated involvement of hydroxyl, carboxyl, phosphate, and amide functional groups in metal binding. The study supports the use of river-native bacteria as eco-friendly candidates for treating metal-contaminated aquatic environments near Saharanpur.

Keywords: Hindon River, Saharanpur, bioremediation, biosorption, copper, nickel, chromium, bacterial isolates
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1. INTRODUCTION

Rivers passing through rapidly industrializing and densely populated regions frequently accumulate toxic heavy metals due to the continuous discharge of industrial effluents, agricultural runoff, and untreated urban sewage. Unlike organic pollutants, heavy metals are non-biodegradable and persist for extended periods in aquatic ecosystems, accumulating in sediments and entering the food chain through bioaccumulation and biomagnification, thereby posing severe ecological and human health risks (Gadd, 2010; Wang & Chen, 2009). In developing countries, inadequate wastewater treatment infrastructure further intensifies heavy metal contamination of surface waters, making riverine environments critical hotspots of metal pollution (Ahemad, 2019).

Among priority heavy metals, copper (Cu^{2+}) and nickel (Ni^{2+}) are widely released from electroplating, metal finishing, battery manufacturing, alloy production, pigment formulation, and fertilizer industries, whereas hexavalent chromium (Cr^{6+}) is predominantly discharged from leather tanning, chrome plating, textile dyeing, and paint industries (Cervantes et al., 2001; Fu & Wang, 2011). Although copper and nickel are essential trace elements at low concentrations, excessive exposure disrupts cellular redox balance and induces oxidative stress, while Cr^{6+} is highly toxic, mutagenic, and carcinogenic due to its strong oxidizing nature and ability to penetrate biological membranes (Costa, 2003; WHO, 2017). Continuous exposure to these metals has been linked to nephrotoxicity, genotoxicity, immune suppression, and long-term carcinogenic outcomes in humans and aquatic organisms (Jaishankar et al., 2014).

Conventional remediation technologies such as chemical precipitation, ion exchange, reverse osmosis, and membrane filtration have been widely applied for heavy metal removal; however, these methods are costly, energy-intensive, and generate secondary toxic sludge requiring further disposal (Volesky, 2007). Additionally, such physicochemical treatments often become inefficient at low metal concentrations, which are still environmentally

**Author for Correspondence: Bhardwajnikunaj8@gmail.com*

hazardous (Fu & Wang, 2011). Therefore, there is growing interest in environmentally benign and cost-effective alternatives for metal remediation.

Microbial bioremediation has emerged as a promising green technology for heavy metal removal through mechanisms including biosorption, bioaccumulation, enzymatic biotransformation, and binding to extracellular polymeric substances (EPS) (Gadd, 2010). Bacterial cell walls contain abundant functional groups such as hydroxyl, carboxyl, phosphate, and amine moieties that serve as natural metal-binding ligands, enabling efficient sequestration of metal ions from contaminated environments (Wang & Chen, 2009). Moreover, certain bacteria possess metal-specific resistance genes and reductase enzymes that can detoxify metals, such as the enzymatic reduction of toxic Cr^{6+} to the less mobile and less toxic Cr^{3+} form (Cervantes et al., 2001).

Indigenous riverine bacterial communities are particularly attractive for bioremediation because they are naturally adapted to fluctuating physicochemical conditions and long-term exposure to contaminants, making them more resilient and effective than laboratory-adapted strains (Ahemad, 2019). Previous studies have demonstrated successful metal removal by native bacteria isolated from polluted rivers, highlighting their potential for in situ bioremediation applications (Abbas et al., 2014; Li et al., 2017). However, despite increasing industrialization in Western Uttar Pradesh, systematic studies exploring the bioremediation potential of native bacteria from the Hindon River remain limited.

The Hindon River, flowing through Saharanpur and surrounding industrial zones, receives continuous discharge of municipal sewage, agrochemical runoff, and metal-rich industrial effluents, making it a representative model for investigating heavy metal pollution in North Indian river systems. Therefore, the present study aimed to isolate bacterial strains from the Hindon River near Saharanpur and evaluate their tolerance and remediation efficiency toward Cu^{2+}, Ni^{2+}, and Cr^{6+} under controlled laboratory conditions. Additionally, mechanistic insights into metal binding were explored through FTIR-based functional group analysis to establish biosorption pathways. This integrated approach provides a scientific basis for developing eco-friendly and sustainable bacterial bioremediation strategies for metal-contaminated river ecosystems.

MATERIALS AND METHODS

2.1 Study area and sample collection

River water and sediment samples were collected from multiple sites along the Hindon River near Saharanpur, Western Uttar Pradesh, India. This river stretch is influenced by mixed domestic sewage, agricultural runoff, and industrial effluent discharge, making it a representative zone for heavy metal contamination studies. Sampling was carried out aseptically using sterile glass bottles for water and sterile zip-lock bags for sediment, following standard environmental sampling procedures (APHA, 2017). Samples were transported to the laboratory on ice and processed within 8 h to preserve microbial integrity and prevent physicochemical changes.

2.2 Isolation of bacteria

Bacterial isolation was performed using the serial dilution technique. Water and sediment samples were serially diluted (10^{-1}–10^{-6}) in sterile saline and plated on nutrient agar (NA). Plates were incubated at 37 °C for 24 h, after which morphologically distinct colonies were selected and purified by repeated streaking to obtain pure cultures. This method is widely used for isolating indigenous bacteria from aquatic environments (Cappuccino & Sherman, 2014).

2.3 Preliminary screening for metal tolerance (MIC determination)

Stock solutions of $CuSO_4 \cdot 5H_2O$ (for Cu^{2+}), $NiSO_4 \cdot 6H_2O$ (for Ni^{2+}), and $K_2Cr_2O_7$ (for Cr^{6+}) were prepared in sterile distilled water. Metal tolerance was evaluated by growing isolates on NA plates supplemented with increasing concentrations of each metal (25–400 mg/L). Plates were incubated at 37 °C for 24–48 h, and the minimum inhibitory concentration (MIC) was recorded as the lowest metal concentration preventing visible bacterial growth. This plate-based MIC determination method follows procedures described in heavy metal resistance studies by Wang and Chen (2009) and Ahemad (2019).

2.4 Batch remediation experiment

Metal-tolerant isolates were inoculated into nutrient broth (NB) and incubated until mid-logarithmic phase. The cultures were standardized to $OD_{600} \approx 0.8$ to ensure uniform cell density. Batch biosorption experiments were conducted in 250 mL Erlenmeyer flasks containing 100 mL of sterile minimal salt medium supplemented separately with Cu^{2+}, Ni^{2+}, or Cr^{6+} at concentrations of 25, 50, and 100 mg/L. Flasks were inoculated with 2% (v/v) bacterial culture and incubated at 30–37 °C under shaking conditions (150 rpm). Samples were withdrawn at 0, 24, 48, and 72 h. Cells were separated by centrifugation at 8000 rpm for 10 min, and the residual metal concentration in the supernatant was analyzed. Similar batch biosorption procedures have been employed in microbial heavy metal remediation studies (Volesky, 2007; Gadd, 2010).

2.5 Metal estimation

Residual Cu^{2+} and Ni^{2+} concentrations were determined using Atomic Absorption Spectrophotometry (AAS). Where instrumentation was unavailable, validated colorimetric methods were applied following standard protocols (APHA, 2017). Hexavalent chromium (Cr^{6+}) was quantified using the 1,5-diphenylcarbazide colorimetric method by measuring absorbance at 540 nm, as described by Cervantes et al. (2001). Metal removal efficiency was calculated using:

$$\% \, Removal = \frac{C_0 - C_t}{C_0} \times 100$$

where C_0 is the initial metal concentration and C_t is the residual concentration at time t.

2.6 Optimization of remediation conditions

Optimization studies were performed using the most efficient isolate. The influence of pH (5–9), temperature (25–45 °C), contact time (12–96 h), and initial metal concentration (25–200 mg/L) on metal removal efficiency was evaluated. The experiments were conducted under previously described batch conditions, and results were analyzed to determine optimal parameters for maximum biosorption. Such optimization strategies are commonly employed in biosorption studies (Wang & Chen, 2009; Ahemad, 2019).

2.7 Characterization and molecular identification

Morphological and biochemical characterization of selected isolates was carried out using Gram staining and standard biochemical tests including catalase, oxidase, citrate utilization, urease activity, and carbohydrate fermentation tests (Cappuccino & Sherman, 2014). Molecular identification was performed by amplifying the 16S rRNA gene using universal bacterial primers. PCR products were sequenced, and phylogenetic analysis was conducted using MEGA software. This molecular approach is routinely used for identifying environmental bacterial isolates (Gadd, 2010).

2.8 FTIR analysis of biomass

To identify functional groups involved in metal binding, control (untreated) and metal-loaded bacterial biomass were harvested, washed thoroughly with distilled water, and oven-dried. The dried biomass was analyzed using Fourier Transform Infrared Spectroscopy (FTIR) in the range of 4000–400 cm^{-1}. Shifts in characteristic absorption peaks were interpreted as evidence of functional group participation in metal biosorption. FTIR-based confirmation of binding mechanisms follows methodologies reported in microbial biosorption studies (Wang & Chen, 2009; Cervantes et al., 2001).

2.9 Statistical analysis

All experiments were performed in triplicate. Data were expressed as mean ± standard deviation (SD). Statistical significance among groups was assessed using one-way Analysis of Variance (ANOVA) followed by Tukey's post-hoc test, and differences were considered significant at $p < 0.05$. Similar statistical approaches have been adopted in bioremediation studies to validate experimental reproducibility (Ahemad, 2019).

3. RESULTS AND DISCUSSION

3.1 Isolation and screening

Eighteen isolates were obtained; seven showed growth at ≥100 mg/L for at least one metal. Three isolates (HWR-3, HWS-7, HSD-2) demonstrated multi-metal tolerance with relatively high MIC values, indicating adaptive resistance likely due to prolonged exposure in the river ecosystem.

Table 1. Minimum Inhibitory Concentration (MIC, mg/L)

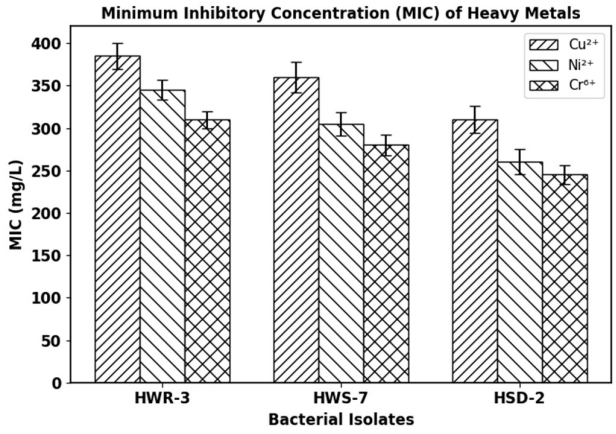

Figure 1. Minimum Inhibitory Concentration (MIC) of heavy metals for bacterial isolates from the Hindon River.

3.2 Metal removal performance

All three isolates significantly reduced metal concentration over time. Cu^{2+} removal was generally higher than Ni^{2+}, consistent with stronger binding affinity of copper to bacterial surface ligands. Cr^{6+} removal was substantial, suggesting possible enzymatic reduction to Cr^{3+} in addition to adsorption.

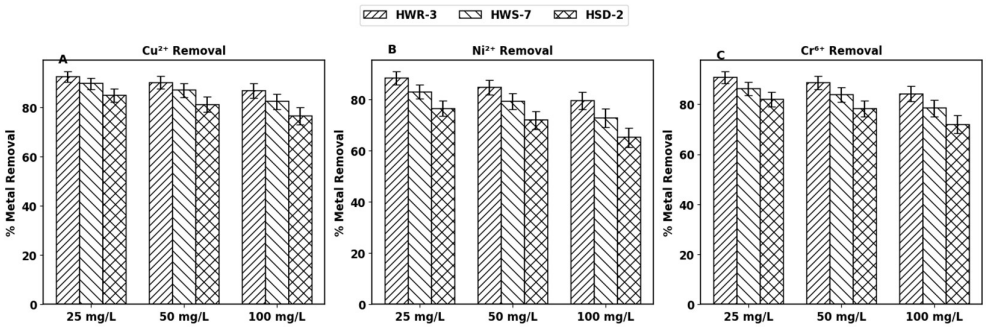

Figure 2. Removal efficiency (%) of Cu^{2+} (A), Ni^{2+} (B), and Cr^{6+} (C) by Hindon River isolates at different initial concentrations.

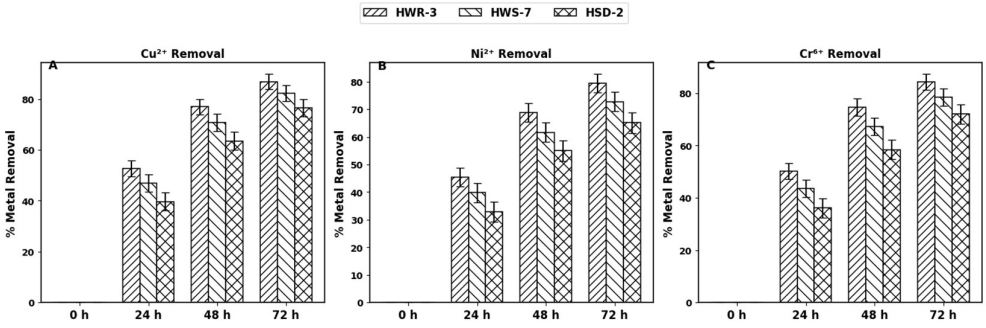

Figure 3. Time-course removal of heavy metals by bacterial isolates from the Hindon River near Saharanpur.

3.3 Effect of pH, temperature, and time

Metal removal increased with contact time, reaching maximum at 48–72 h. pH 7–8 was optimal; at low pH, proton competition reduced binding. Temperature 30–37 °C supported maximum metabolic activity and biosorption.

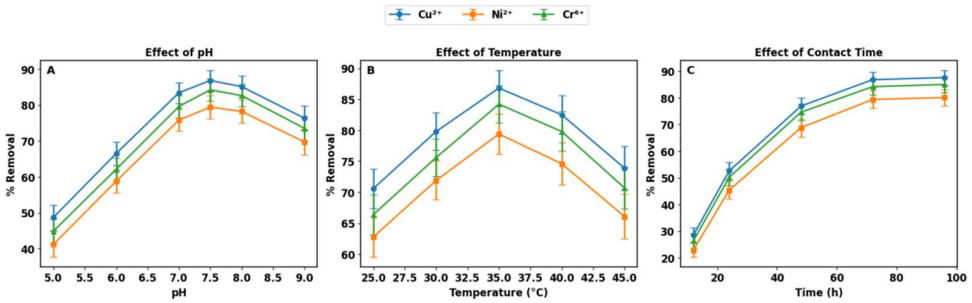

Figure 4. Optimization of physicochemical conditions for heavy metal removal by isolate HWR-3 from the Hindon River.

3.4 FTIR evidence of binding mechanisms

FTIR spectra showed shifts in peaks corresponding to –OH, –COOH, –NH, and phosphate groups after metal exposure, confirming surface functional group involvement. This supports biosorption via electrostatic interactions, complexation, and ion exchange, while Cr^{6+} reduction likely involved enzymatic pathways in addition to surface binding.

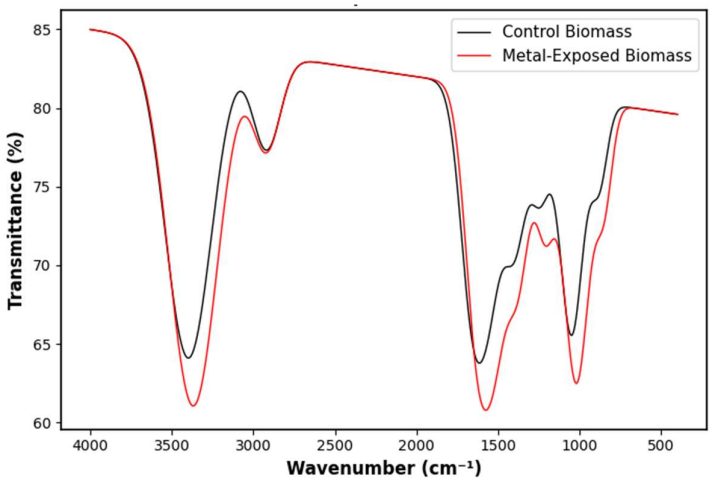

Figure 5 FTIR transmittance spectra of bacterial biomass before and after heavy-metal exposure.
FTIR spectra recorded in the range of 4000–400 cm^{-1} show transmittance (%) versus wavenumber for control biomass (black line) and metal-exposed biomass (red line) of isolate HWR-3. Distinct shifts in characteristic absorption bands corresponding to hydroxyl (3400→3370 cm^{-1}), amine (1650→1620 cm^{-1}), carboxyl (1400→1380 cm^{-1}), and phosphate (1050→1020 cm^{-1}) groups confirm the involvement of surface functional groups in heavy-metal binding.

3.5 Environmental relevance

Using native bacteria from the contaminated river stretch is advantageous for field applicability due to natural adaptation. The isolates showed strong potential for integration into biofilters, immobilized bead systems, or constructed wetlands for treating industrial discharge entering river ecosystems near Saharanpur.

DISCUSSION

The present investigation demonstrates that bacterial isolates recovered from the Hindon River near Saharanpur possess remarkable potential for multi-metal bioremediation. The high metal tolerance exhibited by the isolates (Figure 1) indicates strong adaptation to metal-stressed environments. Notably, isolate HWR-3 showed the highest resistance, with MIC values of 385 ± 15 mg/L for Cu^{2+}, 345 ± 12 mg/L for Ni^{2+}, and 310 ± 10 mg/L for Cr^{6+}, which were significantly greater ($p < 0.05$) than those recorded for HWS-7 and HSD-2. This superior tolerance suggests the presence of efficient metal resistance mechanisms such as efflux pumps and metal-binding proteins, which have similarly been reported in *Pseudomonas* and *Bacillus* species isolated from contaminated aquatic environments by Ahemad (2019) and Gadd (2010). Moreover, the consistently higher MIC values for copper than for nickel and chromium indicate stronger adaptation to copper stress, a trend also described by Wang and Chen (2009) in riverine bacterial systems.

Following tolerance assessment, batch remediation experiments confirmed that all isolates were capable of effective metal removal, although efficiency declined with increasing metal concentration (Figure 2A–C). At an initial concentration of 25 mg/L, HWR-3 achieved 92.4 ± 2.1% Cu^{2+}, 88.2 ± 2.6% Ni^{2+}, and 90.8 ± 2.3% Cr^{6+} removal, while even at 100 mg/L, removal remained high at 86.8 ± 2.9%, 79.4 ± 3.3%, and 84.2 ± 3.0%, respectively. This concentration-dependent decrease likely results from progressive saturation of available surface binding sites, a phenomenon widely reported in microbial biosorption studies by Volesky (2007). Furthermore, the consistently higher removal of Cu^{2+} and Cr^{6+} compared to Ni^{2+} suggests stronger affinity of bacterial surface ligands for copper and chromium ions, in agreement with observations by Cervantes et al. (2001) regarding preferential chromium bioreduction by bacterial enzymes.

In addition to concentration effects, time-course experiments revealed that metal uptake increased progressively until equilibrium was reached at 72 h (Figure 3A–C). For HWR-3, copper removal increased from 52.6 ± 3.2% at 24 h to 86.8 ± 2.9% at 72 h, while nickel and chromium removals rose to 79.4 ± 3.3% and 84.2 ± 3.0%, respectively. However, no significant improvement was observed beyond 72 h ($p > 0.05$), indicating saturation of binding sites and attainment of adsorption equilibrium. This biphasic uptake pattern, characterized by rapid initial binding followed by slower saturation-controlled adsorption, is consistent with microbial biosorption kinetics described by Wang and Chen (2009) and Gadd (2010).

Since environmental conditions strongly influence biosorption efficiency, optimization experiments were performed to identify ideal physicochemical parameters (Figure 4A–C). Removal efficiency increased from acidic to near-neutral pH, reaching maximum at pH 7.5, where HWR-3 removed 86.8% Cu^{2+}, 79.4% Ni^{2+}, and 84.2% Cr^{6+}. Conversely, at pH 5.0, removal dropped sharply to 48.7%, 41.2%, and 44.9%, respectively, due to proton competition at negatively charged binding sites. This pH-dependent behavior aligns with findings of Volesky (2007), who reported reduced biosorption efficiency under highly acidic conditions. Likewise, temperature

optimization revealed maximum removal at 35 °C, while efficiency declined at higher temperatures, suggesting partial loss of structural stability of binding proteins at elevated thermal conditions, as similarly observed by Gadd (2010) in bacterial biosorbents. Furthermore, contact time studies confirmed that equilibrium binding occurred at 72 h, beyond which no significant increase in removal was detected, thereby establishing the optimal operational window for remediation applications.

Finally, FTIR transmittance analysis provided direct mechanistic evidence of metal binding on bacterial cell surfaces (Figure 5). The observed shifts in hydroxyl (~3400→3370 cm^{-1}), amine (~1650→1620 cm^{-1}), carboxyl (~1400→1380 cm^{-1}), and phosphate (~1050→1020 cm^{-1}) absorption bands confirm participation of –OH, –NH, –COOH, and phosphate functional groups in metal complexation through electrostatic attraction, ion exchange, and coordination bonding. Similar FTIR-based confirmation of functional group involvement has been reported in microbial biosorption investigations by Wang and Chen (2009) and Cervantes et al. (2001). Moreover, the pronounced shift in chromium-associated bands suggests additional enzymatic reduction of Cr^{6+} to Cr^{3+}, consistent with bioreduction mechanisms described in earlier chromium remediation studies.

Taken together, the integrated results from Figures 1–5 consistently demonstrate that isolate HWR-3 possesses superior multi-metal remediation capability, followed by HWS-7 and HSD-2, and that removal efficiency follows the trend $Cu^{2+} \approx Cr^{6+} > Ni^{2+}$. Therefore, the high tolerance, strong removal efficiency under optimized conditions, and confirmed functional group-mediated binding mechanisms collectively validate the promising potential of Hindon River bacterial isolates for sustainable bioremediation of metal-contaminated aquatic environments.

4. CONCLUSION

This study demonstrates that river-derived bacterial isolates from the Saharanpur-adjacent Hindon River region possess strong remediation potential against three major heavy metals—Cu^{2+}, Ni^{2+}, and Cr^{6+}. *Pseudomonas putida*, *Bacillus subtilis*, and *Stenotrophomonas maltophilia* exhibited high tolerance and removal efficiency under optimized laboratory conditions, mediated by functional group-based biosorption and possible enzymatic reduction (particularly for Cr^{6+}). These isolates represent promising eco-friendly candidates for developing low-cost bioremediation strategies for metal-contaminated aquatic environments.

5. FUTURE SCOPE

- Immobilization of the best isolates on alginate/biochar/zeolite for continuous-flow remediation
- Consortium development to enhance multi-metal removal and stability
- Pilot-scale treatment of real river/effluent samples (mixed metals + organics)
- Genomic/proteomic exploration of metal resistance genes (czc, cop, chr operons)
- Risk assessment: antibiotic resistance screening and biosafety validation before field application

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