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# Harnessing heavy metal-tolerant bacteria and phytotoxicity assessment for ecofriendly treatment of industrial effluents

Sharmila Shashikant Ghangale[1]*, Ramling Sidaramappa Saler[2],
Sharad Ratan Khandelwal[3], Dilip Vishwanath Handore[4], Anita Vishwanath Handore[4]

[1] Department of Biotechnology, Changu Kana Thakur Arts, Commerce and Science College, New Panvel, India
[2] Department of Environmental Science, K.T.H.M. College, Nashik, India
[3] Department of Microbiology, Phytoelixir Pvt. Ltd., Nashik, India
[4] Research and Development Department, Phytoelixir Pvt. Ltd., Nashik, India

* Corresponding author E-mail: gssharmi@yahoo.com

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**Abstract**
This study treated heavy-metal pollution in industrial wastewater by isolating and characterizing heavy metal-tolerant bacterial strains for bioremediation of industrial wastewater. The effluent samples were sterilized and aseptically inoculated with isolated heavy metal-tolerant bacteria such as *Bacillus lichniformis, Pseudomonas aeruginosa, and Stenotrophomonas maltophilia*. The samples were subjected to physicochemical analysis, and titrimetric analysis. Further, Atomic Absorption Spectroscopy (AAS) was used for Heavy metal analysis. Phytotoxic assay was carried out by seed germination tests and pot culture study. Over 96-hour period, improvement featuring reduced turbidity, a stabilized neutral pH, and a substantial 72% decrease in total solids was observed. The application of the bioremediation techniques using heavy metal tolerant bacteria resulted in a significant reduction of heavy metal concentrations, surpassing 85%. Moreover, seed germination rates in the treated industrial effluent were higher than the untreated samples. Pot culture tests further revealed diminished toxicity (2.5%) in treated roots compared to untreated ones (36.7%), accompanied by increased root and shoot growth in the treated pots. The noticeable enhancement in germination rates and the improved growth of plants in the treated effluent underscore the promising potential for the sustainable application of these eco-friendly bacteria, making them a cost-effective microbial bioremediation solution for industrial wastewater.

**Keywords**
*Eco-friendly, Industrial wastewater, Heavy metals, Microbial Bioremediation, Phytotoxicity.*

## Introduction

The presence of heavy metals in industrial effluents poses a considerable threat to aquatic ecosystems, human health, and overall ecological balance (Tayang and Songachan, 2021). Conventional wastewater treatment methods often prove inadequate in effectively addressing the intricate composition of industrial effluents. Consequently, there is a pressing need to shift towards ecologically conscious and economically viable alternatives (Ianeva, 2009).

In light of the escalating industrial activities and the consequent release of diverse pollutants into aquatic ecosystems, innovative and sustainable approaches are urgently required to tackle the environmental challenges posed by industrial effluents (Samadi et al., 2015). This study aims to tackle the issue of heavy metal pollution in industrial wastewater by isolating and characterizing heavy metal-tolerant bacterial strains. The primary obje-

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ctives include identifying bacterial isolates, enriching them, applying them in the bioremediation process, assessing enzymatic activity, and exploring how variations in pH and temperature influence the efficacy of these heavy metal-tolerant strains in microbial bioremediation.

The study explores the promising realm of microbial bioremediation as a pivotal strategy for mitigating the impact of industrial effluents on water quality (Iwasaki et al., 2009). Focusing on metal-tolerant microbial isolates, the study delves into their potential applications in restoring water ecosystems, offering hope for sustainable water management in eco-friendly way (Ayaz et al., 2020). It not only demonstrates the bioremediation potential of bacterial isolates uniquely adapted to thrive in metal-contaminated environments but also extends its scope to Phytotoxicity assessment(Ji and Silver, 1995). Recognizing the intricate interplay between microbial activity and plant health, the research explores the potential effects of metal-tolerant bacterial isolates on plant physiology (Tolbatov et al., 2020). By evaluating the Phytotoxicity of these isolates, the study aims to provide a holistic understanding of their environmental impact, contributing valuable insights for the development of sustainable water management practices (Hobman and Brown, 1996). As the world grapples with the imperative to safeguard water resources and preserve ecological integrity, the findings presented in this manuscript represent a significant stride towards achieving these objectives (Manisha et al, 2011; Tayang and Songachan, 2021; Yumpu, 2023).

By unravelling the potential of metal-tolerant bacterial isolates in the bioremediation of industrial effluents and concurrently assessing their Phytotoxicity, this research seeks to provide a robust foundation for the development of eco-friendly and efficient wastewater treatment strategies, fostering a harmonious coexistence between industrial progress and environmental sustainability (Manisha et al, 2011; Tayang and Songachan, 2021; Yumpu, 2023). Therefore, this study emphasizes the promising potential for the sustainable utilization of these eco-friendly bacterial strains for the microbial bioremediation of industrial wastewater.

## Materials and Methods

### Collection and bioremediation of industrial effluent

Samples were collected in sterile glass bottles from Metallurgical Industries, Nashik. These samples were sterilized and aseptically inoculated with isolated heavy metal-tolerant bacteria i.e., *Bacillus lichniformis, Pseudomonas aeruginosa,* and *Stenotrophomonas maltophilia*. Further, allowed to incubate at 200 rpm. on a rotary shaker at room temperature. Sterilized industrial effluent was kept in control.

### Physiochemical analysis

The pH, temperature, and electrical conductivity readings were recorded at the sample collection site using respective portable handheld instruments. Total Dissolved Solids (TDS) and Total Suspended Solids (TSS) were determined by gravimetric analysis using the following equations respectively as,

$$\text{Total Dissolved Solids (TDS)} = \frac{\{[\text{Weight of dried residue + evaporating dish (mg)}] - [\text{Weight of the evaporating dish (mg)}]\} * 100}{\text{Sample volume}} \quad [1]$$

$$\text{Total Suspended Solids (TSDS)} = \frac{\{[\text{Weight of the Whatman filter paper after filtration (mg)}] - [\text{Weight of the Whatman filter paper before filtration (mg)}]\} * 100}{\text{Sample volume}} \quad [2]$$

And Total Solids (mg/L) were calculated by summation of TDS and TSS values.

Thereafter, Dissolved Oxygen (DO) in the industrial effluent sample was assessed using the Winkler's titrimetric method and Biological Oxygen Demand (BOD) was determined using the 5-day incubation method prescribed by the American Public Health Association (APHA) standard. Further, BOD values were computed using the formula: BOD (5d) $O_2$ mg/L = 16 (V1–V2), where V1 and V2 denote the amounts of $Na_2S_2O_3$ required for the sample before and after incubation on the 5th day.

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Chemical Oxygen Demand (COD) was determined by the APHA standard titrimetric analysis. COD values were computed using the formula: (A-B)/L = sample (ml) × M × 8000, where A and B represent the amounts of FAS required for the blank and sample, M is the molarity of FAS, and 8000 is the milliequivalent weight of oxygen × 1000 ml/L.
Heavy metal analysis of the industrial effluent sample was performed using Atomic Absorption Spectroscopy (AAS).

**Phytotoxic assay: Seed germination test for treated industrial effluent**

Seeds of various dicot and monocot plants viz.maize, green gram, moth beans, cowpea seeds, horse gram, and fenugreek were surface sterilized with 0.1% w/v aqueous solution of Mercuric chloride and rinsed with distilled water. During this test, 10 uniform seeds of each plant were plated on sterilized petri plates with blotting paper, irrigated daily with 5 ml of treated industrial effluent, and incubated at 25 ± 2°C for 3-4 days. Seed germination rates were recorded after every 4 days for treated, untreated, and control groups, providing a systematic evaluation of industrial effluent impact on different plant varieties. Percent germination was calculated as the ratio of germinated seeds by total number of seeds.

**Phytotoxic assay: Pot culture test for treated industrial effluent**

Wheat seeds *(Triticum aestivum* L.) were soaked in sterilized distilled water for 5-6 hours and sown individually in six pots filled with clean soil for each treatment group: treated, untreated, and tap water (control). Daily irrigation with 25 ml of treated or untreated industrial effluents was administered to respective pots, while the control pots were irrigated with tap water. Germination rates and seedling growth were monitored daily for 7-12 days. Growth parameters, including shoot length, root length, and shoot height, were measured and recorded for each pot plant.

**Statistical analysis**

All experiments were conducted in triplicates. Data was employed for calculating descriptive statistical values. All values are represented as Mean (SD), specified otherwise.

## Results

**Physicochemical characteristics, bioremediation, and heavy metal analysis**

The results, summarized in Table 1, reveal significant improvements in industrial effluent samples over a period of 96 hours. The samples exhibited decreased turbidity and samples became odorless with neutral pH. Notably, a substantial 72% reduction in total solids was observed in the effluent samples. Furthermore, the bioremediation treatment was found to be effective in reducing heavy metal concentrations, with remarkable reductions observed for Zinc, Lead, Mercury, Copper, and Manganese (>85% reduction). These findings underscore the success of the bioremediation process in enhancing the quality of the industrial effluent by addressing both physicochemical parameters and heavy metal content. Detailed results are illustrated in Table 1

**Phytotoxic assay: Seed germination test**

As depicted in Fig. 1, and Table 2, Green gram, Cowpea, and Fenugreek seeds exhibited a remarkable 90% germination rate, while Maize, Moth beans, and Horse gram seeds showed 80% germination rate in treated industrial effluent compared to control. In contrast, green gram seeds displayed 30% germination rate, while Maize and Moth beans exhibited 20% germination in untreated industrial effluent compared to the control. Cowpea, Horse gram, and Fenugreek seeds showed only 10% germination rate in untreated industrial effluent compared to control. (Fig, 1 and Table 2).

**Phytotoxic assay: Pot .reulture test**

The results of the pot culture test are represented in Table 3 & Table 4 and Fig. 2. It was observed that the percentage toxicity remarkably varied in treated roots (

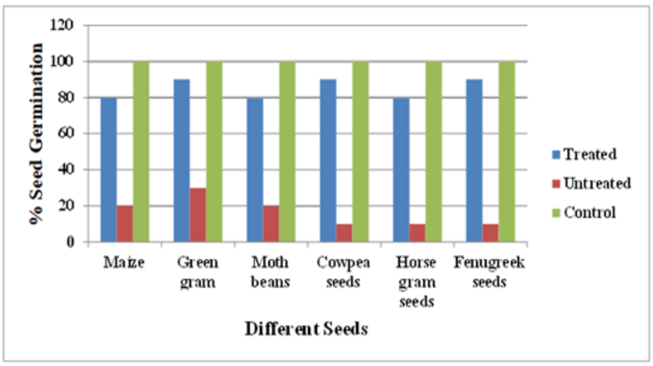


**Figure 1.** *Seed germination(%) by treated and untreated industrial effluent samples*

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**Table 1.** *Physicochemical, bioremediation and heavy metal analysis*

| Parameters | Time in hrs | | | | | % Reduction |
|---|---|---|---|---|---|---|
| | 0 | 24 | 48 | 72 | 96 | |
| Colour | Gray | Gray | Gray | Light Gray | Light Gray | ------- |
| Odor | Fishy | Fishy | Fishy | Odourless | Odourless | ------- |
| Temperature(°C) | 29°C | 28°C | 28°C | 28°C | 28°C | ------- |
| pH | 0.00 | 3.03 | 5.4 | 7.70 | 08.84 | ------- |
| Turbidity (NTU) | 0.00 | 23.56 | 41.24 | 66.60 | 79.14 | 79.14 |
| TDS (mg/L) | 0.00 | 22.06 | 47.97 | 63.50 | 71.18 | 71.18 |
| TSS (mg/L) | 0.00 | 27.54 | 43.37 | 61.05 | 72.91 | 72.91 |
| TS (mg/L) | 0.00 | 24.14 | 46.23 | 62.57 | 71.84 | 71.84 |
| DO (mg/L) | 1.36 | 1.92 | 2.89 | 4.15 | 4.89 | ------- |
| COD (mg/L) | 0.00 | 22.30 | 52.01 | 68.45 | 79.78 | 79.78 |
| BOD (mg/L) | 0.00 | 25.37 | 54.25 | 73.10 | 80.63 | 80.63 |
| Electrical Conductivity (mg/L) | 0.00 | 10.72 | 28.40 | 52.75 | 62.61 | 62.61 |
| Mercury (mg/L) | 0.00 | 19.23 | 46.15 | 76.92 | 88.46 | 88.46 |
| Lead (mg/L) | 0.00 | 29.65 | 54.48 | 80.00 | 91.73 | 91.73 |
| Zinc (mg/L) | 0.00 | 23.43 | 59.37 | 83.85 | 95.31 | 95.31 |
| Copper (mg/L) | 0.00 | 25.47 | 58.49 | 76.41 | 86.79 | 86.79 |
| Chromium (mg/L) | 0.00 | 17.39 | 38.40 | 54.34 | 63.04 | 63.04 |
| Cadmium mg/L) | 0.00 | 14.28 | 41.75 | 60.43 | 72.53 | 72.53 |
| Nickel (mg/L) | 0.00 | 20.83 | 37.66 | 57.14 | 66.23 | 66.23 |
| Iron(mg/L) | 0.00 | 22.56 | 48.17 | 68.29 | 81.71 | 81.71 |
| Manganese (mg/L) | 0.00 | 26.08 | 43.47 | 60.86 | 88.41 | 88.41 |
| Arsenic (mg/L) | 0.00 | 14.77 | 32.95 | 50.00 | 64.77 | 64.77 |

| Sr. No. | Name of the Seeds | Total no of Seeds taken | | | Germinated Seeds | | |
|---|---|---|---|---|---|---|---|
| | | C | T | U | C | T | U |
| 1. | Maize | 10 | 10 | 10 | 10 | 08 | 02 |
| 2. | Green gram | 10 | 10 | 10 | 10 | 09 | 03 |
| 3. | Moth beans | 10 | 10 | 10 | 10 | 08 | 02 |
| 4. | Cowpea seeds | 10 | 10 | 10 | 10 | 09 | 01 |
| 5. | Horse gram seeds | 10 | 10 | 10 | 10 | 08 | 01 |
| 6. | Fenugreek seeds | 10 | 10 | 10 | 10 | 09 | 04 |

T = Treated, U= Untreated, C=Control

**Table 2**
*Seed germination tests for treated and untreated industrial effluent samples*

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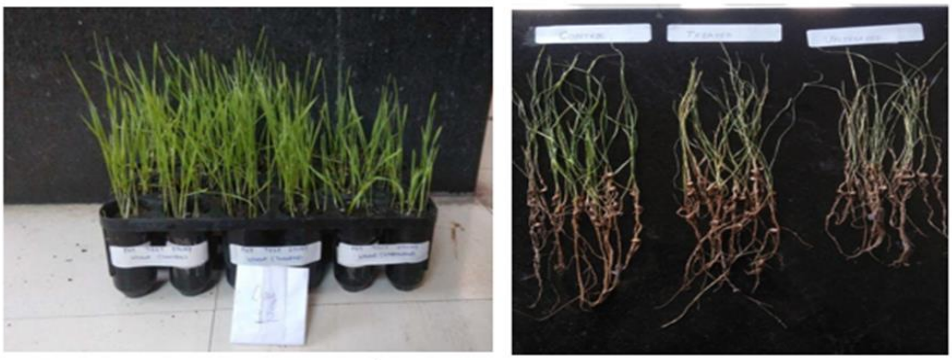
| Sr. No | Control | | Treated | | Untreated | |
|---|---|---|---|---|---|---|
| | Root Length (cm) | Shoot Length (cm) | Root Length (cm) | Shoot Length (cm) | Root Length (cm) | Shoot Length (cm) |
| 1 | 22.5 | 19.2 | 21.6 | 18.8 | 9.5 | 13.3 |
| 2 | 24.5 | 20.1 | 23.5 | 19.7 | 10.0 | 10.5 |
| 3 | 21.9 | 18.2 | 20.6 | 17.4 | 8.5 | 10.7 |
| 4 | 23.1 | 21.4 | 21.1 | 18.8 | 9.0 | 14.6 |
| 5 | 21.7 | 17.1 | 19.5 | 16.9 | 10.8 | 16.5 |
| 6 | 21.3 | 18.2 | 20.6 | 17.2 | 8.7 | 10.8 |
| 7 | 22.5 | 19.2 | 21.8 | 18.6 | 9.5 | 14.4 |
| 8 | 23.8 | 18.1 | 21.8 | 17.8 | 13.5 | 14.8 |
| 9 | 23.2 | 19.2 | 22.5 | 18.9 | 8.5 | 10.2 |
| 10 | 20.5 | 16.4 | 19.1 | 16.1 | 10.5 | 13.1 |

**Table 3**
*Pot culture test for treated and untreated industrial effluent samples*

| Mean value | Water sample | | |
|---|---|---|---|
| | Control | Treated | Untreated |
| Root | 22.50 | 21.21 | 9.85 |
| Shoot | 18.71 | 18.02 | 12.89 |
| Root: Shoot Ratio | 1.20 | 1.17 | 0.76 |
| % Toxicity | 0.00 | 2.50 | 36.66 |

**Table 4**
*Toxicity (%) of treated and untreated industrial effluents*


A] Soaking of wheat B] Growth of seedlings on 5th Day



C] Growth of seedlings on 12th Day D] Measurement of root and shoot length

**Figure 2**
*Root and Shoot length during Pot culture test for treated industrial effluent sample*

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2.5%) and untreated roots (36.7%). Similarly, the root and shoot growth was numerically found to be higher in treated pots as compared to untreated pots. One-way analysis of variance (ANOVA) for both, root (0.048) and shoot (0.003) was statistically significant (P<0.5) as shown in Table 5.

**Table 5.** *ANOVA Test for pot culture study*

| Sr. No. | ANOVA (Single factor-Root) | | | | | | |
|---|---|---|---|---|---|---|---|
| 1 | Source of Variation | SS | df | MS | F | P-value | F crit |
| 2 | Between Groups | 27.3045 | 9 | 3.033833 | 3.069128 | 0.047695 | 3.020383 |
| 3 | Within Groups | 9.885 | 10 | 0.9885 | | | |
| | Total | 37.1895 | 19 | | | | |
| **Sr. No.** | **ANOVA (Single factor-Shoot)** | | | | | | |
| 1 | Source of Variation | SS | df | MS | F | P-value | F crit |
| 2 | Between Groups | 27.59 | 9 | 3.065611 | 6.529523 | 0.003545 | 3.02 |
| 3 | Within Groups | 4.7 | 10 | 0.4695 | F | P-value | F crit |
| | Total | 32.2855 | 19 | | | | |

## Discussions

The comprehensive exploration of bio-remediation strategies employing metal-tolerant microbial isolates for the treatment of industrial effluents has yielded noteworthy results, as evidenced by the multi-faceted assessment encompassing physicochemical parameters, heavy metal concentrations, and phyto-toxicity assays.

### Physicochemical characteristics and bioreme-diation

The physicochemical analysis of the industrial effluent samples before and after the bio-remediation process revealed substantial impro-vements. The observed reduction in turbidity, neutralization of pH, and the remarkable 72% decrease in total solids indicated the efficacy of the employed microbial isolates in enhancing the overall quality of the industrial effluent. These findings align with the anticipated outcomes of successful bio-remediation, where microbial activity contri-butes to the breakdown of organic and inorganic pollutants, leading to improved water clarity and reduced suspended solids. Moreover, the bioreme-diation treatment demonstrated its prowess in dimi-nishing heavy metal concentrations in industrial effluent samples. Significant reductions of over 85% were noted for Zinc, Lead, Mercury, Copper, and Manganese. This outcome underscores the potential of the selected metal-tolerant bacterial strains (*Bacillus lichniformis, Pseudomonas aeruginosa, and Stenotrophomonas maltophilia*) in facilitating the removal or transformation of heavy metal pollutants, thereby ameliorating the environmental impact of industrial discharges.

### Phytotoxic assays

The seed germination test and pot culture test provided valuable insights into the phytotoxicity of the treated and untreated industrial effluents. Green gram, Cowpea, and Fenugreek seeds displayed a remarkable 90% germination rate in treated industrial effluent, emphasizing the positive impact of the bio-remediation process on seed viability. Conversely, untreated industrial effluent exhibited adverse effects on germination rates, with significantly lower percentages observed for various seeds. The pot culture study validated the positive influence of bio-remediation on plant growth. Treated roots demonstrated only 2.5% toxicity, in stark contrast to untreated roots with a greater

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toxicity rate (36.7%). This finding underscores the potential of metal-tolerant bacterial strains in mitigating the phytotoxic effects of industrial effluents, promoting healthier root development.

**Statistical Analysis:**

Statistical analyses, including ANOVA, validated with the significance of observed differences in root and shoot growth between treated and untreated samples. The low p-values ($p<0.5$) reinforce the statistical robustness of the results, further supporting the positive impact of the bio-remediation process on plant physiology.

**Implications and Future Directions**

The successful application of metal-tolerant bacterial strains in the bio-remediation of industrial effluents holds promising implications for sustainable water management. The findings not only contribute to the understanding of microbial interactions with pollutants but also it could provide a practical framework for development of eco-friendly and efficient water treatment strategies. Future research avenues may explore the long-term stability and resilience of the introduced bacterial strains in real-world industrial effluent scenarios. Additionally, investigating the microbial community dynamics during bio-remediation and their interaction with different pollutants could further enhance the comprehension of the intricate processes involved.

## Conclusion

This study highlights the effectiveness of utilizing isolated heavy metal-tolerant bacterial strains for the bioremediation of industrial wastewater. This approach leads to improvements in physicochemical parameters and a reduction in heavy metal concentrations. The noticeable enhancement in germination rates and the improved growth of plants in the treated effluent underscore the promising potential for the sustainable application of these eco-friendly bacteria, making them a cost-effective microbial bioremediation solution for industrial wastewater. Consequently, this research represents a significant advancement in the pursuit of sustainable solutions to alleviate the environmental impact of industrial activities. The incorporation of metal-tolerant microbial isolates into bioremediation processes emerges as a viable and effective strategy, striking a harmonious balance between industrial progress and environmental preservation.

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