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Characterization of industrial effluent samples and evaluation of heavy metal removal by *Pseudomonas plecoglossicida* and *Serratia marcescens* isolated from extreme environmental conditions

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ABSTRACT

Industrial wastewater contains metals that require effective removal before discharge, making bacterial treatment a relevant approach for investigation. This study characterized effluents from rice mill and automobile industries in Tiruchirappalli and food processing and dyeing industries in Coimbatore, Tamil Nadu, India, and evaluated metal removal by two bacterial isolates. Physicochemical parameters were analysed using standard procedures, and chromium, copper and zinc concentrations were measured by atomic absorption spectrometry. The isolates, identified through partial 16S rRNA gene sequencing as *Pseudomonas plecoglossicida* KMJ01 (C1) and *Serratia marcescens* KMJ03 (C2), were applied separately to each effluent at 5% (v/v) and incubated for 24 h at 150 rpm. Effluent pH ranged from 6.3 to 10.6, total solids from 350 to 4,450 mg/L, and chemical oxygen demand from 840 to 1,760 mg/L. Zinc removal ranged from 94.3% to 97.5% with C1 and from 89.6% to 98.5% with C2. C1 achieved 98.9% chromium removal in food processing effluent, while C2 achieved 97.8% in automobile effluent. Both isolates removed 99.3% of copper from rice mill effluent. In dyeing effluent, copper removal differed markedly, reaching 96.8% with C1 and 25.9% with C2. These findings identify both isolates as candidates for further evaluation, with performance depending on the metal and wastewater matrix. Controlled, replicated studies are needed to establish removal mechanisms and practical treatment feasibility.

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INTRODUCTION

Water is essential to industrial operations, including processing, washing, cooling and the transport of materials. These activities generate wastewater whose composition varies with the raw materials, production processes and chemicals used. Metal contamination has been investigated in several industrial settings, including mining, pulp and paper production, printed circuit board manufacturing, petroleum refining, tanning and textile processing (Wei *et al.*, 2018; Sharma *et al.*, 2022; Wang *et al.*, 2021; Thorat and Sonwani, 2022; Nur-E-Alam *et al.*, 2020; Khan *et al.*, 2023). When inadequately treated, these discharges can introduce metals and other contaminants into receiving waters, creating a continuing need for effective wastewater characterization and treatment.

Metal-bearing wastewater presents a particular challenge because metals persist even when their chemical forms and environmental availability change. Industrial effluents may contain chromium, copper, zinc, nickel, cadmium, lead and other potentially harmful elements, with their occurrence and concentrations depending on the source (Shrestha *et al.*, 2021). Their environmental effects are influenced by concentration, speciation, exposure conditions and interactions with other wastewater constituents. Metals may also accumulate in environmental compartments beyond the original discharge location. Although Zhang *et al.* (2012) examined traffic-associated contamination rather than industrial wastewater, their findings illustrate the spatial variability of metal accumulation in soils. Such variability reinforces the importance of assessing contamination within its specific environmental setting.

Industrial wastewater is rarely a simple solution of individual contaminants. Dissolved salts, suspended particles, organic matter and metals occur together and can influence both environmental behaviour and treatment performance. Reviews of microbial metal remediation and algae-based wastewater treatment describe approaches for addressing aspects of this complexity (Volarić *et al.*, 2021; Jayaraman *et al.*, 2024). Other treatment studies target specific constituents, such as nitrate removal from textile wastewater using an aloe vera coagulant (Mustapha and Obayomi, 2025). These different treatment targets highlight the need to define which wastewater constituents are being measured and which outcomes a treatment is intended to achieve. Water-quality assessment also provides an important basis for understanding contamination within receiving environments (Das, 2025).

The term heavy metals is widely used in environmental research, although the elements grouped under this term differ in their biological functions and toxicological properties (Fulke *et al.*, 2020). Some metals participate in biological processes at low concentrations but become harmful when exposure exceeds the capacity of organisms to regulate them. Excess metal concentrations can interfere with cellular components and metabolic functions (Tripathi *et al.*, 2019). Chromium, copper and zinc are therefore relevant treatment targets, but their removal should be evaluated individually rather than assumed to follow a common pattern. Research on metal-binding materials and microalgal treatment further demonstrates that removal depends on the properties of the target element, the sorbent and the surrounding medium (Ahmed and Ahmaruzzaman, 2016; Sibi, 2019).

Effective wastewater management requires both appropriate treatment technologies and the institutional capacity to implement them. Broader water-management challenges have been discussed in relation to cooperation over shared resources and wastewater treatment practices (Nanni, 2016; Wang *et al.*, 2014). At the treatment level, biological systems offer several options for managing wastewater constituents. Investigations of integrated anaerobic and trickling-filter systems and reviews of agricultural wastewater treatment illustrate the range of available biological approaches (Tessema *et al.*, 2024; Abonyi *et al.*, 2024). Their suitability nevertheless depends on wastewater composition and the treatment objective; removal of organic matter does not necessarily establish effective removal of metals.

Bacteria have attracted interest as potential agents for metal remediation because their cells and associated extracellular materials can interact with metal ions. Reported processes include surface binding, intracellular accumulation and transformations that alter metal mobility or toxicity (Roy *et al.*, 2024). Extracellular polymeric substances may also contribute to metal retention through interactions with their constituent functional groups (Sonkar *et al.*, 2026). These processes redistribute or transform metals rather than degrade the elements themselves. Moreover, a decrease in metal concentration in a treated liquid does not, without additional measurements, identify the mechanism responsible.

Biological treatment has been investigated using both individual isolates and mixed microbial communities. Sghaier *et al.* (2019), for example, reviewed microbial consortia for textile wastewater treatment. Findings from such systems cannot be transferred directly to individual bacterial strains or from dye removal to metal removal. Evaluating isolates separately is therefore useful for identifying differences in their performance against particular metals and wastewater matrices. Environmental origin may provide a rationale for selecting candidate isolates, but their treatment potential must be established experimentally.

For industrial application, an important question is whether a bacterial isolate maintains its removal performance across wastewaters with different physicochemical characteristics. Rice mill, automobile, food processing and dyeing effluents provide contrasting matrices in which differences in pH, solids, organic load and dissolved constituents may influence measured metal removal. Comparing the same isolates across these effluents can help identify combinations that merit more detailed investigation, without assuming that performance in one wastewater will predict performance in another.

This study therefore characterized the physicochemical properties of industrial effluents collected from Tiruchirappalli and Coimbatore, Tamil Nadu, India, and evaluated chromium, copper and zinc removal by *Pseudomonas plecoglossicida* KMJ01 (C1) and *Serratia marcescens* KMJ03 (C2). Rice mill and automobile effluents were obtained from Tiruchirappalli, while food processing and dyeing effluents were obtained from Coimbatore. The isolates were characterized using biochemical tests and partial 16S rRNA gene sequence analysis, and their removal performance was assessed separately in each effluent. The objective was to compare metal removal across the four wastewater matrices and identify isolate–effluent combinations suitable for further controlled evaluation.

MATERIALS AND METHODS

Study area and effluent collection

Industrial effluents were collected from four industrial sources in Tamil Nadu, India. Rice mill and automobile industry effluents were obtained from Tiruchirappalli, while food processing and dyeing industry effluents were collected from industrial outfalls in Coimbatore. The geographical locations of the study areas are shown in Fig. 1.



Fig. 1. Study areas

Samples were collected in sterile screw-capped bottles, transported to the laboratory and stored at 4 °C until analysis. Each effluent was characterized for its physicochemical properties and chromium (Cr), copper (Cu) and zinc (Zn) concentrations before bacterial treatment.

Physicochemical characterization

The physicochemical characteristics of the effluents were determined following the procedures described

in *Standard Methods for the Examination of Water and Wastewater* (APHA, 2005). The parameters examined were pH, electrical conductivity, temperature, turbidity, total solids, total dissolved solids, total suspended solids, chloride, total hardness, calcium, magnesium, total acidity, total alkalinity, biochemical oxygen demand (BOD) and chemical oxygen demand (COD).

Total hardness, calcium and magnesium were determined using EDTA titrimetric procedures, and chloride was measured by argentometric titration. The remaining parameters were analysed using the corresponding procedures in APHA (2005). Temperature was expressed in °C, electrical conductivity in µS/cm and turbidity in nephelometric turbidity units (NTU). Concentrations of solids and other chemical constituents were expressed in mg/L, with the reporting basis for hardness, calcium, magnesium, acidity and alkalinity specified as appropriate to the analytical method. These measurements provided the baseline characteristics presented in Fig. 2 and 3.

Bacterial isolates and biochemical characterization

Two bacterial isolates, designated C1 and C2, were evaluated separately for their ability to remove Cr, Cu and Zn from the industrial effluents. The isolates were identified in the manuscript as *Pseudomonas plecoglossicida* strain KMJ01 (C1) and *Serratia marcescens* strain KMJ03 (C2). Their phenotypic characteristics were examined by Gram staining and the indole, methyl red, Voges–Proskauer, citrate utilization and urease tests.

Molecular identification and sequence analysis

The isolates were further characterized by partial sequencing of the 16S rRNA gene. Genomic DNA was extracted using a HiMedia DNA extraction kit according to the manufacturer's instructions. PCR amplification was performed in a reaction volume of 20 µL containing 10 µL of 2× PCR master mix, 1 µL each of the forward and reverse primers, 2 µL of template DNA and 6 µL of sterile double-distilled water.

The amplification programme consisted of an initial denaturation at 95 °C for 5 min, followed by 30 cycles of denaturation at 95 °C for 1 min, annealing at 58 °C for 1 min and extension at 72 °C for 1 min. A final extension was performed at 72 °C for 10 min. Amplification products were examined by electrophoresis on a 1% agarose gel.

The resulting partial 16S rRNA gene sequences were compared with reference sequences in the NCBI nucleotide database using BLAST. Multiple sequence alignment was performed using Clustal W (Thompson *et al.*, 1994). The sequence relationships presented in Fig. 4 were used to support the identification of C1 and C2. The manuscript reports GenBank accession numbers PX481392 for strain KMJ01 and PX482431 for strain KMJ03.

Treatment of industrial effluents

Each isolate was grown separately in 100 mL of Luria–Bertani (LB) broth to prepare the inoculum. The cultures were subsequently added individually at an inoculation rate of 5% (v/v) to 250 mL Erlenmeyer flasks containing 100 mL of the respective industrial effluent supplemented with LB medium. Each of the four effluents was therefore treated separately with C1 and C2.

The inoculated flasks were incubated for 24 h on an orbital shaker at 150 rpm under aerobic conditions. Following incubation, the suspensions were centrifuged to separate the bacterial biomass from the liquid phase, and the supernatants were collected for residual metal analysis.

Determination of chromium, copper and zinc

Metal concentrations were determined before treatment and in the supernatants recovered after treatment. Samples were digested with concentrated nitric acid (HNO_3), diluted to a final volume of 100 mL with distilled water and filtered through Whatman No. 1 filter paper. Chromium, copper and zinc were quantified using an iCE 3000 series atomic absorption spectrometer (Thermo Scientific). The measurements were performed at the National

College Instrumentation Facility, National College (Autonomous), Tiruchirappalli, India.

Because the post-treatment measurements were made on separated supernatants, they represented metal remaining in the recovered liquid fraction. Differences between initial and final concentrations were used to evaluate apparent metal removal under the experimental conditions; they did not independently establish the mechanism responsible for that removal.

Calculation of metal removal efficiency

The removal efficiency for each metal was calculated from its concentration before treatment and its residual concentration after 24 h:

Metal removal efficiency (%)={(C_i-C_f)/C_i}×100

Where C_i is the initial metal concentration and C_f is the residual metal concentration after treatment, both expressed in mg/L. Concentrations used in the calculation must refer to a comparable sample-volume basis after accounting for analytical dilution and any dilution introduced by the inoculum or LB supplementation. Removal efficiencies were calculated separately for each isolate, metal and effluent type, corresponding to the comparisons presented in Fig. 5.

RESULTS

Distribution of industrial effluent sources

The four industrial effluent sources were located in two districts of Tamil Nadu, India. Rice mill and automobile industry effluents originated from Tiruchirappalli, whereas food processing and dyeing industry effluents originated from Coimbatore (Fig. 1).

Physicochemical characteristics of the effluents

The pH values differed among the four effluents. Rice mill and automobile effluents had pH values of 7.3 and 7.8, respectively, while food processing effluent recorded the lowest pH of 6.3. Dyeing effluent had the highest pH of 10.6 (Fig. 2A).

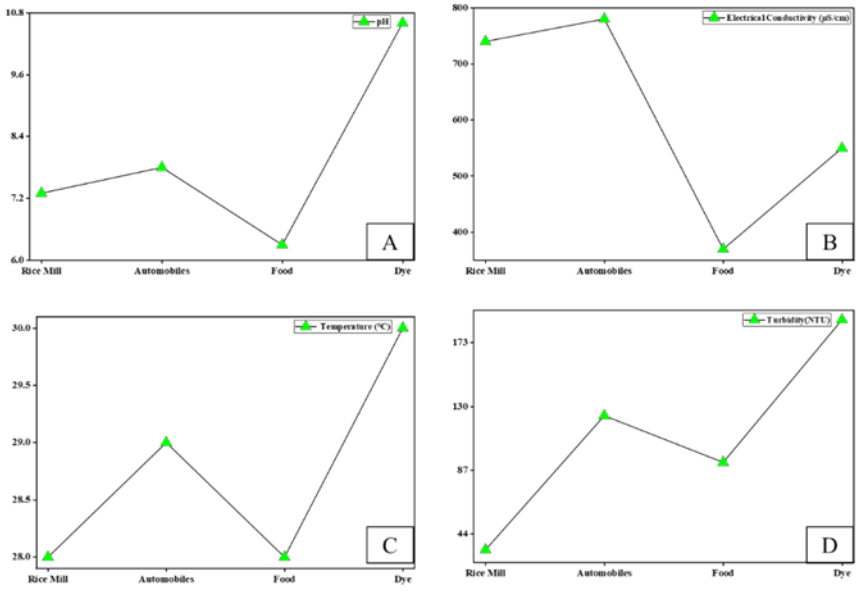


Fig. 2. A-pH, B-Electrical conductivity, C-Temperature and D-Turbidity of the Effluent samples

Electrical conductivity ranged from 370 to 780 µS/cm. Automobile effluent recorded the highest value of 780 µS/cm, followed by rice mill effluent at 740 µS/cm and dyeing effluent at 550 µS/cm. Food processing effluent had the lowest conductivity of 370 µS/cm (Fig. 2B).

Temperatures ranged from 28 to 30 °C. Rice mill and food processing effluents both recorded 28 °C, compared with 29 °C for automobile effluent and 30 °C for dyeing effluent (Fig. 2C). Turbidity was highest in dyeing effluent at 188.4 NTU, followed by

automobile effluent at 123.8 NTU and food processing effluent at 92.4 NTU. Rice mill effluent had the lowest turbidity of 33.5 NTU (Fig. 2D).

Total solids concentrations were 350 mg/L in rice mill effluent, 1,400 mg/L in automobile effluent, 3,850 mg/L in food processing effluent and 4,450 mg/L in dyeing effluent. Total dissolved solids followed a different distribution, with concentrations of 200, 650, 550 and 2,150 mg/L, respectively. Total suspended solids concentrations were 150 mg/L in rice mill effluent, 750 mg/L in automobile effluent, 3,300 mg/L in food processing effluent and 2,300 mg/L in dyeing effluent (Fig. 3).

Chloride concentrations ranged from 250 to 1,450 mg/L. Rice mill and automobile effluents contained 250 and 350 mg/L, respectively, compared with 1,350 mg/L in food processing effluent and 1,450 mg/L in dyeing effluent. Total hardness was highest in food processing effluent at 2,400 mg/L, followed by automobile effluent at 1,000 mg/L. Rice mill and dyeing effluents each recorded a total hardness of 800 mg/L (Fig. 3).

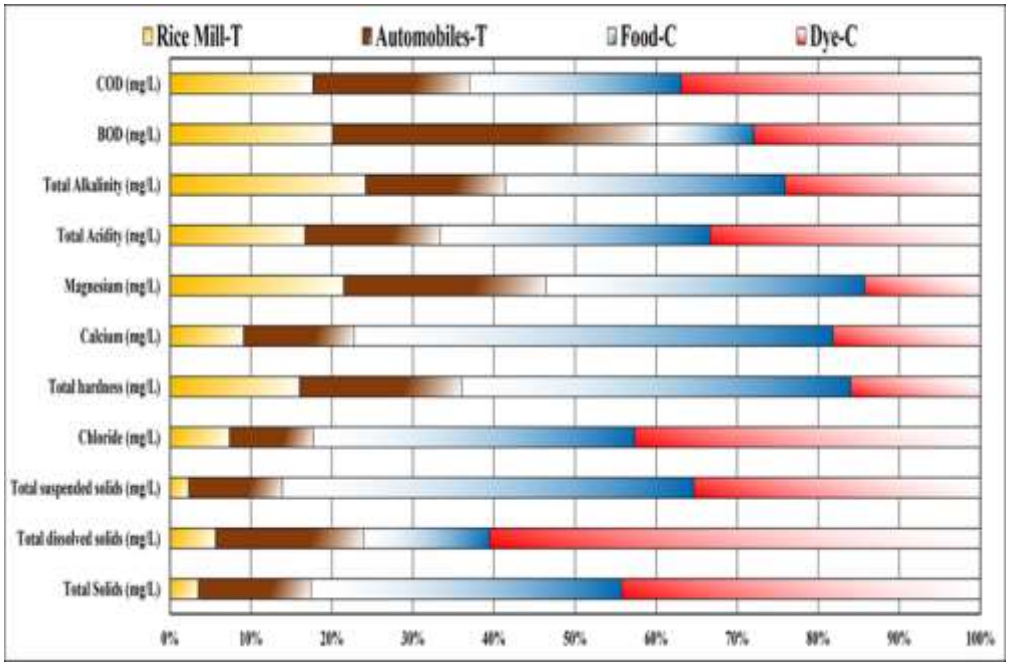


Fig. 3. Physicochemical characterization of all the four effluent samples (T–Tiruchirappalli; C–Coimbatore)

Food processing effluent had the highest reported calcium concentration of 1,300 mg/L, while dyeing effluent contained 400 mg/L. Magnesium concentrations were 600 mg/L in rice mill effluent, 700 mg/L in automobile effluent, 1,100 mg/L in food processing effluent and 400 mg/L in dyeing effluent.

Total acidity was 500 mg/L in both rice mill and automobile effluents and 1,000 mg/L in both food processing and dyeing effluents. Total alkalinity was highest in food processing effluent at 5,000 mg/L. Rice mill and dyeing effluents each recorded 3,500 mg/L, whereas automobile effluent recorded 2,500 mg/L (Fig. 3).

BOD was highest in automobile effluent at 333 mg/L, followed by dyeing effluent at 233 mg/L and rice mill effluent at 167 mg/L. Food processing effluent had the lowest BOD of 100 mg/L. COD concentrations were 840 mg/L in rice mill effluent, 920 mg/L in automobile effluent, 1,240 mg/L in food processing effluent and 1,760 mg/L in dyeing effluent (Fig. 3).

Sequence relationships of the bacterial isolates

The sequence tree for isolate C1, designated KMJ01, placed the isolate among *Pseudomonas* reference sequences, including *P. plecoglossicida* strains YL3 and S1. The tree for isolate C2, designated KMJ03,

placed the isolate alongside *Serratia marcescens* reference sequences, including strains MH6, ELP1.10, BP2, SASK1000, KS10 and ZSF02 (Fig. 4).

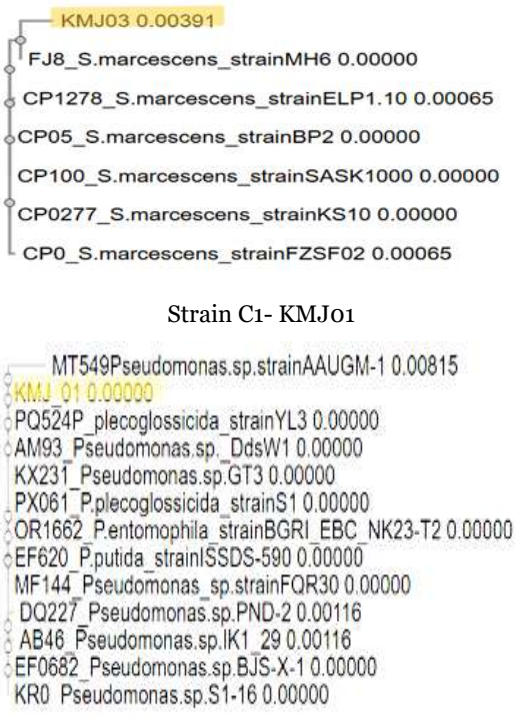


Fig. 4. Phylogenetic tree based on 16S rRNA analysis of strains C1 & C2

Heavy metal removal efficiencies

In rice mill effluent, chromium removal was 28.4% with C1 and 79.4% with C2. Copper removal was 99.3% with both isolates, while zinc removal reached 97.5% with C1 and 98.1% with C2. The difference between the isolates was greatest for chromium, for which C2 exceeded C1 by 51.0 percentage points (Fig. 5).

In automobile effluent, C1 removed 47.9% of chromium, 92.8% of copper and 96.4% of zinc. Corresponding removal efficiencies with C2 were 97.8%, 91.0% and 98.5%. Chromium removal was 49.9 percentage points higher with C2, whereas copper removal was 1.8 percentage points higher with C1 (Fig. 5).

In food processing effluent, chromium removal reached 98.9% with C1 and 93.7% with C2. Copper removal was 61.1% with C1 and 58.1% with C2, while zinc removal was 94.3% and 94.5%, respectively. C1 recorded higher chromium and copper removal than C2, while zinc removal differed by 0.2 percentage points (Fig. 5).

In dyeing effluent, C1 removed 74.1% of chromium, 96.8% of copper and 94.4% of zinc. Removal efficiencies with C2 were 45.1% for chromium, 25.9% for copper and 89.6% for zinc. C1 exceeded C2 by 29.0 percentage points for chromium, 70.9 percentage points for copper and 4.8 percentage points for zinc (Fig. 5).

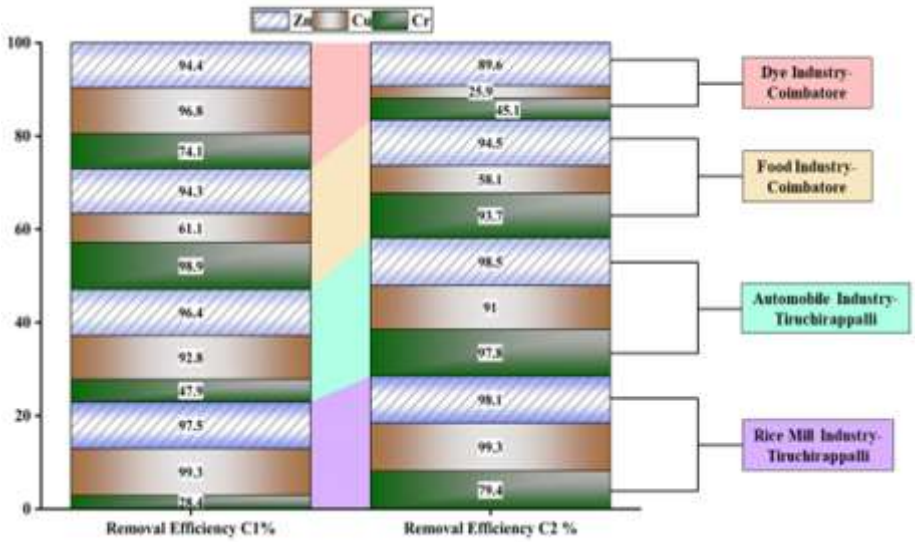


Fig. 5. Heavy metal removal efficiency of C1 and C2 strains

Across the four effluents, chromium removal ranged from 28.4% to 98.9% with C1 and from 45.1% to 97.8% with C2. Copper removal ranged from 61.1% to 99.3% with C1 and from 25.9% to 99.3% with C2. Zinc removal ranged from 94.3% to 97.5% with C1 and from 89.6% to 98.5% with C2 (Fig. 5).

DISCUSSION

The four industrial effluents differed in their physicochemical characteristics and in the removal efficiencies achieved by the two bacterial isolates. Although both isolates removed chromium, copper and zinc, their performance varied with the metal and effluent type. Zinc removal remained comparatively high across all four effluents, whereas chromium and copper removal showed greater differences between C1 and C2. These findings indicate that the performance of the isolates should be considered in relation to the composition of each wastewater rather than expressed as a single overall removal efficiency.

The pH values of the rice mill and automobile effluents were 7.3 and 7.8, respectively, compared with 6.3 in food processing effluent and 10.6 in dyeing effluent. This variation is important because pH can influence both bacterial activity and the chemical forms in which metals occur. The rice mill effluent can be considered alongside the physicochemical characterization reported by Pradhan and Sahu (2004), while Khan *et al.* (2025) provide a comparison for wastewater from vehicle service stations. Differences between these studies and the present findings may reflect variation in industrial operations, water sources, cleaning practices and sampling conditions. Consequently, the characteristics of an individual sample should not be treated as representative of all wastewater generated by the same industrial sector.

Electrical conductivity was higher in the rice mill and automobile effluents than in the food processing and dyeing effluents. Previous investigations of automobile wastewater and rice mill effluent also provide evidence of variation in conductivity between industrial sources (Sudhir and Prasanna, 2023; Choudhary *et al.*, 2015). In the present study, conductivity did not follow the same order as total dissolved solids. The reported conductivity units, measurement conditions and solids determinations therefore warrant checking before a relationship between these parameters is inferred. Conductivity alone also cannot establish which dissolved constituents contributed to the measured values.

The solids measurements showed a clear distinction between the effluents. Food processing and dyeing effluents contained substantially more total solids than rice mill and automobile effluents, while suspended solids were highest in food processing effluent. Rice mill wastewater has previously been investigated using electrocoagulation for the removal of suspended solids and COD, demonstrating the relevance of these parameters to its treatment (Karichappan *et al.*, 2013). In the present samples, suspended material may have affected the distribution of metals between particulate and liquid fractions. However, the contribution of particle-associated metals was not measured separately. Chloride, hardness and alkalinity further differentiated the wastewater matrices, and their combined effects cannot be inferred from any one parameter. The broader importance of characterizing industrial discharges and their receiving waters has been discussed by Kanu and Achi (2011), while the treatment implications of wastewater composition are addressed by Metcalf and Eddy (2014).

The dyeing effluent had the highest pH, turbidity, total solids, total dissolved solids and COD among the four samples. Its alkaline pH is consistent with the variability in textile wastewater composition described by Yaseen and Scholz (2019). Studies involving textile effluents from different treatment settings likewise provide a basis for comparison, including those of Buscio *et al.* (2015), Punzi *et al.* (2015), Tomei *et al.* (2016), Bhuvaneswari *et al.* (2015) and Almazán-Sánchez *et al.* (2016). These comparisons should nevertheless account for differences in production processes and whether the wastewater was untreated, mixed or partially treated. The treatment options reviewed by Kaur and Sharma

(2015) also concern dye removal, which should be distinguished from the metal removal evaluated here. COD exceeded BOD in every effluent, with BOD/COD ratios of approximately 0.20 for rice mill effluent, 0.36 for automobile effluent, 0.08 for food processing effluent and 0.13 for dyeing effluent. These ratios indicate that the oxygen demand measured by the BOD test represented only part of the chemically oxidizable load. They do not, by themselves, identify the compounds responsible or establish their toxicity. The low ratio in food processing effluent is particularly relevant because its industrial origin alone would not justify assuming that the sampled wastewater was readily biodegradable. The differences reinforce the need to evaluate the actual wastewater composition when considering biological treatment (Metcalf and Eddy, 2014).

The sequence analyses placed C1 among *Pseudomonas* reference sequences and C2 among *Serratia marcescens* reference sequences. Their different removal profiles are consistent with the possibility of strain-specific responses to metals and wastewater constituents. Metal resistance among *Pseudomonas* and related bacteria has been examined by Matyar *et al.* (2010) and Ceylan and Ugur (2012), providing a basis for investigating these organisms in contaminated environments. However, resistance and removal are different traits: the ability to tolerate a metal does not necessarily imply a high capacity to retain it. Moreover, partial 16S rRNA gene relationships do not establish the presence of particular resistance genes, extracellular polymers or metal-transforming enzymes.

Chromium removal showed the largest differences between the isolates in rice mill and automobile effluents. C2 achieved 79.4% removal in rice mill effluent and 97.8% in automobile effluent, compared with 28.4% and 47.9%, respectively, for C1. The pattern differed in food processing and dyeing effluents, where C1 achieved higher chromium removal. Thus, neither isolate consistently outperformed the other across all wastewater types. Studies of other bacteria support the wider potential for chromium removal, although their experimental conditions differ. Imron *et al.* (2024), for example, reported chromium removal of 86.17% by live *Bacillus nitratireducens* cells at an initial concentration of 100 mg/L. Javed *et al.* (2024) investigated cadmium and chromium biosorption using *Bacillus xiamenensis* and *Bacillus cereus*. Such studies provide useful context, but percentage removal alone is insufficient for ranking organisms tested at different metal concentrations, biomass loadings and contact times.

The wider bacterial remediation literature includes biosorption by dry bacterial biomass, resistance mechanisms in environmental isolates and biofilm-associated chromium detoxification (Rizvi *et al.*, 2020; Arroyo-Herrera *et al.*, 2021; Aththanayake *et al.*, 2025). These processes offer possible explanations for metal removal, but the present measurements do not distinguish among them. In particular, the decline in chromium concentration should not be described as evidence of Cr(VI) reduction to Cr(III). Although bacterial chromium reduction is discussed by Pradhan *et al.* (2017), establishing that transformation in the present isolates would require chromium speciation and supporting biochemical evidence. The current findings demonstrate a decrease in chromium remaining in the recovered liquid fraction.

Copper removal was nearly complete in rice mill effluent, reaching 99.3% with both isolates. In dyeing effluent, however, removal reached 96.8% with C1 but only 25.9% with C2. This difference suggests that copper removal depended on the interaction between isolate identity and wastewater composition. Surface binding is a plausible contributor, given the established use of microbial biomass for copper sequestration. Bilal *et al.* (2013) reviewed copper removal using a range of waste-derived biosorbents, while Hansda *et al.* (2016) distinguished the contributions of biosorption and bioaccumulation in microbial systems. The present results are compatible with these processes, but they do not identify the dominant pathway. Differences in pH, metal

complexation, competing ions or cellular responses remain possible explanations rather than demonstrated causes.

Zinc removal was more consistent than chromium or copper removal. C1 achieved efficiencies of 94.3–97.5%, while C2 achieved 89.6–98.5% across the four effluents. This comparatively narrow range suggests that zinc removal was less variable across the tested wastewater matrices under the reported conditions. It does not establish that zinc was preferentially adsorbed, because removal percentages also depend on initial concentrations and the amount of available biomass. The broader potential of bacterial systems for removing metals from wastewater has been discussed by Sreedevi *et al.* (2022), but identifying the basis of the present zinc removal would require measurements of biomass-associated metal and the corresponding liquid and solid fractions.

The performance of C2 also merits comparison with earlier work on *Serratia*. Marrero *et al.* (2009) investigated nickel and cobalt removal by native metal-resistant bacteria, including *S. marcescens*. That study provides relevant precedent for examining metal removal in this species, although its findings cannot be transferred directly to chromium, copper and zinc in mixed industrial effluents. Likewise, Choi and Yun (2004) examined lead biosorption by *Corynebacterium glutamicum* biomass. Together, these studies illustrate the diversity of bacterial materials investigated for metal sequestration, while emphasizing that performance remains specific to the organism, metal and experimental system.

Bacterial cell surfaces contain functional groups capable of interacting with metal ions, making biosorption a reasonable hypothesis for part of the observed removal (Ayangbenro and Babalola, 2017; Igiri *et al.*, 2018). Previous work has examined metal retention by an *Escherichia coli* biofilm supported on zeolite, living and non-living *Pseudomonas aeruginosa* cells, and free or immobilized *Aspergillus niger* biomass (Quintelas *et al.*, 2009; Gabr *et al.*, 2008; Tsekova *et al.*, 2010). Xu *et al.* (2020) specifically investigated cadmium biosorption by a *Pseudomonas* isolate and identified a contribution from cell-wall-associated metal. These studies support the plausibility of biomass-mediated retention, but they do not demonstrate the same mechanism in C1 or C2. Surface characterization, biomass digestion and metal mass-balance measurements would be needed to establish where the removed metals accumulated.

Metal sequestration should also be distinguished from the degradation of organic pollutants. Zeng *et al.* (2013) characterized extracellular peroxidases involved in lignin biodegradation, while Zhao *et al.* (2015) examined fungal growth and lignin metabolism under cadmium stress. These studies concern biological activity in chemically complex environments, but they do not establish metal degradation. Similarly, Fashola *et al.* (2016) reviewed bacterial resistance strategies in relation to gold-mine contamination rather than demonstrating the specific automobile-effluent treatment claimed in the original discussion. For the present study, the defensible interpretation is that metal concentrations decreased in the analysed supernatants; the metals themselves were not degraded.

Several features of the experimental design limit how far the removal results can be attributed to the inoculated isolates. The manuscript does not clearly document uninoculated controls, replication, treatment pH or corrections for dilution caused by inoculum and LB supplementation. Consequently, the contributions of bacterial activity, medium components, precipitation, indigenous microorganisms and physical separation remain unresolved. This is particularly relevant to the alkaline dyeing effluent. Because the initial effluent and post-treatment supernatant may represent different particulate fractions, comparable sample preparation is also necessary to distinguish treatment-associated removal from the effects of centrifugation. These limitations do not negate the observed concentration changes, but they restrict conclusions about mechanism and reproducibility.

The findings support further evaluation of C1 and C2 as separate bacterial treatments, particularly for zinc removal and for the combinations showing high chromium or copper removal. Their practical value will depend on performance under controlled and reproducible conditions, as well as biomass recovery, metal disposal or recovery, and the resources required for treatment. Saravanan *et al.* (2021) provide a broader context for evaluating wastewater treatment technologies. Vocciante *et al.* (2019), however, examined graphene-based adsorption for oil-spill remediation and therefore cannot substantiate the scalability of these bacterial treatments. Continuous-operation studies, comparisons with appropriate controls and an assessment of treatment costs are needed before industrial applicability or environmental superiority can be established.

CONCLUSION

The four industrial effluents differed in their physicochemical characteristics and in the metal removal achieved by *Pseudomonas plecoglossicida* KMJ01 (C1) and *Serratia marcescens* KMJ03 (C2). Zinc removal was consistently high, ranging from 94.3% to 97.5% with C1 and from 89.6% to 98.5% with C2. Chromium and copper removal varied more widely across the effluents. C1 achieved the highest chromium removal in food processing effluent (98.9%), whereas C2 achieved 97.8% chromium removal in automobile effluent. Both isolates removed 99.3% of copper from rice mill effluent, but their performance differed markedly in dyeing effluent, where copper removal reached 96.8% with C1 and 25.9% with C2.

These findings identify both isolates as candidates for further investigation, with their suitability depending on the target metal and wastewater composition. The measured decreases in supernatant metal concentrations do not establish the removal mechanisms or demonstrate chromium speciation changes. Further studies incorporating appropriate controls, independent replication, metal mass balances and defined treatment conditions are needed to confirm performance and assess the feasibility of application in industrial wastewater treatment.

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