IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

Investigating Heavy Metal-Induced Stress On Microbial Populations And Their Response To Adaptions And Growth

Bacterial Tolerance and Growth under Lead and Mercury Stress

[1]Shambhavi. C. Balagali, [2]Ashrith Kumar A, [3]Ann Irene D

[1]Student, [2]Assistant Professor, [3]Assistant Professor

[1]Department of Life Science,

[1]Garden City University, Bangalore, India

Abstract: Heavy metal contamination is a significant environmental issue. Toxic metals like lead (Pb) and mercury (Hg) do not break down and build up in living organisms. They disrupt how microbes function and can affect ecosystems, even in small amounts. This study looked at how stress from heavy metals impacts bacterial populations isolated from industrial soil samples. We also assessed their tolerance and growth responses to lead acetate ($Pb(C_2H_3O_2)_2$) and mercury chloride ($HgCl_2$). Soil samples were collected from two industrial sites in Bengaluru. We obtained bacterial isolates using serial dilution and culture methods. Pure isolates underwent disc diffusion assays, heavy metal-supplemented sub-culturing, and broth exposure experiments to examine their responses based on metal type and concentration. The disc diffusion assay showed that mercury chloride had strong bacteriostatic activity. It created large zones of inhibition at all tested concentrations (5 mg, 10 mg, and 15 mg) and completely stopped bacterial growth. In contrast, lead acetate created smaller inhibition zones, with bacteria continuing to grow at all concentrations. This indicates partial tolerance and suggests adaptive mechanisms like efflux, sequestration, or biosorption. Broth culture experiments confirmed that the inhibition was dependent on concentration with lead exposure. However, there was no growth in mercury-supplemented broth. Luria broth did not support bacterial growth for either heavy metal, and we consistently observed precipitation, likely due to chemical interactions reducing nutrient availability. Screening on Eosin Methylene Blue agar showed no growth, suggesting that the isolates were non-lactose fermenters. Gram staining confirmed the presence of Gram-positive cocci and bacilli, indicating a diverse bacterial community. Overall, the results showed that bacterial tolerance to heavy metals varies by metal type and concentration. Isolates were more sensitive to mercury but showed greater tolerance to lead. These findings shed light on how microbes adapt to heavy metal stress and underline the potential use of lead-tolerant bacterial strains in bioremediation efforts for contaminated environments. There is a need for more molecular characterization and resistance gene profiling to better understand adaptive mechanisms and improve microbial applications for environmental restoration.

Keywords: Heavy metal contamination, bacterial tolerance, lead acetate, mercury chloride, bioremediation.

I. INTRODUCTION

Heavy metal contamination is a lasting environmental issue worldwide. Metals like lead (Pb), cadmium (Cd), mercury (Hg), arsenic (As), chromium (Cr), and nickel (Ni) are toxic and non-biodegradable. Unlike organic pollutants, heavy metals do not break down into safe compounds. They can stay in soils, sediments, and water for long periods, leading to bioaccumulation in organisms and biomagnification through food chains. This creates serious risks for both ecosystems and human health [3,16]. Major sources of heavy metal release

include industrial activities, mining, smelting, electroplating, burning fossil fuels, using agrochemicals, and poor waste management. These contribute to widespread contamination [14,8].

Microorganisms are crucial for nutrient cycling and soil health, but they are very sensitive to metal stress. Heavy metals affect microbial function by binding to proteins, damaging DNA, altering cell membranes, and producing reactive oxygen species (ROS). These factors can hinder growth and metabolism [13]. As a result, microbial biomass and diversity decline significantly. A recent global meta-analysis found reductions of up to 42% in microbial biomass carbon and 66% in dehydrogenase activity when exposed to heavy metals [7]. This compromises important ecological processes like nitrogen fixation, phosphate solubilization, and organic matter breakdown, ultimately lowering soil fertility and ecosystem resilience [12,6].

Despite these harmful effects, microorganisms have developed various resistance mechanisms. These include efflux pumps, storing metals inside cells, metal-binding proteins, biosorption, biomineralization, and enzymatic changes [11,9]. Horizontal gene transfer helps spread resistance quickly, and co-selection with antibiotic resistance genes raises public health concerns [13]. These adaptive strategies are the basis for bioremediation, which offers an eco-friendly and cost-effective alternative to traditional chemical or physical methods. Recent advancements like biofilm-based techniques, microbial consortia, and omics-driven microbial engineering are improving the effectiveness and range of bioremediation [4,18].

Considering the ecological dangers of heavy metals and the potential of microbial adaptations, this study investigates how bacteria respond to stress from lead and mercury. The goal is to gain insights into microbial tolerance mechanisms and how they can be applied in bioremediation strategies.

Heavy metal contamination is a major global concern due to the persistence, toxicity, and bioaccumulative nature of metals such as cadmium, lead, mercury, and chromium. Anthropogenic activities including mining, industrial effluents, and agricultural practices are the primary contributors to soil and water contamination [7,2]. Once released, these metals disrupt microbial activity, enzyme function, and nutrient cycling, leading to reduced soil fertility and posing risks to agricultural productivity and food chain safety [7]. Traditional remediation strategies such as chemical precipitation or soil washing are costly and often generate secondary waste, whereas biological approaches like phytoremediation and microbial bioremediation are more sustainable and eco-friendly [5,1].

Heavy metals negatively affect microbial populations by inhibiting enzyme activity, damaging cell membranes, and inducing oxidative stress [3,6]. This toxicity is concentration-dependent; for example, cadmium is highly toxic even at low levels, whereas zinc, though essential, becomes inhibitory at elevated concentrations [11]. Such stress reduces microbial growth, diversity, and metabolic activity, ultimately altering microbial community structure [6]. Nevertheless, bacteria have evolved diverse resistance mechanisms, including efflux pumps, intracellular sequestration, enzymatic detoxification, extracellular precipitation, and biofilm formation [11,9]. These adaptations are often plasmid-mediated, facilitating horizontal transfer of resistance traits in contaminated environments [9]. Several studies have documented tolerant genera such as *Pseudomonas*, *Bacillus*, and *Staphylococcus*, which demonstrate survival under heavy metal stress through stress protein induction and enhanced enzyme production [6,5]. Such organisms hold significant potential in bioremediation, where naturally resistant bacteria are employed to detoxify and remove heavy metals from contaminated environments in an eco-friendly and cost-effective manner [5,4].

II. Materials and Methodology

3.1 Materials:

Table.1: Materials Utilized

Category	Materials
Culture media	Nutrient Agar (NA), Nutrient Broth (NB), Luria Bertani Agar (LBA), Luria Broth (LB), Potato Dextrose Agar (PDA), Eosin Methylene Blue (EMB) Agar, Simmon's Citrate Agar, Indole broth, MR-VP broth
Heavy metals	Lead acetate ($(Pb(C_2H_3O_2)_2$), mercury chloride ($HgCl_2$)
Staining Reagents	Crystal violet, Gram's iodine, Safranin, Lacto-Phenol Cotton Blue
Biochemical Reagents	Kovac's reagent (Indole), Barritt's reagents A & B (VP), Citrate indicator

3.2 METHODOLOGY

3.2.1 Sampling

Soil samples were collected from various locations within the Pennya Industrial Area, Bengaluru (Sample-1; 13.0085°N, 77.4996°E), focusing on sites showing industrial activity and waste accumulation, which are likely to have elevated Lead and Mercury levels. At each site, subsamples were collected from a 10-15 cm depth using sterile spatulas and placed into clean glass jars.

Additional samples were collected from Aspen Steel Pvt. Ltd., Medahalli, Bengaluru (Sample-2; 13.0135°N, 77.7310°E). All samples were properly labeled, transported under chilled and aseptic conditions, and stored at 4 °C. Microbial analysis was carried out within 24 hours using serial dilution techniques to ensure sample viability.

3.2.2 Primary isolation of microorganisms

Microbial isolation was carried out using the serial dilution and spread plate method. One gram of soil was suspended in 9 mL of sterile saline solution, and serial dilutions were prepared up to 10^{-9}. Appropriate aliquots from these dilutions were inoculated onto Nutrient Agar (NA), Luria-Bertani Agar (LBA), and Potato Dextrose Agar (PDA) plates using the spread plate technique. The plates were incubated at 28–37 °C for 24–72 hours. Morphologically distinct colonies appearing on NA and LBA plates were selected and sub-cultured into Nutrient Broth (NB) for further growth and characterization.

3.2.3 Secondary inoculation and Heavy Metal Exposure

In the secondary incubation phase, purified bacterial isolates from primary isolation were sub-cultured onto Nutrient Agar (NA) and Luria-Bertani Agar (LBA) plates supplemented with lead (Pb) and mercury (Hg) at 50 ppm. Selected colonies from the primary isolation plates were streaked onto these heavy-metal-supplemented media to evaluate their growth under metal-induced stress. Plates were incubated at 37 °C for 24–72 hours, and microbial growth was monitored.

Additionally, isolates were inoculated into Nutrient Broth (NB) containing 50 ppm of Pb and Hg to assess growth in liquid media. This procedure allowed determination of the maximum tolerance concentration (MTC) for each isolate across different media and metal exposures.

3.2.4 Selective Media Screening and Gram's Staining and Biochemical Characterization of HM-Resistant bacteria isolates

Following secondary incubation on heavy-metal-supplemented media, purified bacterial isolates were subjected to selective media screening to differentiate and preliminarily identify microbial species. Isolates were streaked onto Eosin Methylene Blue (EMB) agar to identify lactose-fermenting gram-negative bacteria. Gram staining was performed to determine gram reaction and cellular morphology, and the catalase test was conducted to detect catalase enzyme activity. Additionally, IMViC tests (Indole, Methyl Red, Voges-Proskauer, and Citrate utilization) were performed for biochemical characterization of the isolates.

Detailed colony characteristics were recorded for all purified isolates grown on Nutrient Agar (NA), Luria-Bertani Agar (LBA), Potato Dextrose Agar (PDA), and heavy-metal-supplemented plates. Observations included colony size, shape, color, surface texture, elevation, margin, opacity, and edge, which were used to differentiate and categorize the bacterial isolates.

3.2.5 Preparation of Heavy Metal Stocks and Discs for Tolerance Testing

Heavy metal stock solutions were prepared using analytical-grade salts of Mercury Chloride and Lead Acetate to evaluate microbial tolerance under concentration-dependent conditions. Each stock solution was prepared by dissolving 8 g of the respective salt in 60 mL of distilled water, yielding a working concentration of 1.3 mg/mL. From these stocks, discs containing 5 mg, 10 mg, and 15 mg of each heavy metal were prepared by dispensing 5 mL, 10 mL, and 15 mL of solution, respectively.

Sterile Whatman No. 1 filter paper discs (6 mm diameter) were used for disc preparation. The discs were immersed in the heavy metal solutions and placed on a shaker at 203 rpm for 2 h to ensure uniform absorption, followed by overnight soaking under aseptic conditions. The next day, the discs were dried under sterile laminar airflow to eliminate excess surface moisture while retaining the absorbed metal content. A negative control disc was prepared by soaking in 15 mL sterile saline under identical conditions.

For antimicrobial sensitivity testing, actively growing bacterial cultures were swabbed uniformly on Nutrient Agar (NA) and Luria-Bertani Agar (LBA) plates to obtain confluent lawns. The prepared heavy metal discs (5 mg, 10 mg, and 15 mg) were aseptically placed on the inoculated plates using sterile forceps. Plates were incubated at 37 °C for 24 h, after which zones of inhibition were measured (in mm) to assess

bacterial sensitivity or resistance to each concentration of Lead Acetate and Mercury Chloride. This disc diffusion assay provided a comparative evaluation of microbial tolerance against increasing concentrations of the test metals.

3.2.6 Subculturing with Heavy Metal Stock

Bacterial isolates were sub-cultured in Nutrient Broth (NB) and Luria Broth (LB) supplemented with heavy metals to assess concentration-based microbial tolerance. The heavy metals used were Lead acetate ($Pb(C_2H_3O_2)_2$) and Mercury Chloride ($HgCl_2$). The media were supplemented with graded concentrations of each heavy metal (0.05, 0.06, 0.07, 0.08, and 0.09 mg/mL; equivalent to 50, 60, 70, 80, and 90 µg/mL) to study bacterial growth under increasing metal exposure. The inoculated NB and LB vials were incubated at 37 °C for 24–72 hours.

III. RESULTS AND DISCUSSION

4.2.1 Sampling: Serial dilutions of the collected soil samples were prepared up to 10^{-9}, and aliquots from each dilution were plated on Nutrient Agar and Luria Bertani Agar for microbial enumeration and also utilized for primary isolation of bacterial colonies.

4.2.2 Primary isolation of Microorganisms

Microbial colonies were successfully isolated from soil samples using the serial dilution and spread plate method, with visible growth observed on Nutrient Agar (NA) and Luria-Bertani Agar (LBA) plates. Colonies were characterized based on morphology, size, color, texture, and elevation, revealing distinct patterns across different dilutions.

For Sample-1 (NA plates), colonies at the 10^{-3} dilution were circular and spread, creamy to off-white, opaque, with dense and uniform growth and a smooth, moist surface, though elevation was not specified. At the 10^{-2} dilution, colonies were circular, creamy and opaque, with dense growth, smooth creamy texture, and raised elevation. At the 10^{-1} dilution, colonies were irregular and lobate, creamy and opaque with some translucent areas, exhibiting dense growth with mucoid, sticky texture and raised to flat elevation.

In contrast, Sample-2 (NA plates) showed circular colonies at all dilutions; at 10^{-1}, colonies were raised with smooth, entire edges, uniform surface, and yellowish-white color; at 10^{-2}, colonies were convex, smooth, and whitish-grey; and at 10^{-3}, colonies were convex with smooth, even edges and creamy or white color, displaying uniform surface and texture.

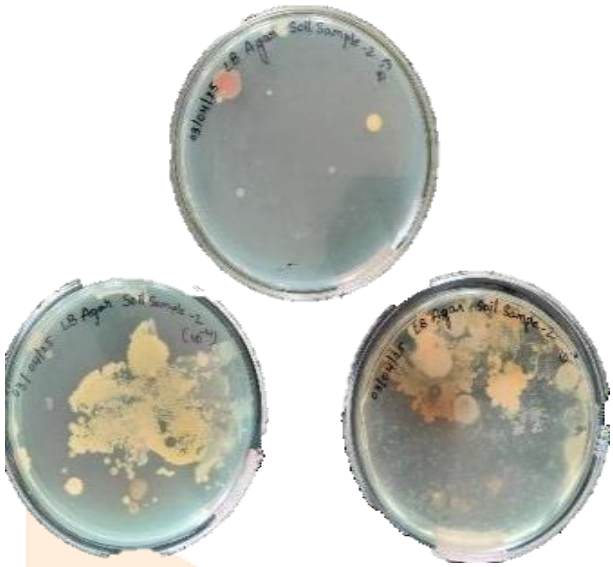
Fig.1& 2: The culture grown in the nutrient agar from the sample 1 and 2

Sample-2 Bacterial Colony Characteristics on LBA Plates

For Sample-2 grown on Luria-Bertani Agar (LBA) plates, colonies exhibited circular to irregular shapes across dilutions. At the 10^{-1} dilution, colonies were small pinpoint in size, yellowish, orange, or brown in color, smooth with slight slime, with smooth, flower-like, lobed margins, and raised or convex elevation. At the 10^{-3} dilution, colonies ranged from pinpoint to large, yellow, orange, or creamy-white, smooth and sticky in texture, with smooth, lobed, ciliate margins, and raised elevation. At the 10^{-4} dilution, colonies were

pinpoint, punctiform, or large, orange, white, or cream in color, smooth with slight slime, lobate margins, and elevation varying from some raised to flat.

Fig.3: Cultures grown in LBA from the sample -2



4.2.3 Secondary inoculation and Heavy Metal Exposure

In the secondary inoculation phase, purified bacterial isolates from sample-1 and sample-2 were sub-cultured onto Nutrient Agar (NA) plates supplemented with varying concentrations of Lead (Pb), and Mercury (Hg), to assess bacterial tolerance under heavy metal stress.

In sample-1, bacterial growth was observed on Lead-supplemented plates at all tested concentrations (0.05g, 0.10g, and 0.15g per 100ml). Although colony size and density decreased with increasing concentrations, bacterial growth persisted, indicating a moderate level of tolerance to Lead. However, no bacterial growth was observed on any of the Mercury- supplemented Nutrient Agar plates at any concentration, suggesting that the isolates from sample-1 were highly sensitive to these two metals and could not tolerate even the lowest concentration tested.

In contrast, sample-2 exhibited growth only on Lead-supplemented plates. Bacterial colonies were present at all concentrations of Lead, though reduced colony size and density were noted at higher concentrations. No bacterial growth was observed on the Mercury- supplemented plates in sample-2, indicating complete growth inhibition and very low tolerance to these metals. Overall, the results indicate that both samples demonstrated the highest tolerance to Lead, while Mercury was highly toxic to the isolates.

Table. 2: Morphological Colony Characteristics of Sample-1 grown on Heavy metals NA plates

Heavy metal agar (in dilution)	**Shape**	**Size**	**Color**	**Texture**	**Edge**	**Growth**
Lead Acetate (10^{-3})	Circular	Small colonies	Brown, creamy white	Smooth	Shiny, smooth	Growth
HgCl2	-	-	-	-	-	No Growth

Table. 3: Morphological Colony Characteristics of Sample- 2 grown on Heavy metal NA plates

Heavy metal Agar (in dilution)	Shape	Size	Color	Texture	Edge	Growth
Lead Acetate (10^{-3})	Circular, irregular colonies	Small colonies	Pigmented yellow and orange color	Smooth, rough and shiny	-	Growth
HgCl2	-	-	-	-	-	No Growth

4.2.4 Selective Media Screening and Gram's Staining and Biochemical Characterization of HM-Resistant bacteria isolates

Purified bacterial isolates were further characterized using selective media and staining techniques. The isolates were streaked onto Eosin Methylene Blue (EMB) agar to screen for lactose-fermenting, gram-negative bacteria; however, no growth was observed for isolates from either Sample-1 or Sample-2, suggesting they were either gram-positive or non-lactose-fermenting. Gram staining of the purified isolates revealed the presence of both gram-positive and gram-negative bacteria. Gram-positive cells appeared purple, while gram-negative cells stained pink or red. Morphological examination indicated a mix of spherical and bacilli-shaped bacteria (cocci and rods) among both groups, highlighting the diversity of bacterial populations capable of surviving under heavy metal stress.

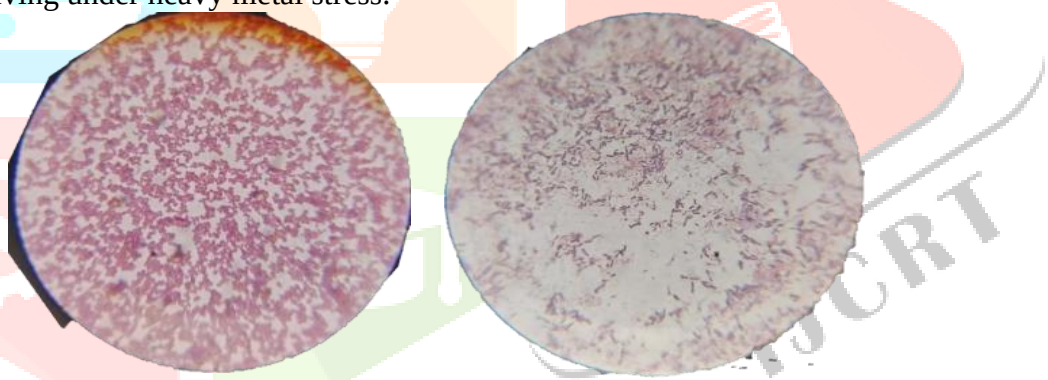


Fig. 4&5: Gram staining of the culture from heavy metal agar plates

The IMViC test was performed to biochemically characterize bacterial isolates exposed to heavy metals. Growth and biochemical reactions were observed only in lead acetate-treated cultures, while mercury chloride-treated cultures showed no growth or color change, indicating complete inhibition by mercury. The lead-tolerant isolate exhibited a negative indole test (green color), a negative methyl red test (yellow ring), a positive Voges-Proskauer reaction, and a positive citrate utilization test (greenish-blue color), yielding an overall IMViC pattern of – – + + which is characteristic of certain gram-negative enteric bacteria such as *Enterobacter* or *Klebsiella* species. A distinct brown coloration in the lead-supplemented broth was also observed, likely due to interactions between bacterial metabolites and lead ions or formation of lead precipitates. These results suggest that the isolate is metabolically adapted to tolerate lead, whereas mercury exposure completely inhibited bacterial growth and biochemical activity.

Table. 4: IMVIC test of Lead- resistant isolate

Biochemical Test	Observation of Lead-resistant isolate
Indole	- (Brown and Green color)
Methyl Red (MR)	-(Yellow color ring)
Voges-Proskauer (VP)	+ (dark red ring)
Citrate	+ (Greenish Blue)

4.2.5 Disc diffusion Assay for Heavy Metal Tolerance

In the disc diffusion assay conducted to assess bacterial tolerance to heavy metals, distinct concentration-dependent inhibition patterns were observed across Lead acetate and Mercury Chloride treatments. In the case of Lead Acetate, both sample 1 and sample 2 demonstrated moderate tolerance, with small inhibition zones around the 5mg discs and progressively larger zones at 10mg and 15mg concentrations, suggesting that Lead exerted a measurable inhibitory effect but allowed some bacterial survival, particularly at lower concentrations. In case of Mercury chloride produced large and clear inhibition zones at all concentrations, and no bacterial growth was observed inside the zones, confirming that the bacteria were completely sensitive to mercury.

Overall, the results revealed that Lead showed the highest tolerance across both samples and mercury chloride exhibited stronger toxicity that Lead and that disc diffusion assay effectively demonstrated the inhibitory effects of the two heavy metals in a concentration-dependent manner. The assay confirmed that bacterial sensitivity to heavy metals was concentration- dependent, with larger zones of inhibition consistently forming at higher metal concentrations, and no bacterial growth was observed withing the inhibition zones across all tested metals, validating the effectiveness of the disc diffusion method in evaluating metal tolerance.

The summarized disc diffusion assay results reveal distinct bacterial responses to different heavy metals across varying concentrations. Lead acetate produced consistent zones of inhibition around 6.6 mm to 8.2 mm, indicating moderate sensitivity and suggesting the potential for bacterial resistance mechanisms to develop under prolonged exposure. In contrast, Mercury chloride produced clear and larger zones of inhibition, ranging approximately from 9.2 mm to 12.0 mm, with no bacterial growth observed inside the zones. This indicates that the tested bacteria were highly sensitive to mercury, and the metal exhibited strong bacteriostatic activity at all concentrations tested. These results demonstrate that mercury is more toxic to the tested bacteria than lead and that the inhibitory effect of both metals increases with concentration. Overall, Mercury chloride demonstrated the highest toxicity, followed by Lead, confirming the differential tolerance of bacteria to heavy metal stress and their potential adaptation to sub-lethal concentrations.

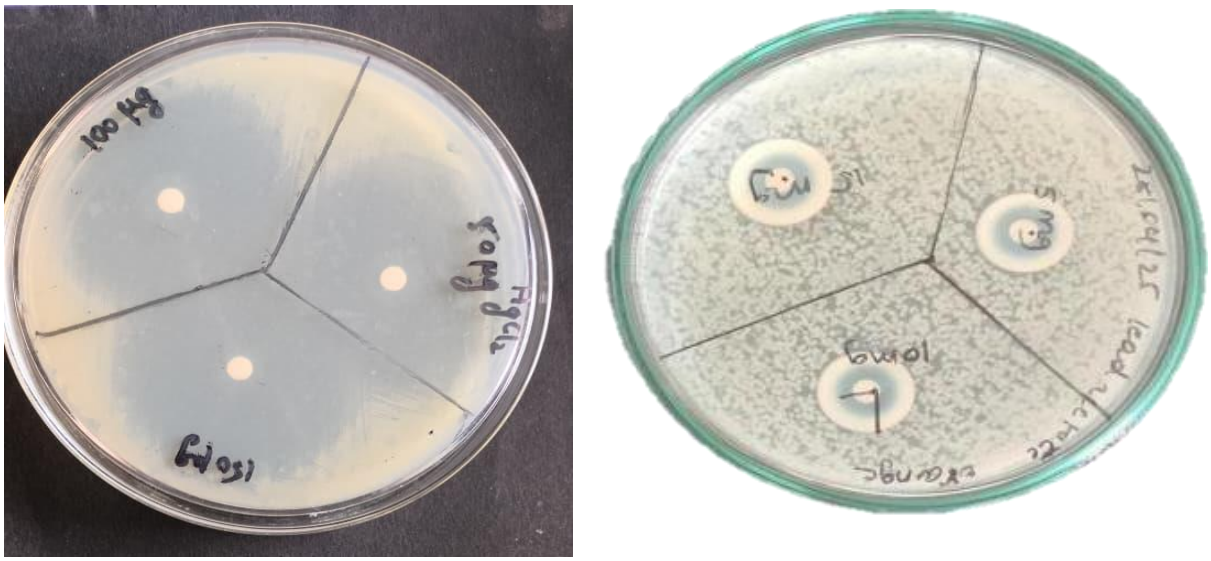
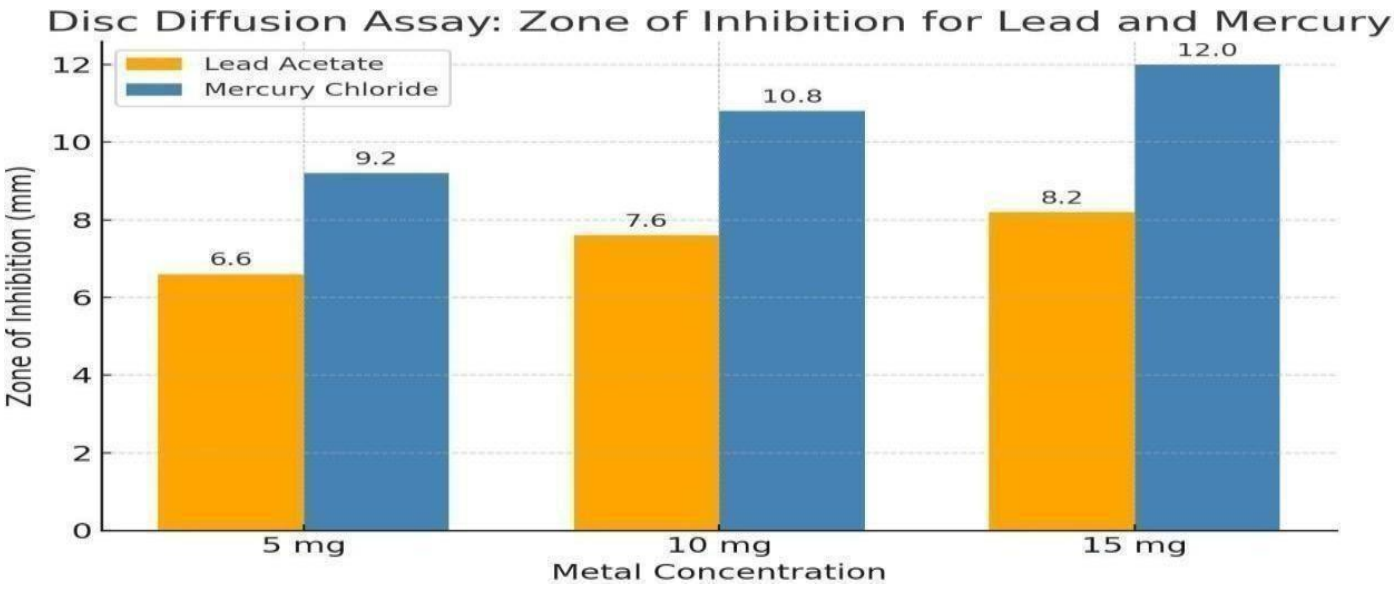


Fig. 6: Zone of Inhibition for Mercury and Lead respectively

Fig.7: The graph showing the zone of inhibition of Lead acetate and Mercury chloride



4.2.6 Subculturing with Heavy Metal Stock

Bacterial isolates were successfully sub-cultured in Nutrient Broth (NB) and Luria Broth (LB) supplemented with Lead Acetate (Pb(C2H3O2)2) and Mercury chloride (HgCl2) at concentrations of 5mg, 6mg, 7mg, 8mg, and 9mg per 100 ml. In Nutrient broth, bacterial growth was observed across all concentrations for Lead, with reduced turbidity at higher concentrations, indicating partial tolerance and a concentration-dependent inhibitory effect. No visible growth was noted in Nutrient Broth supplemented with Mercury suggesting strong toxicity even at lower concentrations.

In contrast, no bacterial growth was observed in any of the Luria Broth (LB) vials across both heavy metals and concentrations. Instead, precipitate was observed forming at the bottom of the LB tubes, indicating chemical interactions or salt precipitation, which may have contributed to complete growth inhibition in LB. These findings suggest that Nutrient Broth supported limited bacterial survival and adaptation under metal-induced stress, while LB conditions were unfavorable, possibly due to media composition or metal-ligand interactions adversely affecting bacterial physiology.

Table .5: Sub cultured in vials containing NB and LB at different concentrations from 5mg- 10mg. (G-growth, NG- Non growth, Ppt- Precipitation)

Nutrient Broth	Conc. 5mg (S1)	Conc .5mg (S2)	Conc. 6mg (S1)	Conc. 6mg (S2)	Conc. 7mg (S1)	Conc. 7mg (S2)	Conc. 8mg (S1)	Conc. 8mg (S2)	Conc. 9mg (S1)	Conc. 9mg (S2)
Lead	G	G	G	G	G	G	G	G	G	G
Mercury	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG

Luria Bertni Broth	Conc. 5mg (S1)	Conc .5mg (S2)	Conc. 6mg (S1)	Conc. 6mg (S2)	Conc. 7mg (S1)	Conc. 7mg (S2)	Conc. 8mg (S1)	Conc. 8mg (S2)	Conc. 9mg (S1)	Conc. 9mg (S2)

Lead	Ppt	Ppt	Ppt	Ppt	Ppt	Ppt	LG	NG	G	NG
Mercu r y	Ppt	Ppt	Ppt	Ppt	Ppt	Ppt	Ppt	Ppt	Ppt	Ppt

4.2.7 Discussion

The present study focused on evaluating the effect of heavy metal-induced stress on bacterial populations isolated from industrial soil samples, with particular emphasis on their tolerance and growth response in the presence of Lead Acetate (Pb(C2H3O2)2) and Mercury Chloride (MgCl2). Through disc diffusion assays, heavy metal-supplemented sub-culturing, and broth-based exposure, the bacterial isolates exhibited clear metal-specific and concentration-dependent tolerance patterns [1,15].

The disc diffusion assays revealed that Mercury Chloride was highly toxic to all tested bacterial isolates. Both sample 1 and sample 2 showed large zones of inhibition surrounding mercury discs at all tested concentrations (5 mg, 10 mg, and 15 mg), indicating complete sensitivity and an inability to withstand mercury exposure. Mercury's known ability to disrupt cellular enzyme activity, protein structure, and DNA integrity may explain the total inhibition of bacterial growth observed in this study [2,6]. These toxic effects result in complete growth inhibition, which aligns with the present study's findings.

In contrast, Lead Acetate was the most tolerated heavy metal among the three tested. Both sample-1 and sample-2 demonstrated growth at all concentrations, although the size of the inhibition zones increased with increasing lead concentrations. These results suggest that the bacterial isolates possess some level of resistance or adaptive capacity to lead exposure, which may be due to efflux or sequestration mechanisms that reduce intracellular lead toxicity [18,10].

Sub-culturing in Nutrient Broth (NB) and Luria Broth (LB) further confirmed the differential tolerance patterns. Bacterial growth was observed in Nutrient Broth supplemented with Lead Acetate, although turbidity decreased with increasing concentrations, indicating concentration-dependent growth inhibition. Notably, no bacterial growth was observed in Luria Broth across both heavy metals and concentrations, and precipitation was consistently seen in the LB tubes or vials. This precipitation may have resulted from interactions between the heavy metal salts and broth components, which could alter the availability of essential nutrients or create an unfavorable environment, leading to complete growth inhibition. These findings suggest that the media composition plays a critical role in supporting or restricting bacterial growth under metal stress [1].

Selective media screening using Eosin Methylene Blue (EMB) Agar showed no bacterial growth, which may indicate that the isolates were non-lactose fermenters and potentially Gram-positive bacteria. Gram staining results confirmed the presence of Gram-positive bacteria among the isolates, indicating a diverse bacterial population. Sub-cultured in Nutrient Broth for further incubation and observed aerobic and anaerobic organisms. Overall, the results of this study clearly demonstrate the bacterial tolerance to heavy metals is both metal-specific and concentration-dependent. The isolates exhibited the highest sensitivity to Mercury, variable sensitivity, and the greatest tolerance to Lead [10]. Furthermore, the distinct growth patterns observed between solid (Nutrient Agar) and liquid (NB and LB) media emphasize the importance of environmental and nutritional factors in bacterial survival under metal-induced stress [1]. This study provides valuable evidence for understanding bacterial adaptation to heavy metal contamination and highlights the potential for identifying metal-tolerant strains that could contribute to future bioremediation applications [17].

IV. Conclusion and Scope for Future Study

The present study systematically evaluated the concentration-dependent effects of heavy metal exposure on bacterial isolates obtained from industrial soil samples. The results demonstrated that bacterial tolerance to heavy metals is highly variable and metal-specific, with distinct susceptibility patterns observed for Mercury Chloride ($HgCl_2$) and Lead Acetate ($Pb(C_2H_3O_2)_2$). Mercury Chloride exhibited the most potent bacteriostatic effect, resulting in complete growth inhibition across all tested concentrations, whereas Lead Acetate showed the highest tolerance levels, with bacterial proliferation persisting even under increased metal stress. The differential responses observed between solid and liquid media emphasized the influence of nutrient composition and environmental conditions on microbial adaptability. The absence of bacterial growth in Luria Broth supplemented with heavy metals, accompanied by precipitate formation, highlights potential physicochemical interactions affecting bacterial survival. The presence of both aerobic and anaerobic bacterial populations in Nutrient Broth further indicates the diversity of microbial responses to heavy metal stress.

These findings provide valuable insights into bacterial stress response mechanisms under heavy metal contamination and establish a foundational understanding for the potential application of heavy metal-tolerant bacteria in bioremediation and ecological restoration of polluted environments. The observed concentration-dependent inhibition patterns suggest that heavy metal exposure can selectively suppress sensitive strains while allowing resistant populations to thrive, highlighting the role of environmental factors, previous exposure history, and potential genetic adaptations in bacterial survival. The selective pressure exerted by metals like Mercury may drive the evolution of resistance mechanisms, with significant implications for microbial community structure in contaminated ecosystems.

The study also opens promising avenues for future research in microbial bioremediation. Lead-tolerant bacterial isolates can be further explored for their practical application in both in-situ and ex-situ bioremediation processes aimed at reducing heavy metal concentrations in soils and water bodies. Future studies should focus on assessing metal uptake capacity, biosorption potential, and overall bioremediation efficiency under field and laboratory conditions. Developing microbial consortia combining different metal-tolerant bacteria could enhance remediation efficiency, particularly at sites contaminated with multiple heavy metals. Integration with phytoremediation techniques, where metal-tolerant bacteria support plant growth and metal absorption, offers additional opportunities for eco-friendly and effective site restoration. Long-term studies evaluating the stability, adaptability, and biofilm-forming ability of these isolates in natural contaminated environments will help identify the most suitable candidates for sustained remediation activities. Comparative analyses across different industrial sites, soil types, and aquatic ecosystems will provide broader insights into the versatility and practical application of these microbes.

Collectively, this research contributes to a better understanding of how microbial populations respond to environmental contaminants and lays the groundwork for developing cost-effective, sustainable, and scalable microbial solutions for the remediation of heavy metal-contaminated environments, thereby supporting environmental protection and pollution management strategies.

Acknowledgment

The authors gratefully acknowledge Garden City University for providing the essential infrastructure and academic support required for this study. I also extend our sincere appreciation to Mr. Ashrith Kumar A and Ms. Ann Irene D for their expert guidance, critical insights, and constructive suggestions, which were instrumental in the successful execution and completion of this research.

References

1. Ali, S., & Khan, E. (2018). Heavy metal tolerance in bacteria isolated from industrial soils. *Journal of Environmental Biology, 39*, 123–135. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC12370754
2. Barkey, D., & Olson, M. (2003). Mercury toxicity and microbial inhibition: Mechanistic insights. *Environmental Toxicology and Chemistry, 22*(12), 2872–2879. https://academic.oup.com/femsre/article/27/2-3/355/615559
3. Fowler, B. A. (2016). Monitoring human populations for early markers of cadmium toxicity: A review. *Toxicology and Applied Pharmacology, 311*, 64–72. https://doi.org/10.1016/j.taap.2016.09.009
4. Gadd, G. M. (2004). Microbial influence on metal mobility and application for bioremediation. *Geoderma, 122*(2-4), 109–119. https://doi.org/10.1016/j.geoderma.2004.01.002
5. Gadd, G. M. (2010). Metals, minerals and microbes: Geomicrobiology and bioremediation. *Microbiology, 156*(3), 609–643. https://doi.org/10.1099/mic.0.037143-0
6. Liu, P., Wang, L., & Chen, J. (2024). Mercury-induced stress in soil bacteria: Effects on growth and DNA integrity. *Ecotoxicology and Environmental Safety, 252*, 114487. https://www.sciencedirect.com/journal/ecotoxicology-and-environmental-safety/vol/271/suppl/C
7. Liu, Y., Zhang, X., Chen, J., & Wang, P. (2025). Global meta-analysis of heavy metal effects on soil microbial biomass and enzyme activities. *Science of the Total Environment, 924*, 171567. https://doi.org/10.1016/j.scitotenv.2018.08.294
8. Martinez, C., & Lee, J. (2019). Sources and impacts of heavy metal pollution: A comprehensive review. *Environmental Chemistry Letters, 17*(2), 729–754. https://doi.org/10.1007/s10311-018-00823-6
9. Mathivanan, K., Rajaram, R., & Balasubramanian, V. (2019). Microbial detoxification of heavy metals: Mechanisms and impact on environmental bioremediation. *International Journal of Environmental Science and Technology, 16*(4), 2079–2092. https://www.researchgate.net/publication/363033111_Mechanism_of_Microbial_Detoxification_of_Heavy_Metals_A_Review

10. Nazir, R., Qureshi, R. A., & Khan, M. S. (2020). Bacterial adaptation to lead contamination: Growth response and tolerance. *BMC Microbiology*, *20*, 88. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7175174/
11. Nies, D. H. (1999). Microbial heavy-metal resistance. *Applied Microbiology and Biotechnology*, *51*(6), 730–750. https://doi.org/10.1007/s002530051457
12. Patel, R., & Zhang, L. (2019). Microbial communities and heavy metal stress: Implications for soil fertility. *Soil Biology and Biochemistry*, *131*, 50–68. https://doi.org/10.1016/j.soilbio.2019.01.012
13. Sharma, R., & Gupta, A. (2023). Co-selection of heavy metal and antibiotic resistance in bacteria: Implications for human health. *Frontiers in Microbiology*, *14*, 115206. https://pmc.ncbi.nlm.nih.gov/articles/PMC11253441/
14. Smith, K., Jones, D., & Taylor, M. (2021). Industrial sources of heavy metal contamination: A global review. *Environmental Pollution*, *279*, 116898. https://doi.org/10.1016/j.envpol.2021.116898
15. Sun, Y., Zhang, Q., & Li, H. (2020). Impact of heavy metals on soil bacteria: Resistance and adaptation. *Frontiers in Microbiology*, *11*, 567890. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC12370754/
16. Thompson, L. A., & Turner, A. (2020). Heavy metals in aquatic ecosystems: A review of risks to organisms and human health. *Environment International*, *140*, 105750. https://doi.org/10.1016/j.envint.2020.105750
17. Vats, P., Gupta, S., & Sharma, A. (2021). Bioremediation potential of metal-tolerant bacteria: Applications in contaminated soils. *Journal of Hazardous Materials*, *416*, 125746. https://www.sciencedirect.com/science/article/pii/S2590182624000109
18. Ventosa, A., & Ruiz-Berraquero, F. (2013). Mechanisms of heavy metal resistance in bacteria. *Microbiology and Molecular Biology Reviews*, *77*(2), 123–145. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC12370754/