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Assessment of Heavy Metal Tolerance and Detoxification Capacity of Microorganisms Isolated from Industrial Wastewater

[1] Sushmita S. Pawar, [2] Neha K. Vishwakarma, [2] Manisha T. Pawar, [2] Neha N. Gavale, [2] Esha R. Borkar and [2] Sakshi D. Wangujar

[1:] Assistant Professor, [2:] Students. Department of Microbiology and Biotechnology, Deogiri College, Chhatrapati Sambhajinagar - 431005, Maharashtra, India.

Email - pawarsushmita787@gmail.com

Abstract: *Heavy metal pollution in industrial effluents poses a significant threat to environmental and public health due to the non-biodegradable and toxic nature of heavy metals. This study focuses on the isolation, screening, and characterization of heavy metal-tolerant microorganisms from industrial wastewater and evaluates their potential for bioremediation. Initial isolation from effluent samples was performed using the serial dilution and spread plate technique on Nutrient Agar, revealing a diverse microbial load. Screening against four heavy metals - zinc (Zn), copper (Cu), chromium (Cr), and cadmium (Cd) identified multiple resistant strains. The Minimum Inhibitory Concentration (MIC) studies further quantified these tolerances, with selected isolates exhibiting growth at concentrations as high as 1000 ppm, particularly for Copper and Zinc. A colorimetric metal reduction assay was developed using green tea extract as a complexing agent to monitor metal utilization. Time-course analysis demonstrated rapid reduction kinetics, with substantial metal removal occurring within the first 2 days. To validate the detoxification efficiency, the treated water was subjected to phytotoxicity assessments. These findings highlight the potential of these indigenous microbial isolates as effective, eco-friendly candidates for the bio restoration of metal-contaminated industrial sites.*

Key Words: *Bioremediation, Heavy Metal Tolerance, Industrial Effluent, MIC, Green Tea Extract, Phytotoxicity.*

1. INTRODUCTION:

Industrialization has led to the discharge of massive quantities of untreated or partially treated effluents into aquatic ecosystems, introducing a mixture of hazardous substances [2]. Among these, heavy metals such as zinc (Zn), copper (Cu), chromium (Cr), and cadmium (Cd) are particularly notorious. Unlike organic pollutants, these metals are non-biodegradable and tend to accumulate in biological systems, eventually leading to severe physiological and neurological disorders in humans [1].

Heavy metals are defined by their high atomic weights and densities at least five times greater than that of water. In industrial environments, metals like chromium (Cr) are widely used in tanning and electroplating, while zinc (Zn) is essential for galvanizing processes. However, when concentrations exceed permissible limits, they become highly toxic [20]. For example, Chromium is a known carcinogen and mutagen that easily penetrates biological membranes [2]. Zinc and copper are essential trace elements, but at high concentrations they cause oxidative stress and inhibit enzymatic activities [1]. Cadmium is known for its long biological half-life, it primarily targets renal and skeletal systems [20].

The presence of heavy metals in industrial sites exerts a selective pressure on local microbial populations. To survive, indigenous microorganisms develop specialized resistance mechanisms, including extracellular sequestration, biosorption to cell walls, and active efflux systems [1, 20].

Bioremediation utilizes these metal-tolerant microbes to remove or detoxify pollutants. This biological approach is increasingly favoured over traditional chemical methods because it is cost-effective, avoids the production of toxic sludge, and can be applied *in situ* [2]. Strains isolated from polluted sites often show a "Multi-Metal Resistance" (MMR) profile, making them ideal candidates for broad-spectrum effluent treatment.

Accurate quantification of metal ions is crucial for monitoring remediation. While traditional methods like Atomic Absorption Spectroscopy (AAS) are highly sensitive, they involve high operational costs and complex instrumentation [21].

Recent advancements in green analytical chemistry emphasize the use of natural reagents. Green tea (*Camellia sinensis*) extract has emerged as a sustainable chromogenic agent. The polyphenols and catechins in green tea react with metal ions to form-colored complexes, allowing for spectrophotometric estimation at specific wavelengths (lambda max). This "green" method aligns with sustainability goals by reducing the use of hazardous synthetic chemicals in the laboratory [9].

The ultimate goal of wastewater treatment is to ensure the safety of the discharged water for agricultural and environmental use. Phytotoxicity assays, such as seed germination and root elongation tests using *Allium cepa* (onion), serve as critical bio-indicators [6, 20]. These tests provide a direct measure of the bioavailability of residual metals and the overall success of the detoxification process [2].

The present study focuses on the assessment of heavy metal tolerance and detoxification capacity of microorganisms isolated from industrial wastewater of Chhatrapati Sambhajinagar, Maharashtra (India).

2. MATERIAL AND METHOD:

Collection of Water Samples:

Different locations within industrial areas of Chhatrapati Sambhajinagar District, Maharashtra, India, were selected as sampling sites for the collection of heavy metal-contaminated wastewater for the isolation of heavy metal-tolerant microbial strains. Clean screw-cap bottles were used for sample collection. The bottles were carefully opened at the sampling site, and wastewater effluent samples were collected aseptically to minimize the risk of external contamination. Immediately after collection, the bottles were tightly sealed, properly labeled, and transported aseptically to the microbiology laboratory under appropriate conditions. The collected samples were stored in refrigerator until isolation of heavy metal-tolerant microorganisms and further microbiological studies.

Isolation of Microbial Strains from Industrial Effluent Water:

For isolation and cultivation of microbial strains from industrial effluent, nutrient agar was used. The isolation was conducted using the serial dilution and spread plate technique. After spreading, all plates were incubated in an inverted position at 37°C. The growth was monitored periodically between 24 to 48 hours until distinct, well-developed colonies observed. After incubation, plates containing well-isolated and morphologically distinct colonies were selected for further study.

Preliminary Identification of Isolates:

Colony morphology and Gram's staining was performed for all isolates according to the published protocol outlined by Dubey and Maheshwari (2008). The following biochemical tests were also performed for all isolates, i.e. Catalase Test, Indole Production Test, Methyl Red & Voges-Proskauer Test (MR-VP Test), Citrate Utilization Test, Sugar Oxidation (Glucose, Lactose, Sucrose, Dextrose and Maltose). All the tests were performed according to the published literature (American Society for Microbiology).

Preliminary Screening of Microorganisms for Heavy Metal Tolerance:

This stage of the study aims to identify specific microbial isolates capable of surviving and proliferating in the presence of toxic heavy metals, assessing their potential for bioremediation. The following heavy metal salts were selected for the study - Zinc Sulfate ($ZnSO_4$), Copper Sulfate ($CuSO_4$), Potassium dichromate ($K_2Cr_2O_7$) and Cadmium Chloride ($CdCl_2$) as the source of zinc (Zn), copper (Cu), chromium (Cr), and cadmium (Cd) respectively. The concentration of each metal salts was prepared using distilled water and standardized to 100 ppm (equivalent to 100 mg/L). Nutrient Agar (NA) was used as the basal medium, supplemented with the respective heavy metal salt solutions.

The resistance of the isolates was determined using the agar streaking method. Separate flasks of nutrient agar media were prepared, each containing a specific heavy metal salts at a final concentration of 100 ppm. After sterilization, metal-supplemented agar plates were prepared. A control plate (without heavy metal) was also prepared to verify the media sterility. Each microbial isolate was aseptically streaked onto the surface of the individual metal-supplemented agar plates using a sterile inoculating loop. The plates were incubated at 37°C for 24 to 48 hours. After incubation, the metal tolerance of each isolate was recorded. Growth (+) indicates the isolate is tolerant to the specific heavy metal at 100 ppm, no growth (-) indicates sensitivity to the heavy metal.

Determination of Minimum Inhibitory Concentrations (MICs):

Following the preliminary screening, the degree of resistance for each metal-tolerant isolate was quantified by determining the minimum inhibitory concentration (MIC). This study identifies the lowest concentration of a heavy metal that completely inhibits the visible growth of a microorganism. The experiment was conducted using an incremental concentration gradient of heavy metal salts to evaluate the tolerance thresholds of the isolates.

Each metal salt ($ZnSO_4$, $CuSO_4$, $K_2Cr_2O_7$ and $CdCl_2$) was incorporated into the separate nutrient agar media at the following standardized concentrations: 150 ppm (0.15 mg/mL), 250 ppm (0.25 mg/mL), 400 ppm (0.40 mg/mL), 500 ppm (0.50 mg/mL) and 1000 ppm (1.00 mg/mL). The MIC was determined using the agar dilution method to observe

the growth patterns across the concentration gradient. Each heavy metal-tolerant isolate was streaked onto the series of plates containing increasing concentrations of the specific metal. The plates were incubated at 37°C for 24 to 48 hours. The plates were observed for the presence or absence of microbial colonies. The concentration at which no visible growth was recorded as the MIC for that specific isolate and metal.

Heavy Metal Reduction Assay:

This assay is designed to quantify the utilization or reduction of heavy metals by the selected microbial isolates. The method utilizes a green tea-based reagent for the colorimetric detection of metal ions in the residual broth.

A total three isolates were selected based on their MIC value for further study. Green tea extract serves as the complexing agent due to its high polyphenol content, which reacts with metal ions to produce a measurable color change. Eight grams of green tea powder were added to 100 mL of purified water. The mixture was stirred on a magnetic stirrer for 10 to 30 minutes to ensure maximum extraction of active compounds. The extract was filtered through Whatman filter paper to remove particulate matter. The final volume was adjusted back to 100 mL using purified water to maintain a consistent concentration.

For λ max estimation, 25 mL of nutrient broth with heavy metal was prepared separately. Also prepared one 25 mL of nutrient broth without heavy metal to use as a blank. For metal reduction assessment**,** prepared 25 mL nutrient broth with heavy metal (3 flasks of each heavy metal). Sterilised all the flasks and after sterilization used for estimation of lambda max and metal reduction assessment.

Procedure for Estimation of Lambda Max (λ max):

To ensure high sensitivity, the optimum wavelength for the metal-polyphenol complex was determined. A mixture of 1 mL plain nutrient broth and 2 mL green tea extract was used to adjust the spectrophotometer to zero. 1 mL of the metal supplemented broth was mixed with 2 mL of green tea extract. The absorbance was scanned across the UV-Visible range (200 nm to 700 nm). The wavelength of maximum absorption (λ max), was selected for all subsequent measurements.

Procedure for Metal Reduction Assessment:

The ability of the isolates to reduce metal concentration in the medium was measured as follows. Nutrient broth flasks prepared for metal reduction assessment were inoculated with the selected isolates. Following inoculation, 2 mL of broth was aseptically collected from each flask and centrifuged at 10,000 rpm for 10 Minutes. After centrifugation, 1 mL of the clear supernatant (containing residual metal) was collected in separate tubes. 2 mL of the green tea extract was added to each tube containing 1 mL of supernatant and mixed thoroughly. A mixture of 1 mL plain nutrient broth and 2 mL green tea extract was used to adjust the spectrophotometer to zero. The absorbance was recorded at the previously determined lambda max against the control blank. Same procedure was followed at day 2 to determine the reduction in metal concentration. The percentage of heavy metal utilization or reduction by the microbial isolates is calculated using the following formula;

$$\%\text{Metal Utilization} = \left(\frac{\text{Absorbance}_{\text{initial}} - \text{Absorbance}_{\text{final}}}{\text{Absorbance}_{\text{initial}}}\right) \times 100$$

Time-Course Analysis of Heavy Metal Bioremediation:

To ensure uniform microbial density across all experimental flasks, a concentrated cell suspension was prepared. Each isolated culture was inoculated into 25 mL of sterile nutrient broth and incubated at 37°C for 18-24 hours to reach the exponential growth phase. The broth was centrifuged at 10,000 rpm for 10 minutes, and pellet was collected. The resulting pellet was washed 2-3 times with sterile water followed by centrifugation to remove residual media components that might interfere with the assay. The final pellet was resuspended in 2.5 mL of sterile water to create a concentrated microbial "starter" culture.

The assay was performed in a water-based system to minimize complexation with organic matter. 100 mL of sterile water was supplemented with the required concentration (ppm) of heavy metal salts ($ZnSO_4$, $CuSO_4$, $K_2Cr_2O_7$ and $CdCl_2$). A total of 12 flasks were prepared (3 Isolates × 4 heavy metals). Each flask was inoculated with 0.5 mL of the prepared microbial suspension. 100 mL of sterile water was kept as blank to adjust zero. The reduction of heavy metals was monitored up to 6 days.

A mixture of 1 mL sterile water (without metals) and 2 mL green tea extract was used to adjust the spectrophotometer to zero. Following inoculation, 2 mL of water was aseptically collected from each flask and centrifuged at 10,000 rpm for 10 Minutes. After centrifugation, 1 mL of the clear supernatant was collected in separate tubes. 2 mL of the green tea extract was added to each tube containing 1 mL of supernatant and mixed thoroughly. The absorbance was recorded at the previously determined lambda max. Same procedure was followed up to 6 days to determine the reduction in

metal concentration. The percentage of heavy metal reduction by the microbial isolates was calculated using the following formula;

$$\% \text{ Metal Reduction} = \left(\frac{A_0 - A_t}{A_0} \right) \times 100$$

Where, A_0 is absorbance at initial and A_t absorbance at a specific time point.

Phytotoxicity Assessment by Seed Germination and *Allium cepa* (Onion) Root Assays:

The final phase was to evaluate the biological safety of the treated effluent. By comparing the effects of "microbially treated" metal solutions against untreated controls, it can determine if the microbial process successfully detoxifies the heavy metals for agricultural or environmental release. The heavy metal containing water treated with the selected microbial isolates after absorption was centrifuged at 10,000 rpm for 10 minutes, collected supernatant and used for phytotoxicity tests.

Test Group: Water treated with heavy metals and microbial isolates.

Heavy Metal Control: 100 mL of water with the same metal concentration but no microbial treatment.

Negative Control: 100 mL of normal distilled water.

Seed Germination Assay:

This assay measures the impact of residual metal toxicity on the early developmental stages of *Vigna radiata* (Moong seeds), *Vigna aconitifolia* (Moth seeds) and *Cicer arietinum* (Black Gram). Petri plates were labeled as water control, heavy metal control, and test groups, moisten the filter paper with respective water and placed into the labelled plate. 2 healthy seeds of each type were placed evenly in each plate. The seeds were covered with a second layer of filter paper moistened with the same. Plates were closed and maintained at room temperature for 2 days. Daily records were taken regarding the number of germinated seeds etc.

Onion (*Allium cepa*) Root Elongation Assay:

The onion root assay is a standard bio-indicator for monitoring environmental pollutants and cytotoxicity. Containers were filled with the three experimental waters (Treated, Metal Control, and Normal Water). An onion bulb of uniform size was placed at top of each container. Care was taken to ensure that only the basal disc (root zone) remained in direct contact with the liquid surface. The setup was kept at room temperature for 2 days. The emergence and elongation of the roots were observed.

3. RESULTS AND DISCUSSION:

Collection of Water Samples: A total three waste water samples were collected from different industrial area locations of Chhatrapati Sambhajinagar District, Maharashtra (India).

Figure 1: Collection of Water Samples

Isolation of Microbial Strains from Industrial Effluent Water:

A total of 17 isolates were obtained from all three samples and the isolates are numbered as mentioned in Table 1;

Table 1: Isolate Details

Name of the Sample	Identification Number of Isolate
Sample A	A*
Sample B	A, B, C, D, E, F, G, H and I
Sample C	A, b, c, d, e, f and g

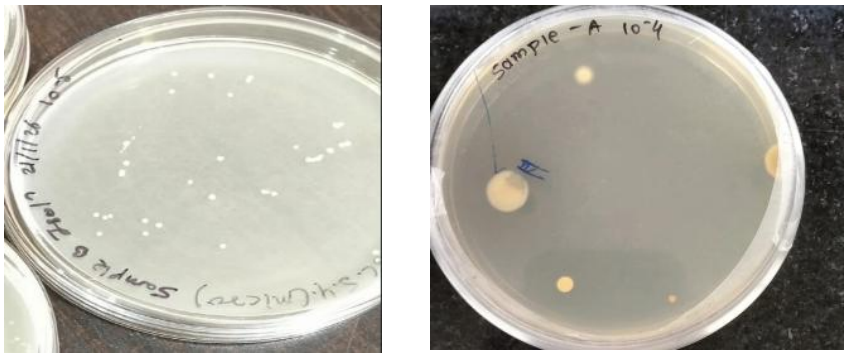

Figure 2: Representative Photographs of Isolated Microbial Strains

Preliminary Screening of Microorganisms for Heavy Metal Tolerance:
Among the 17 isolates tested, most exhibited tolerance to different heavy metal salts, namely $ZnSO_4$, $CuSO_4$, $K_2Cr_2O_7$ and $CdCl_2$ at concentration of 100 ppm, indicating their adaptation to heavy metal polluted environments. Only two isolates (Isolate G and H from Sample B) showed no growth, under the tested conditions, indicating their sensitivity to heavy metal stress at 100 ppm.

Table 2: Isolate Details for Heavy Metal Tolerance

Name of the Sample	Identification number of isolate
Sample A	A*
Sample B	A, B, C, D, E, F and I
Sample C	A, b, c, d, e, f and g

Preliminary Identification of Metal Tolerant Isolates:
Morphological and Microscopic Characterization: Morphological characterization revealed diversity among isolates, including both Gram-positive and Gram-negative bacteria, indicating a heterogeneous microbial population capable of surviving in contaminated environments.

Table 3: Morphological and Microscopic Details of Sample B Isolates

Characters	Isolates A	Isolates B	Isolate C	Isolate D	Isolate E	Isolates F	Isolate G	Isolate H	Isolate I
Size	2mm	1mm	2mm	1mm	3 mm	1mm	1mm	2mm	1mm
Shape	Circular	Circular	Circular	Circular	Circular	Circular	Circular	Circular	Circular
Colour	Creamy White	Pale yellow	White	Cream	Off White	Whitish	Yellow	Light Yellow	Yellow
Margin	Irregular	Entire	Entire	Entire	Entire	Entire	Irregular	Entire	Irregular
Elevation	Raised	Convex	Raised	Raised	Convex	Convex	Raised	Raised	Convex
Opacity	Opaque	Opaque	Opaque	Opaque	Opaque	Opaque	Opaque	Opaque	Opaque
Consistency	Mucoid	Mucoid	Sticky	Mucoid	Smooth	Sticky	Dry	Buttery	Moist
Gram's Nature	G^- Rods	G^+ Rods	G^+ Cocci	G^- Rods	G^- Rods	G^- Rods	G^+ Rods	G^+ Cocci	G^- Rods

Table 4: Morphological and Microscopic Details of Sample A and C Isolates

Characters	Isolates A*	Isolates a	Isolates b	Isolates c	Isolate d	Isolates e	Isolate f	Isolate g
Size	1mm	1mm	1mm	2mm	1mm	3mm	1mm	2mm
Shape	Circular	Circular	Circular	Circular	Circular	Circular	Circular	Circular
Colour	Yellow	Dark Yellow	Whitish	Whitish	Yellow	Orange	Cream	Yellow

Characters	Isolates A*	Isolates a	Isolates b	Isolates c	Isolate d	Isolates e	Isolate f	Isolate g
Margin	Irregular	Irregular	Irregular	Entire	Irregular	Entire	Entire	Entire
Elevation	Flat	Raised	Convex	Raised	Raised	Convex	Raised	Raised
Opacity	Opaque	Opaque	Opaque	Opaque	Opaque	Opaque	Opaque	Opaque
Consistency	Moist	Mucoid	Sticky	Sticky	Mucoid	Sticky	Smooth	Moist
Gram's Nature	G^- Rods	G^- Rods	G^+ Rods	G^+ Rods	G^+ Cocci	G^- Rods	G^- Rods	G^+ Cocci

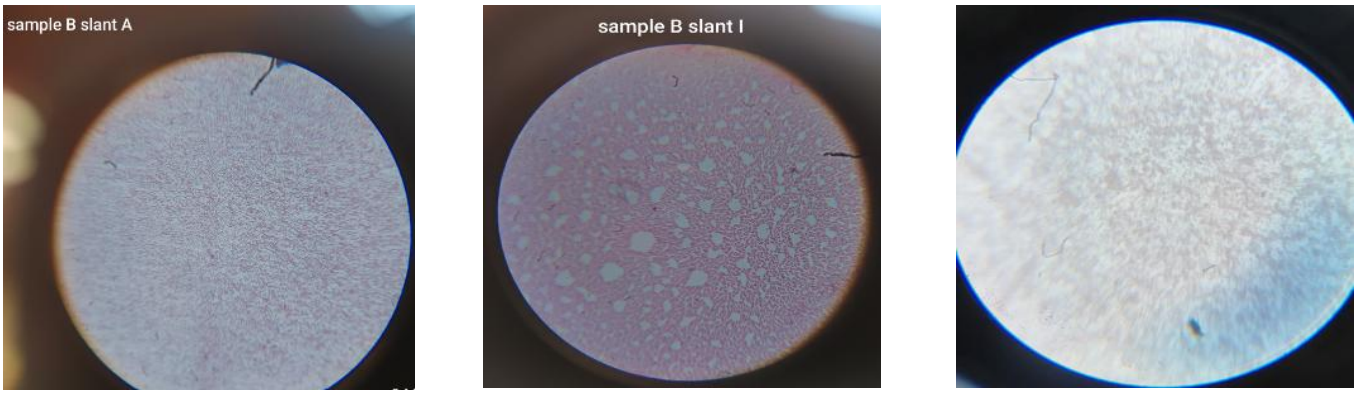

Figure 3: Representative Photographs of Microscopic Observation after Grams Staining

Biochemical Characterisation:

IMViC Test: IMViC contains a series of biochemical tests. It includes four tests: Indole Test, Methyl Red Test & Voges Proskauer Test, Citrate utilization Test.

a. Indole Test: The indole test screens for the ability of an organism to degrade the amino acid tryptophan and produce indole. Indole reacts with Kovac's reagent and produces red color. Based on the observations, most of the isolates were found indole Positive.

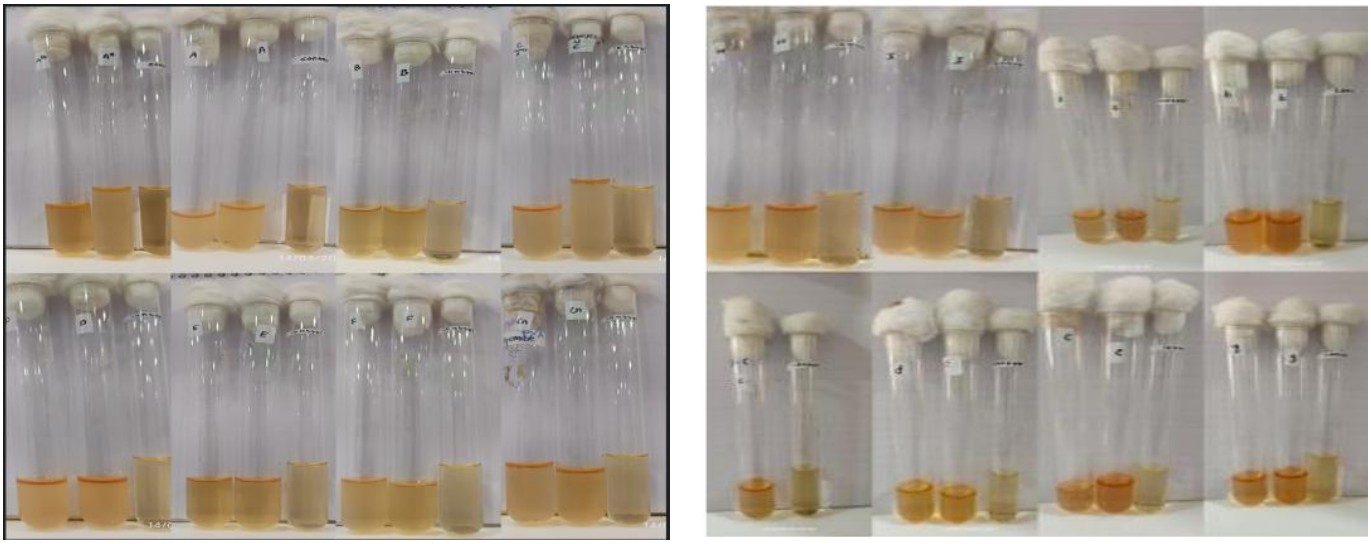

Figure 4: Indole Test of Isolates Isolated from Sample A, B and C

b. Methyl Red and Voges Proskauer Test: The Methyl Red test is based on the ability of some bacteria to perform mixed acid fermentation of glucose, producing stable acids (such as lactic, acetic, and formic acids). These acids significantly lower the pH of the medium. When methyl red indicator is added, it turns red in acidic conditions (positive result) and yellow in less acidic conditions (negative result). The Voges–Proskauer test detects the production of acetoin (a neutral end product) from glucose fermentation. In the presence of oxygen and reagents (α-naphthol and KOH),

acetoin is oxidized to diacetyl, which reacts to produce a pink/red color (positive result). Based on the observations, most of the isolates were found methyl red test positive and VP test negative.

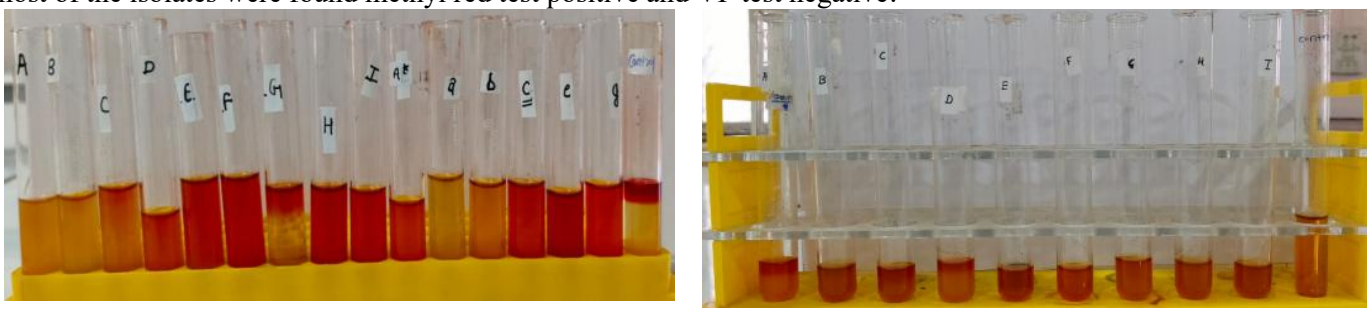

Figure 5: Representative Photograph of Methyl Red and Voges-Proskauer (MR-VP) Test

c. Citrate Test: The citrate test determines the ability of bacteria to use citrate as the sole carbon source and ammonium salts as the nitrogen source. Utilization of citrate produces alkaline byproducts, increasing the pH. This change is detected by bromothymol blue indicator, which turns from green to blue (positive result). Based on the observations, most of the isolates from Sample A and B were found Citrate Positive while Sample C isolates were found Citrate negative.

Figure 6: Citrate Test of Isolates Isolated from Sample A, B and C

Sugar Oxidation Test: Sugar oxidation test is based on the ability of microorganisms to metabolize carbohydrates in the presence of oxygen, produce a small number of weak acids during the Krebs cycle and Entner Doudoroff (glycolysis) and these weak acids to a level that can be detected by bromothymol blue indicator.

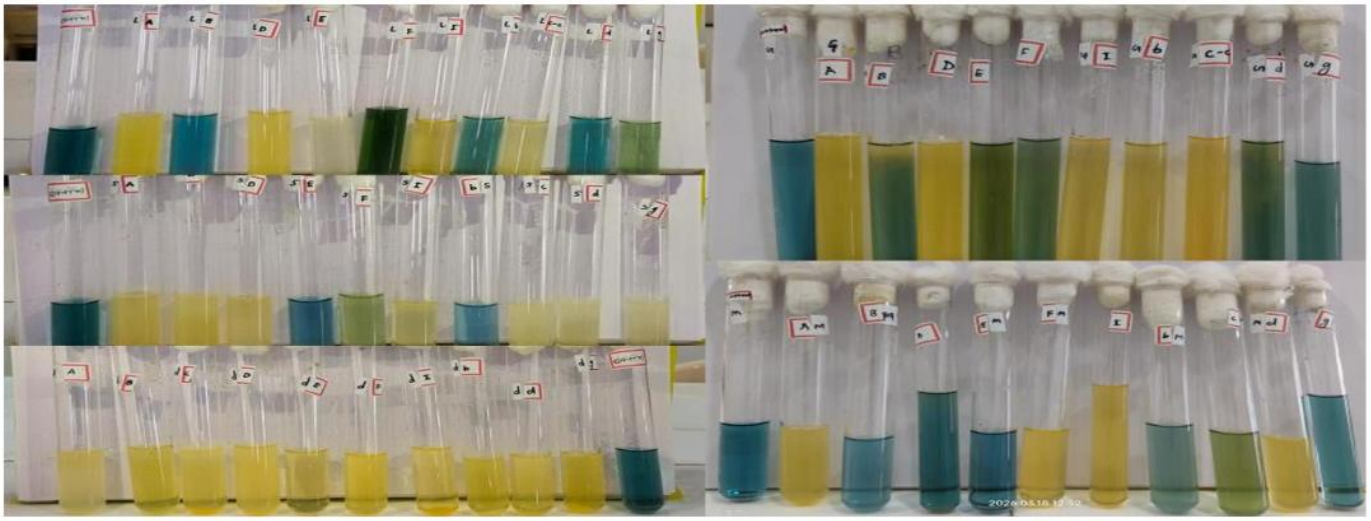

Figure 7: Sugar oxidation test of isolates isolated from sample A, B and C with different sugars (Glucose, Lactose, Sucrose, Dextrose and Maltose)

Catalase Test: The catalase test is an important biochemical test used to detect the presence of the enzyme catalase in bacteria. Catalase-positive bacteria produce the enzyme catalase, which rapidly breaks down hydrogen peroxide into water and oxygen gas. None of the isolates formed visible oxygen bubbles when mixed with 3% hydrogen peroxide indicated that these isolates are catalase negative.

Figure 8: Representative Photograph of Catalase Negative Results

Determination of Minimum Inhibitory Concentrations (MICs):

Each metal tolerant isolates were challenged with different concentrations of heavy metals for determination of the Minimum Inhibitory Concentration (MIC) and the results are as per table 5 to table 7. Based on the observations, three isolates (Isolated A and I from sample B and Isolate c from Sample C) showed tolerance to all tested metals.

The concentrations selected for further study for each isolate were as follows. Isolates A and I: Cadmium chloride at 500 ppm, while zinc sulfate, potassium dichromate, and copper sulfate were maintained at 1000 ppm. Isolate c: Cadmium chloride and zinc sulfate at 1000 ppm, potassium dichromate at 400 ppm, and copper sulfate at 500 ppm.

Table 5: Sample A Isolate Details for Minimum Inhibitory Concentrations (MICs)

Isolate No.	Cadmium Chloride (ppm)					Zinc Sulfate (ppm)					Potassium Dichromate (ppm)					Copper Sulfate (ppm)				
	150	250	400	500	1000	150	250	400	500	1000	150	250	400	500	1000	150	250	400	500	1000
A*	-	-	-	-	-	-	-	-	-	-	+	+	+	-	-	+	+	+	+	+

Table 6: Sample B Isolate Details for Minimum Inhibitory Concentrations (MICs)

Isolate No.	Cadmium Chloride (ppm)					Zinc Sulfate (ppm)					Potassium Dichromate (ppm)					Copper sulfate (ppm)				
	150	250	400	500	1000	150	250	400	500	1000	150	250	400	500	1000	150	250	400	500	1000
A	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
B	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
C	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+
D	-	-	-	-	-	+	+	+	+	+	+	+	+	-	-	+	+	+	+	+
E	-	-	-	-	-	+	+	+	+	-	+	+	+	+	-	+	+	+	+	+
F	-	-	-	-	-	+	+	+	-	-	+	+	+	+	+	+	+	+	+	+
I	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Table 7: Sample C Isolate Details for Minimum Inhibitory Concentrations (MICs)

Isolate No.	Cadmium Chloride (ppm)					Zinc Sulfate (ppm)					Potassium Dichromate (ppm)					Copper sulfate (ppm)				
	150	250	400	500	1000	150	250	400	500	1000	150	250	400	500	1000	150	250	400	500	1000
a	+	-	-	-	-	+	+	-	-	-	+	-	-	-	-	+	+	-	-	-
b	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
c	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	+	+	-
d	+	+	-	-	-	+	-	-	-	-	+	+	+	-	-	+	+	+	+	-
e	-	-	-	-	-	+	+	-	-	-	+	-	-	-	-	+	+	+	-	-
f	+	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
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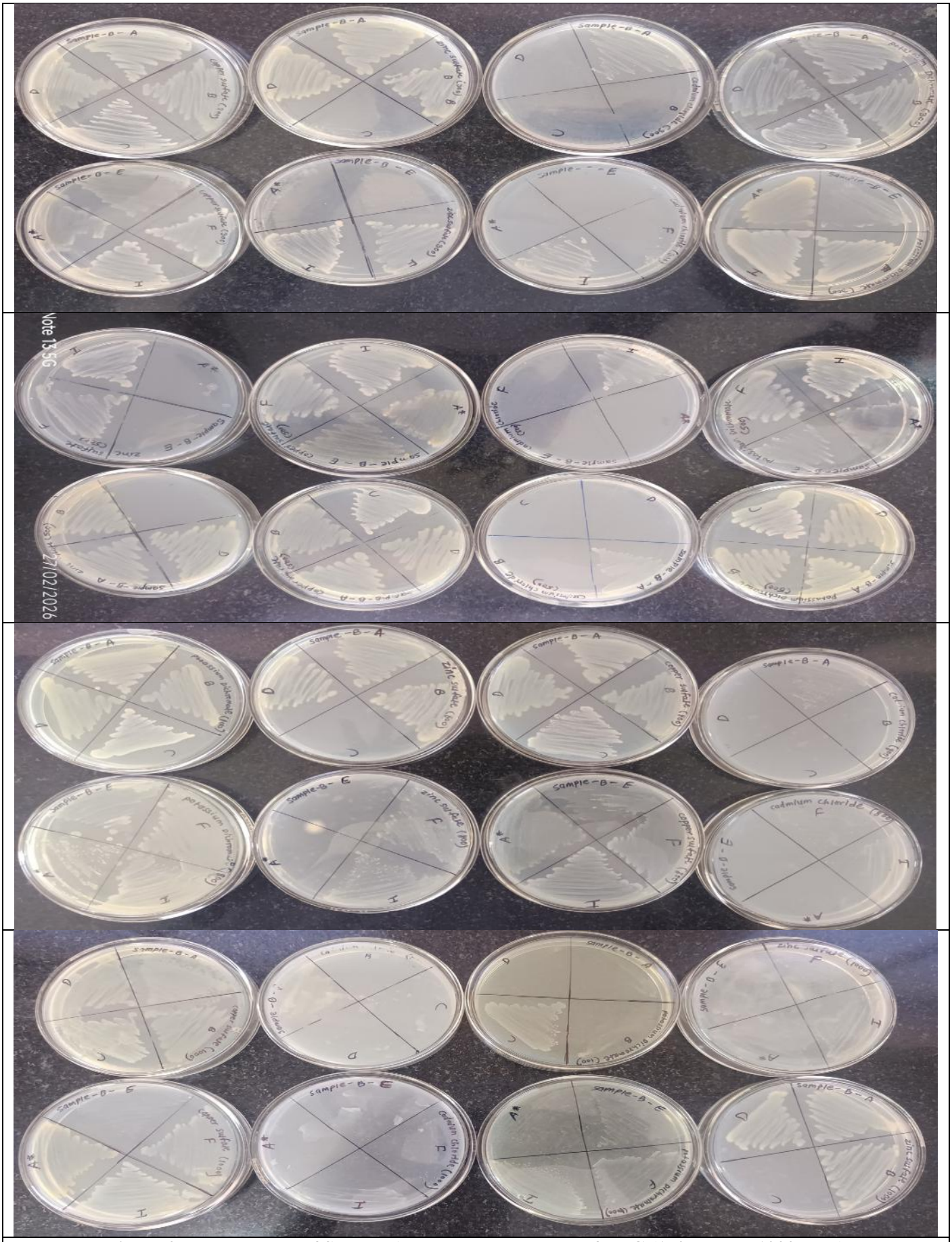

Figure 9: Photographs of Sample A and B Isolate Plates of MIC (150 ppm to 1000 ppm)

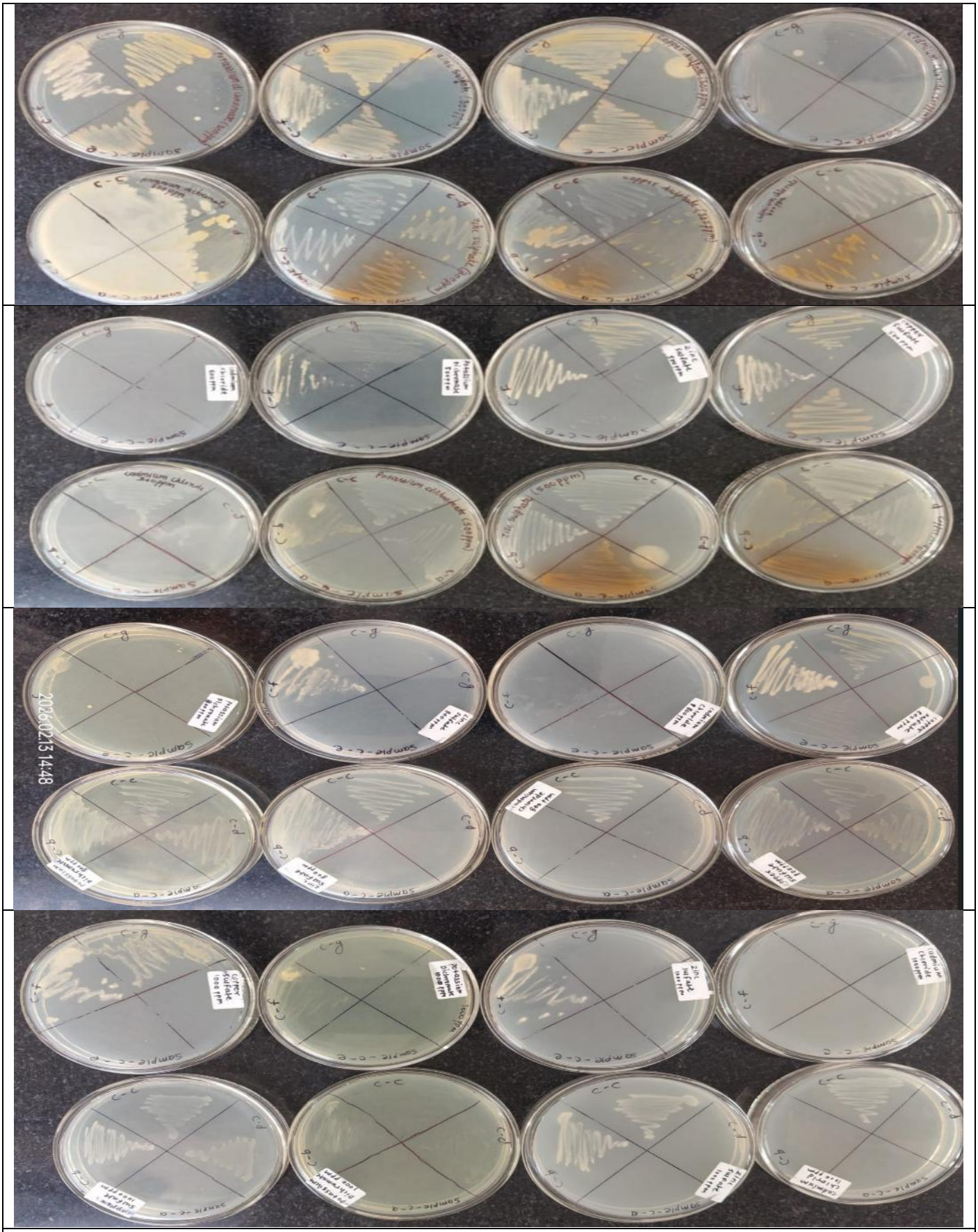

Figure 10: Photographs of Sample C Isolate Plates of MIC (150 ppm to 1000 ppm)

Heavy Metal Reduction Assay:

Estimation of Lambda Max (λ max): Estimated the maximum absorption of all four heavy metals and were as per table 8.

Table 8: Lambda Max Details

Test Details	Cadmium Chloride (nm)	Zinc Sulfate (nm)	Potassium Dichromate (nm)	Copper Sulfate (nm)
λ max	360	230	260	300

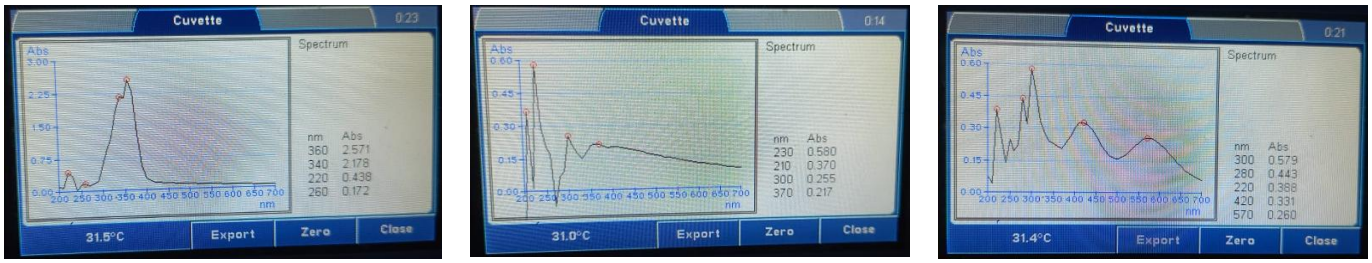

Figure 11: Photographs of Estimation of λ max for Different Metal

Metal Reduction Assessment:

The selected isolates exhibited varying heavy metal reduction efficiencies against $ZnSO_4$, $CuSO_4$, $K_2Cr_2O_7$ and $CdCl_2$. The differences in reduction efficiency might be attributed to isolate-specific metal resistance and may be associated with possible mechanisms such as biosorption, bioaccumulation, extracellular sequestration, or efflux-mediated resistance. Overall, Isolate I demonstrated broad heavy metal removal potential, whereas Isolate c showed particularly high efficiency for cadmium, indicating their potential application in heavy metal bioremediation.

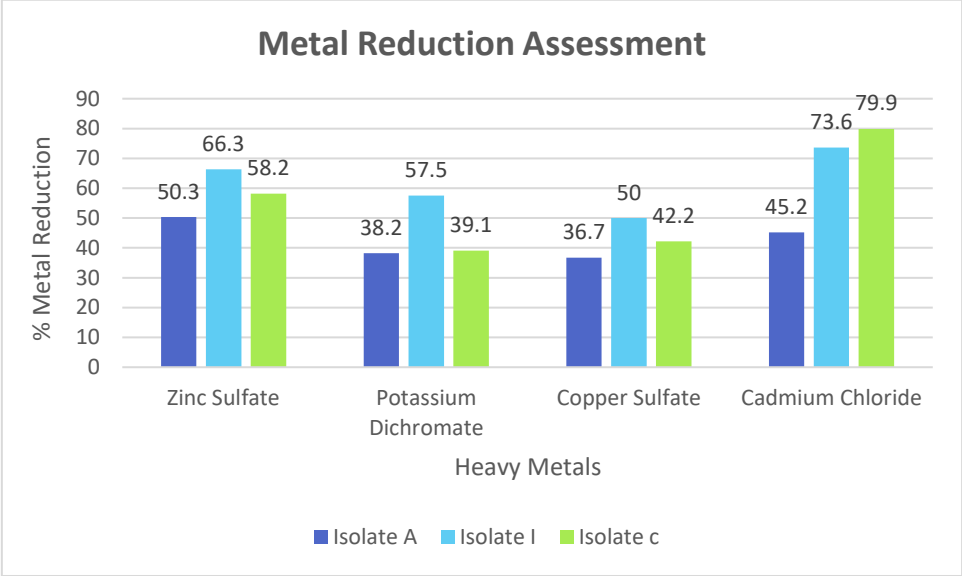

Figure 12: Graphical Representation of % Metal Reduction by Isolate A, I and c

Time-Course Analysis of Heavy Metal Bioremediation:

Heavy metal removal increased progressively from Day 2 to Day 6 for most metals and isolates. Isolate A showed an increase in $ZnSO_4$ removal from 82.1% to 94.2%, while $CuSO_4$ and $CdCl_2$ removal reached 99.5% and 98.7%, respectively, by Day 6 (Figure 13). Isolate I demonstrated the highest $ZnSO_4$ removal (99.9%) and strong $CdCl_2$ removal (99.6%) on Day 6, whereas $K_2Cr_2O_7$ removal remained comparatively low (83.4%) (Figure 14). Isolate c also showed substantial improvement over time, with Day-6 removal reaching 98.1% for $ZnSO_4$, 99.1% for $K_2Cr_2O_7$, 98.0% for $CuSO_4$, and 98.3% for $CdCl_2$ (Figure 15). Overall, the increasing removal efficiency with incubation time indicates effective heavy metal removal by the selected isolates, with $CuSO_4$ and $CdCl_2$ generally showing the highest removal efficiencies. These findings suggest that the selected isolates have promising potential for heavy metal bioremediation.

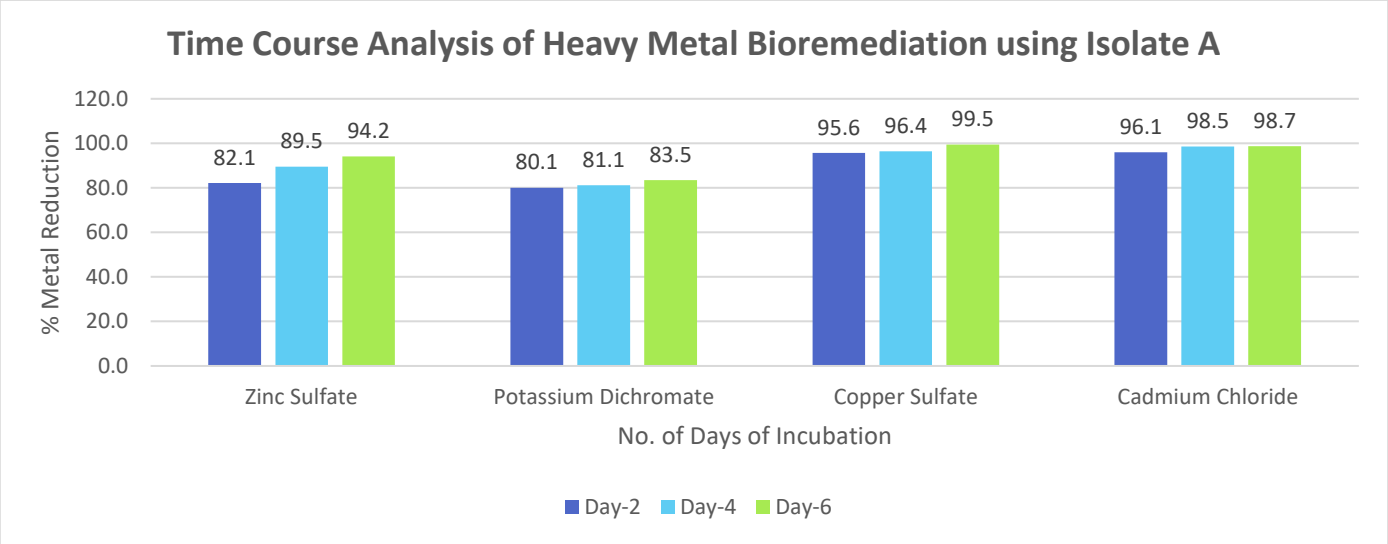

Figure 13: Graphical Representation of % Metal Reduction by Isolate A

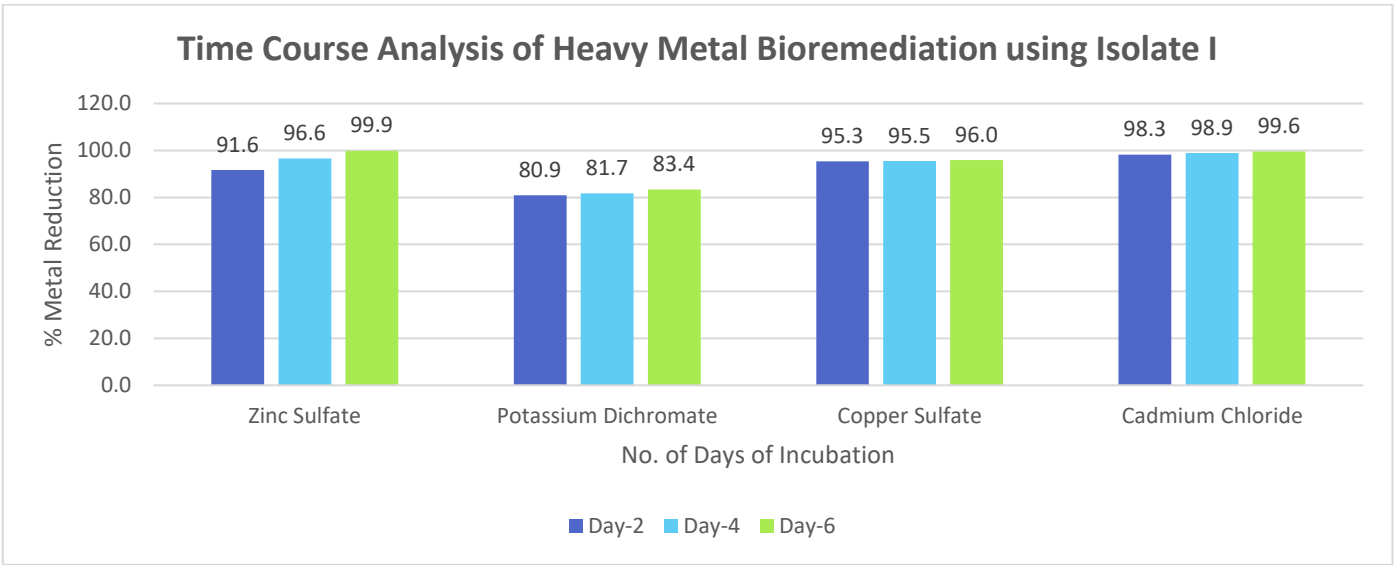

Figure 14: Graphical Representation of % Metal Reduction by Isolate I

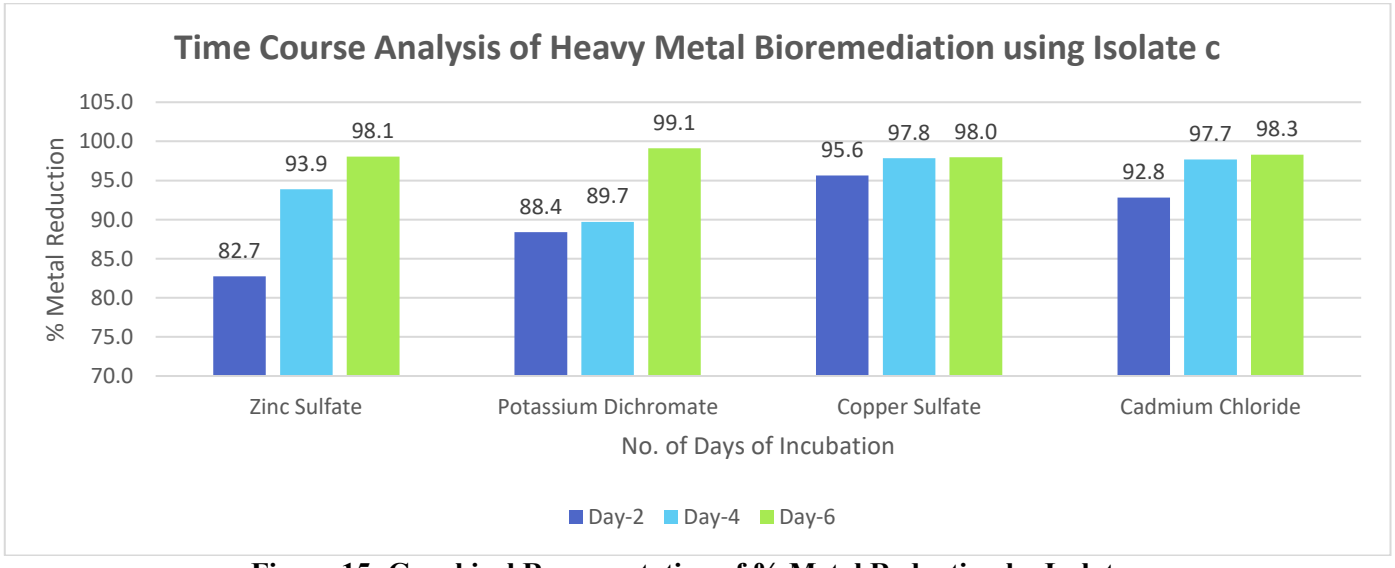

Figure 15: Graphical Representation of % Metal Reduction by Isolate c

Phytotoxicity Assessment: Seed Germination and *Allium cepa* (Onion) Root Assays:

The seed germination and *Allium cepa* root elongation assays showed variable growth responses following microbial treatment of heavy metal containing water.

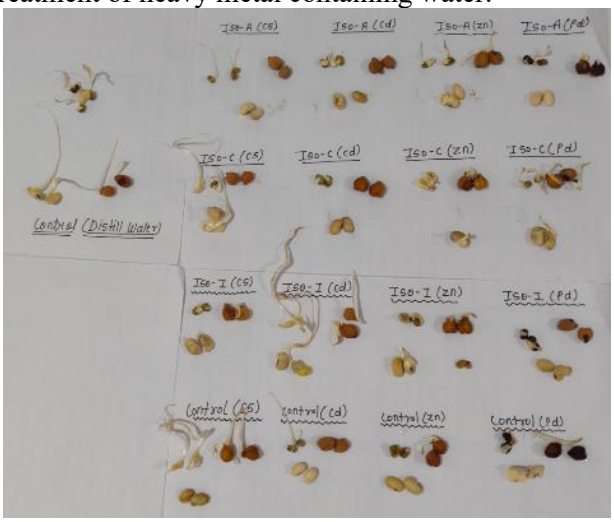

Figure 16: Seed Germination Assay

Figure 17: Onion (*Allium cepa*) Root Elongation Assay

4. CONCLUSION:

The present study successfully demonstrated the isolation and evaluation of heavy metal tolerant microorganisms with potential for the bioremediation from industrial wastewater contaminated with heavy metals. From the 17 microbial strains isolated from industrial effluents, three specific isolates (Isolate A, Isolate I, and Isolate c) exhibited remarkable tolerance to multiple heavy metals including Zinc, Copper, Chromium, and Cadmium. These isolates remained viable at concentrations as high as 1000 ppm for metals such as Zinc, Copper, and Chromium.

The use of green tea extract as a natural chromogenic agent proved to be an efficient, cost-effective and eco-friendly method for the spectrophotometric estimation of heavy metals concentration. The metal reduction assays confirmed that the selected isolates possess bioremediation potential, showing substantial reduction in metal concentration over time.

Time-course studies further revealed that the selected isolates demonstrated substantial heavy metal removal within 48 hours, with removal efficiencies exceeding 80% for most tested metals, indicating rapid and efficient microbial activity. This suggests their suitability for practical wastewater treatment applications.

However, phytotoxicity studies using seed germination and *Allium cepa* root elongation assays indicated mixed results. Although the treated water contains lower heavy metal concentration compared to untreated metal solutions, it did not achieve the safety level equivalent to distilled water. This suggests that while microbial treatment significantly lowers heavy metal concentration, it may not be sufficient as a standalone method to render water completely safe for direct agricultural use.

These findings suggest that the isolated microorganisms have strong potential for application in cost-effective and sustainable bioremediation strategies for industrial wastewater treatment.

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