# Isolation and Characterization of Heavy Metal (Lead)-Resistant Bacteria For Bioremediation of Industrial Wastewater

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A thesis submitted to the Department of Mathematics and Natural Sciences in partial fulfillment of the requirements for the degree of
Bachelor in Science in Biotechnology

Department of Mathematics and Natural Sciences
BRAC University
September, 2025

# Declaration

It is hereby declared that-

1. The thesis submitted is my/our own original work while completing degree at BRAC University.
2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
3. The thesis does not contain material which has been accepted, or submitted, for any other degree or diploma at a university or other institution.
4. We have acknowledged all main sources of help.

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# Approval

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of Summer, 2025 has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Bachelor of Science in Biotechnology on 21 August, 2025.

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## Ethics Statement

This scientific study is done under proper supervision and it is the author's original work. No animal was harmed or injured during the experiments. The article is written in a manner that the writeup does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing. The experiment was done by maintaining all the rules and regulations of the Biotechnology and Microbiology laboratory of the Department of Mathematics and Natural Sciences, BRAC University.

## Abstract:

Lead pollution in industrial wastewater poses an important environmental and health crisis due to its toxicity and persistence. To address this issue, two soil samples were collected from two lead-contaminated industrial zones in Bangladesh, an automobile workshop situated in Mohakhali, Dhaka and an old battery factory area in Comilla. Atomic Absorption Spectroscopy confirmed elevated levels of lead (Pb) (42.6 ppm and 61.5 ppm respectively). Metagenomic analysis of soil samples by bacterial 16s rDNA sequencing revealed diverse microbial populations, dominated by Pseudomonadati and Bacillati phylum, suggesting adaptation to heavy metal stress in natural settings. AAS of the cell free supernatants from isolated bacteria demonstrated remarkable bioremediation efficiency, removing approximately 97% and 95% of lead from 100 ppm lead-supplemented solution. Further analysis by 16S rDNA sequencing of the isolated bacterial strain L1 and L2, revealed morphological adaptations under lead stress and identified both isolates as *Priestia* spp.



**Keywords:** Lead contamination; industrial wastewater; lead resistance; bioremediation; soil bacteria.

# Acknowledgement

First and foremost, all the praise is due to the Almighty, whose blessings, guidance and mercy enabled us to complete our thesis successfully. We are wholeheartedly grateful to our parents for their unconditional love, support and prayers, which are the constant source of strength and motivation for us.

Second of all, we are extremely indebted to our thesis supervisor, Dr. M. Mahboob Hossain (Microbiology Program, Department of Mathematics and Natural Sciences), who guided us efficiently in this journey and without whom, the research would not be possible and helped us to find our true potential as researchers. Our heartiest gratitude goes to Md. Firoze H. Haque, PhD (Chairperson, Department of Mathematics and Natural Sciences) and Munima Haque, PhD (Program Director, Department of Mathematics and Natural Sciences) for their constant support to create an outstanding environment in this department for scientific researches. In addition to it, we are extremely thankful to our concurrent research assistant, Ifthikhar Zaman (Lecturer, Department of Mathematics and Natural Sciences) and Mahdi Moosa, PhD (Assistant Professor, Department of Mathematics and Natural Sciences) whose continuous support, advice, and practical help hugely contributed to the successful completion of this work.

We pay our gratitude to Mehedi Hasan, (Scientific Officer, BCSIR), Kiron Sikdar (Scientific Officer, BCSIR), Trisha Paul (Scientific Officer, BCSIR) and Maksudure Rahman Nayem (Senior Scientific Officer, DNA Solutions Ltd) for their prompt assistance in logistics, chemical analysis, imaging and sequencing respectively. We would like to thank our lab peers, lab assistants and friends - who made this journey so much easier and fruitful by their constant support.

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# List of Acronyms

| | |
|---|---|
| ppb | parts per billion |
| ppt | parts per trillion |
| rDNA | Ribosomal DNA |
| AAS | Atomic Absorption Spectroscopy |
| AAC | 1-aminocyclopropane-1-carboxylate |
| ATP | Adenosine Triphosphate |
| ATPase | Adenosine Triphosphatase |
| BLAST | Basic Local Alignment Search Tool |
| CFU | Colony Forming Unit |
| DNA | Deoxyribonucleic Acid |
| FASTA | FAST-All |
| ICP-MS | ICP Inductively Coupled Plasma Mass Spectrometry |
| NA | Nutrient Agar |
| NB | Nutrient Broth |
| PBS | Phosphate Buffer Solution |
| PSB | Phosphate Solubilizing Bacterium |
| UV | Ultraviolet |

# Chapter 1: Introduction

## 1.1. Industrial Wastewater and Conventional Treatment Methods:

In the context of Bangladesh, industrial wastewater is one of the contributors to environmental pollution. Since industrial wastewater is a mixture of complex chemicals, proper treatment methods and facilities are the preconditions for tackling environmental pollution by industrial wastewater. Among the complex compounds, lead (Pb) poses a greater risk because of its toxicity and longevity. The concentrations of lead in groundwater and surface water in several regions of Bangladesh were 0.04–1167 and 0–230 µg/L. In the deep tube wells, it was reported that the average concentration of lead was 0.04–1167 and 0–230 µg/L (Chowdhury et al., 2022). Even after its toxic effects, lead is used extensively for industrial purposes for various reasons (Raj & Das, 2023). The high demand of Pb can be observed in automobile industries and battery industries. Furthermore, lead is cost-effective, stable, and persistent both chemically and economically. It has become suitable for long-term purposes, although lead has a greater health risk, such as causing neurological, cardiovascular, and reproductive diseases (Chowdhury et al., 2022). Lead can be removed from industrial wastewater using a number of conventional methods, such as membrane separation, ion exchange, and coagulation-flocculation (Chowdhury et al., 2022). However, these methods also have serious limitations, which can minimize their effectiveness. For example, these processes produce a lot of sludge that contains lead, and disposing of it is costly. For instance, these methods generate large amounts of lead-containing sludge, and their disposal is quite expensive. The coagulation-flocculation method is not suitable because this method cannot reduce the required concentration of lead set by the regulatory guidelines (Chowdhury et al., 2022). As for the ion exchange method, it is very expensive, and during the treatment process, clogging by organic

matter can occur. Membrane disfiguring, membrane fouling, low persistence, and high operational costs are the main reasons why membrane separation is used at a lower rate, even though it has high removal rates. As a result, the need for sustainable, cost-effective alternatives is greater. Biotechnology-based materials are most popular because of their effectiveness and compatibility.

## 1.2. Bioremediation:

Microbial bioremediation is one of them, in which microorganisms such as bacteria can degrade or remove harmful compounds, including heavy metals, in the environment. Microorganisms can detoxify hazardous materials (Volaric et al., 2021). The unique feature of using microorganisms is that they do not create any secondary pollution. Therefore, it is very useful for removing heavy metals like lead. There are several ways bacteria can tolerate heavy metals, especially lead. Some bacteria can bind to heavy metals by using their cell walls, or sometimes, heavy metals bind to their extracellular structures. Hence, bacteria either absorb or adsorb. This is known as biosorption (Volaric et al., 2021). It can be done by either living or dead bacterial cells. In this mechanism, bacteria use various functional groups such as carboxyl, amino, and phosphate groups in their cell walls or extracellular structures. Another one is the efflux method, where bacteria reduce the concentration of lead, which reduces their toxicity. Later, bacteria pump them out. In the intracellular sequestration method, bacteria use cysteine-rich peptides called metallothioneins to accumulate lead ions inside of their cells, thus reducing toxicity. For extracellular sequestration, lead binds to the extracellular polymeric substances, or sometimes, bacteria use siderophores and precipitate lead outside the cell. Lastly, in the oxidation/reduction method, bacteria can change the oxidation form of Pb, which makes them less toxic. For industrial purposes, there are two types of bioremediation known as in-situ and ex-situ (Volaric et al., 2021). When the contaminants are treated directly at the contaminated

site without removing them, it is known as in-situ bioremediation. In this process, microbial populations are stimulated to degrade the pollutants by providing nutrients and oxygen (Volaric et al., 2021). In-situ bioremediation is used for treating contaminated soils, polluted groundwater, etc. In-situ bioremediation is of two types, which are intrinsic in-situ bioremediation and engineered in situ bioremediation (Volaric et al., 2021). When an existing microbial population is used by providing them necessary nutrients, that process is known as intrinsic in-situ bioremediation but when only specific microorganisms are introduced through stimulation so that the degradation can be accelerated, it is known as engineered in-situ bioremediation. The limitation of in-situ bioremediation is that often its effectiveness is limited by site conditions such as temperature, moisture, and pH. One of the crucial limitations is that reduced lead can reoxidize and become toxic again (Volaric et al., 2021). Furthermore, in-situ bioremediation is much slower than conventional methods. In ex-situ bioremediation, the contaminants are removed from the site, and they are treated in a well-prepared, engineered environment, such as in a bioreactor. Therefore, the environment can be better controlled than in situ bioremediation. There is a solid-phase system in ex-situ bioremediation where contaminants are treated by excavation. In this system, landfarming, biopiles, and composting are used (Volaric et al., 2021). But in the slurry phase system of ex-situ bioremediation, the contaminants are mixed with water, and then they are broken down. Techniques such as bioventing and bioaugmentation are used in ex-situ bioremediation (Volaric et al., 2021). Ex-situ bioremediation can provide better control, ensure faster results, and offer higher scalability. But there are also some limitations (Volaric et al., 2021). Apart from higher cost, disposal of treated materials is also an issue. So, more energy and resources are much needed.

## 1.3. The Importance of Bioremediation of Pb (Lead):

Recent anthropogenic activities have increased the level of lead (Pb) in the environment. Therefore, it is necessary to contain this so that it does not become a crisis in the future. Bioremediation of lead using bacteria can be an important alternative to create a sustainable solution. There are many reasons why this solution must be implemented. Bacteria such as *Bacillus subtilis* have shown their ability to reduce the Pb toxicity from soil by biosorption **(Naik & Dubey, 2013)**. 76% of lead (Pb) was adsorbed from the contaminated agricultural field using *Bacillus subtilis*. Lead is also non-biodegradable, which is why this compound is very effective in accumulating in the environment. Therefore, a long-term negative impact on ecology becomes expected but bacteria can immobilize lead through biosorption and bioremediation. For example, in Bangladesh, leaching is a very common phenomenon, and through this, lead can pollute the groundwater. Bacteria like *Pseudomonas fluorescens* can stop this because it can immobilize lead by creating lead phosphate, which has a reduced bioavailability compared to lead **(Park et al., 2011)**. The reduction rate was 60% to 70% through bioprecipitation, which indicates *Pseudomonas fluorescens* will be highly effective for in-situ bioremediation. Moreover, the use of batteries has been increasing in Bangladesh, and most of the batteries are not recycled. Bangladesh also does not have a proper drainage system, which is why the water bodies are getting polluted because of Pb. Sulfate-reducing bacteria such as *Desulfovibrio* can precipitate lead in sulfide form **(Hu et al., 2024)**. The problem with industrial areas is that whenever the factories are shut down, those lands can never be used for housing or for agriculture but the restoration can be achieved through bacterial bioremediation. If *Bacillus cereus* PSB-2 is combined with biochar, the rate of reducing the amount of Pb increases up to 65.06% **(Zhang et al., 2025)**. Because of this synergistic effect, microbial diversity and nutrient conditions of the lands can be restored.

## 1.4. Metabolic Activity of Lead-Resistant Bacteria:

Some bacterial strains, specifically *Salmonella choleraesuis* and *Proteus penneri* strains, exhibit intracellular bioaccumulation of Pb (Naik et al., 2012). Low molecular weight bacterial metallothionein proteins are encoded by SmtAB genes. So, these proteins bind to Pb and reduce its toxicity. Some strains, such as *Pseudomonas aeruginosa*, have plasmids (Naik et al., 2012). These plasmids contain necessary genes that can trigger Pb resistance, and also, they aid smtAB genes in enhancing Pb tolerance. Because of smtAB genes, bacterial strains such as *Salmonella choleraesuis* can sequester lead up to 19 mg per gram dry weight (Naik et al., 2012). *Proteus penneri* has slightly higher sequestration capacity, but both are capable of detoxifying Pb because both strains have effective metabolic pathways for lead sequestration. Bacterial isolates such as *Pseudomonas stutzeri* and *Vibrio harveyi* have metabolic and resistance-related pathways through which they showed high resistance towards Pb. Using the pbrA gene, they produce p-type ATPase, which uses ATP hydrolysis that can pump out Pb ions out of their cells. This pathway is known as the cation transport pathway (Naik et al., 2013). For this environmental response, they use the OrfA gene, which is involved in gene regulation pathways. Both *Pseudomonas stutzeri* and *Vibrio harveyi* showed they can trap Pb externally rather than intracellularly. This mechanism is facilitated by extracellular polymeric substances (Naik et al., 2013). Also, since both of them have the pbrA gene, which encodes P-type ATPase, ATPase actively pumps out Pb when it is cultured in lead nitrate. So, bioaccumulation is minimized through this mechanism. Also, in *Achromobacter xylosoxidan*, a plasmid pA81 is present, which has the pbtTFYRABC gene cluster (Hložková et al., 2013). In that plasmid, pbtB/C is present, which conducts Pb ion detoxification through phosphate precipitation. Also, other genes, such as pbtT, pbtF, pbtR, etc., are involved in sequestration, efflux activity, and transcriptional regulation. In *Alcaligenes pakistanensis*, high tolerance to Pb was observed

because of its strict aerobic metabolism (Abbas et al., 2015). Therefore, it has highly activated detoxification pathways that conduct energy-intensive detoxification processes.

## 1.5. Atomic Absorption Spectroscopy (AAS):

Atomic Absorption Spectroscopy is used for analyzing specimens (either biological or chemical) based on the intensity of absorbed light. While a specimen is included for analysis, the present cloud of atoms allows the light to be passed through it, and the amount of light is quantified at the resonant wavelength accordingly. The light utilized in this machinery remains in the visible or ultraviolet (UV) region of the electromagnetic spectrum usually. The specific wavelengths give the analysis an excellent specificity and detection limit. Moreover, absorption for each element is specific since no other element (with a different number of electrons present in the atom) will be able to absorb this wavelength. According to **Beaty and Kerber (1993)**, an atom of a specific element owns a certain number of negatively charged electrons around its nucleus. The electrons tend to remain at the lowest possible energy for stability. If the electron is excited by acquiring the light energy (at a particular magnitude) by the atom, it will promote itself to a higher orbital from a lower orbital, resulting in acquiring a less stable configuration (excited state). The instability of the excited electron leads it to spontaneously return into its 'ground state' configuration. This movement releases an amount of light energy initially absorbed during the excitation of the electron that is measured for each given sample accordingly. The percentage of intensity is compared to a calibration curve (the absorbance versus concentration of the element), from which the amounts of specific materials in a sample can be determined. The interface of an AAS is arranged with some fundamental compartments, such as an atomizer, radiation source, and spectrometer.

- **Atomizer:** It transforms a sample remaining in the liquid state to the gaseous state by breaking the chemical bonds for facilitating access to free atoms. Moreover, it removes the solvent so that individual atoms can absorb light. There are several types of atomizers, such as flame atomizers (like air-acetylene), electrothermal atomizers (graphite furnaces), etc.
- **Radiation Source:** This source provides monochromatic radiation (a light of specific wavelength), which is absorbed by the free atoms. A hollow cathode lamp or electrodeless discharge lamp, in general, is utilized to provide radiation to the element of interest.
- **Spectrometer:** This compartment determines the amount of light absorbed by the free atoms in the atomized sample that is directly connected to the concentration of the element. It includes a monochromator and a detector as well for the analysis.

Typically, an AAS is applied for quantitative metal concentrations in a given solution, analysis of lead in paint, monitoring of trace metals in industrial effluent streams, trace elements in product/raw materials along with ICP-MS (Inductively Coupled Plasma Mass Spectrometry), analysis of additives and purity in steels and other metal alloys, analysis of low-level contaminants, etc. This scientific study incorporated the application of AAS due to the assessment of collected soil samples and bacterial samples to determine whether heavy metals are available and in which concentrations those heavy metals might be available. Regarding the bacterial AAS, this aids the scientists in identifying the bioremediating character of the bacteria by measuring the amount of the heavy metal sequestered by the bacterial strain. Contrarily, the AAS of soil samples aids in assessing pollution by any toxic element. Soil may accumulate heavy metals like lead, chromium, nickel, cadmium, etc. from the nearby contact of industrial wastewater. These elements are foreign particles for the bacteria's ecosystem that

can even enter our food chains of humans, animals, and so on. Since AAS is able to rapidly analyze any metal, even in trace levels, with higher sensitivity and specificity, this experiment was applied in the field of assessing the soil (environmental) samples.

## 1.6. Metagenomic Analysis:

Metagenomic analysis is a method where experimental, computational analysis is used to investigate and discover the genomic content and the functional potential of microbial communities. For this analysis, samples such as soil, water, blood, etc. can be used. There are two ways the DNAs are extracted and sequenced. The first one is whole genome shotgun sequencing, and the other one is amplicon-based sequencing **(Navgire et al., 2022)**. So, at the first step, the samples are collected from which DNA will be extracted. The sample collection varies depending on the goal of the research. Next, DNA is isolated. The isolation process also depends on the samples. For example, environmental samples and human-sourced samples have different methods for DNA isolation. Next, the next-generation sequencing library is prepared. This is one of the most critical steps in metagenomic analysis. In this step, a collection of similarly sized DNA fragments with specific adapter sequences added to the 5' and 3' ends is used. In this step, the quantification is done for accurate sequencing. Now for sequencing, 16S rRNA amplicon sequencing can be used for whole genome sequencing. 16S rRNA amplicon sequencing is used for taxonomic profiling and analysis. Fifteen hundred base pairs in size have 9 unique variable regions **(Navgire et al., 2022)**. They are used for primer binding for PCR, but the hypervariable regions are used for identifying sequence diversity. As for whole genome sequencing, it is used for characterizing the abundance of microbial species. This is a PCR-independent method, and a broad range of biomarkers can be used for desired gene identification. The next step is data analysis **(Navgire et al., 2022)**. For taxonomic

analysis, there are bioinformatic tools available. Kraken is a tool for taxonomic classification from metagenomic DNA sequences **(Wood & Salzberg, 2014)**. Lastly, functional analysis can be done using other different bioinformatics tools such as DMAP (Dragon Metagenomic Analysis Platform). If the goal is to analyze more on the taxonomic profiling, Krona can be used, which gives a complete overview based on the taxonomic ranking. Now, 16S rRNA sequencing can be done using second-generation sequencing or third-generation sequencing. For third-generation sequencing such as Oxford Nanopore, filtering must be done extensively to improve data quality. Otherwise, Kraken will show insignificant taxonomic profiling. For filtering, FASTP and Porechop can be used. Porechop eliminates chimeric sequences and ligated adapters. Then, FASTP is used for quality and length filtering.

# Chapter 2: Materials and Methods

## 2.1. Working Place for The Study:

The present research work was performed in the Communicable Disease and Biomarkers (CDAB) Laboratory of the Department of Mathematics and Natural Sciences, BRAC University, Kha 224 Pragati Sarani, Merul Badda, Dhaka-1212, Bangladesh, supervised by Mahboob Hossain, PhD (Professor, Microbiology Program, Department of Mathematics and Natural Sciences, BRAC University) and Akash Ahmed (Senior Lecturer, Microbiology Program, Department of Mathematics and Natural Sciences, BRAC University).

## 2.2. Media, Solutions, and Reagents:

Media, reagents, and solutions that were used in this scientific study were available as a reagent grade, and without further purification, those were used. Nutrient agar (combination of nutrient broth and 2.3% agar) and nutrient broth (both usual and Lead Nitrate supplemented). Lead Nitrate solutions in different concentrations were used for providing the bacteria with the *in-vitro* stress, similar to the stress given by the exposure of free lead ions in the soil environment. On the other hand, 0.1M Phosphate Buffer Solution (PBS), 2.5% Glutaraldehyde, ethanol (with the concentrations of 30%, 50%, 70%, 80%, 90% and 100%) - these reagents were applied in different phases of experiments to achieve the desired results.

## 2.3. Handling of Laboratory Equipment:

Detergents and liquid dishwashing soap were used to wash all the glasswares, falcon tubes and rinsed 4-5 times with tap water. Autoclavable equipment was sterilized by autoclaving at 121°C for 15 minutes at 15 psi. The microbiological works were done inside the biological safety cabinet.

## 2.4. Sample Collection from Different Industrial Sites:

The soil samples were collected from two different locations in Bangladesh. The first sample was collected from a former battery factory area near Bangladesh Small and Cottage Industries Corporation (BSCIC) Office, Comilla, Bangladesh, which is shut down at present. It was run for approximately 30 years where lead batteries were disposed of directly in the open environment. The second sample was collected from an automobile workshop of Mohakhali, Dhaka which has been running for approximately 10 years. The soil from different adjacent zones in one particular collection site were collected in zipped polyethylene bags. The samples were brought to the laboratory after the day of the collection. The site was chosen because it was hypothesized that there might be endogenous microorganisms present which received a persistent period of stress from the excessive amount of free Pb ions available in the soil and might have evolved a lead biodegradation pathway.

## 2.5. Atomic Absorption Spectroscopy (AAS) of Soil Samples:

### 2.5.1. Preparation of Soil Samples for AAS:

A 100-mL aliquot of well-mixed soil sample was transferred to a beaker. Addition of 2 mL of concentrated $HNO_3$ and 5 mL of concentrated HCl was done into the sample for the

heavy metal that was to be analyzed. The sample was covered with a ribbed watch glass and was heated in a steam bath at 90℃ to 95℃ until the sample was fully dried. After that, the beaker was removed and was allowed for cooling. The beaker walls and the watch glasses were washed down with water and, when necessary, the sample was centrifuged to 10,000 rpm for 10 minutes to remove silicates and other insoluble material that probably clogs the nebulizer at usual cases. Lastly, the final volume was adjusted to 100 mL with reagent water.

### 2.5.2. Assessment:

The assessment of the digested soil samples of two collection sites containing Pb (to be analyzed) were assessed by the scientific personnel of Bangladesh Council of Scientific and Industrial Research (BCSIR), Qudrat-I-Khuda Road, New Elephant Road, Dhanmondi, Dhaka-1205, Bangladesh.

## 2.6. Metagenomic Analysis:

The collected soil samples were sent to DNA Solutions Ltd, Dhaka, for extracting metagenome data of the present genetic material (16S rDNA), yielding FASTQ files, which were transformed into a FASTQC file. After that, the adapter trimming and filtering were done on the platform of Porechop. The final filtering was done and the FASTP format files were rendered. Lastly, the data was analyzed using Kraken and Krona Pie Chart Plot to understand the abundance of present bacterial population in the collected soil samples.

## 2.7. Bacterial Isolation:

The soil samples were diluted at different dilutions from the stock, formerly labelled as $10^0$, those were: $10^{-1}$, $10^{-2}$, $10^{-3}$ and $10^{-4}$. The diluted samples from each soil sample were spread

on lead nitrate (100 ppm lead) treated nutrient agar plates and kept for 24 hours incubation at 37℃. After the incubation, the grown colonies were subcultured on the lead nitrate (100 ppm lead) supplemented nutrient agar plates by streaking. After 24 hours of incubation at 37℃, the subcultured isolates were inoculated in the lead nitrate (100 ppm lead) supplemented broth. After that, the bacteria that came from Comilla soil samples and survived in lead-mediated stressed broths were subcultured on lead nitrate (100 ppm lead) supplemented nutrient agar plates again for preserving the master stock, labeled as L1 and L2 respectively.

## 2.8. Atomic Absorption Spectroscopy (AAS) of Bacterial Samples:

### 2.8.1. Preparation of Bacterial Samples for AAS:

The colonies of isolated bacteria, labelled as L1 and L2, previously grown and preserved on nutrient agar plates were taken out from the fridge and kept for approximately 3-4 hours in the incubator with the temperature of 37℃. After that, a loopful of bacteria from each plate were taken and were inoculated for each of the falcons containing 100 ppm lead nitrate supplemented nutrient broth as experimental samples of L1 and L2 and kept in a shaker incubator with the temperature of 37℃ for 24 hours of incubation. Besides that, two controls were made: the positive control was made of nutrient broth supplemented with lead nitrate (100 ppm lead) only (no bacteria inoculated) and the nutrient broth only (lead nitrate and bacteria excluded) was made as the negative control. The controls were incubated in the same condition along with the experimental samples. After the incubation period, the bacterial suspensions of L1 and L2 (experimental samples) were centrifuged for 10 minutes at 10,000 rpm. After that, the cell free supernatant was collected by separating it from the pellet that contained the bacterial cells only. The cell free supernatants of L1 and L2 and the controls were sent to the desired laboratory for the analysis with AAS.

### 2.8.2. Assessment:

The assessment of the cell free supernatants of two isolated bacterial samples containing the $Pb(NO_3)_2$ and other solutes of nutrient broth were assessed by the scientific personnel of Bangladesh Council of Scientific and Industrial Research (BCSIR), Qudrat-I-Khuda Road, New Elephant Road, Dhanmondi, Dhaka-1205, Bangladesh.

## 2.9. Identification of Microbes by 16S rDNA Sequencing:

For 16S rDNA Sequencing of the two isolated bacterial samples, the subculture of the isolated bacteria was delivered to the DNA Solutions Ltd, Bridge Momtaz Heights, Level-5, 15/2, Shyamoli Road, Dhaka-1207, Bangladesh.

# Chapter 3: Results

The scientific study was focused on isolating and characterizing bacteria that are capable of bioremediating lead (heavy metal) from industrial wastewater. As there were two types of samples, soil samples and bacterial samples, different approaches and techniques were used to selectively screen out our bacteria of interest from the available strains.

## 3.1. Atomic Absorption Spectroscopy of Soil Samples:

After the soil samples of two different regions were digested by nitric acid, the samples were analyzed for measuring the existing amount of free lead by AAS, and it was revealed that lead was present in the concentrations of 42.6 ppm and 61.5 ppm for Comilla and Mohakhali, Dhaka samples, respectively.

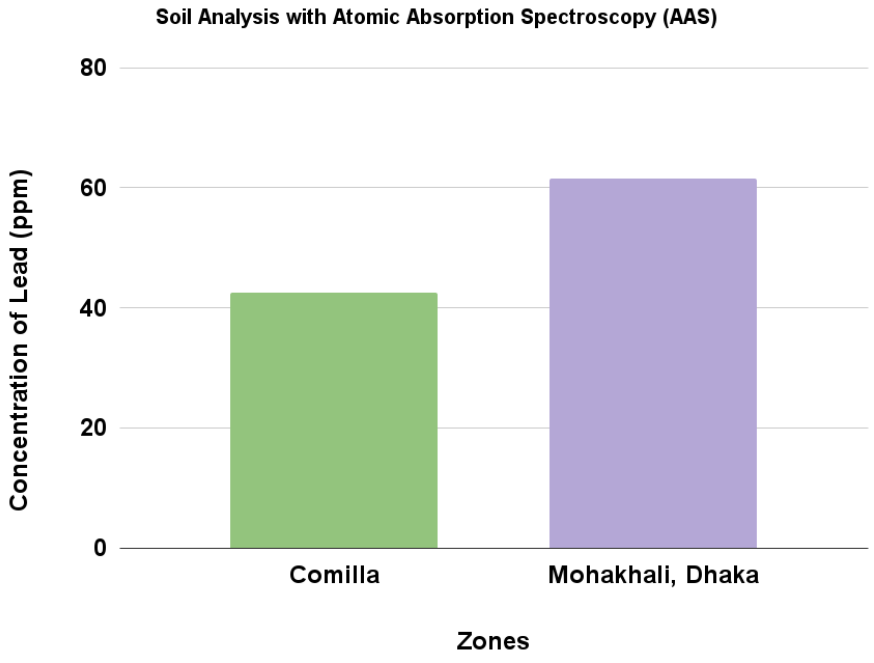



**Figure-1:** Soil Analysis of Comilla and Mohakhali, Dhaka samples with Atomic Absorption Spectroscopy

## 3.2. Metagenomic Analysis of Soil Samples:

After conducting the analysis, the Krona pie charts were found exhibiting that 95% of roots are bacterial populations, and 5% of roots were found with no hits in dataset-3 (from Comilla). Regarding bacterial population, Pseudomonadati bacteria were found as 80% of the bacterial population and Bacillati as 17% of the population as the major phyla in dataset-3. Contrarily, 99% of the roots are bacterial populations, and 1% of the roots were found with no hits in dataset-4 (from Mohakhali, Dhaka). Moreover, Pseudomonadati bacteria were found as 73% of the bacterial population, and Bacillati as 26% of the population, as the major phyla.

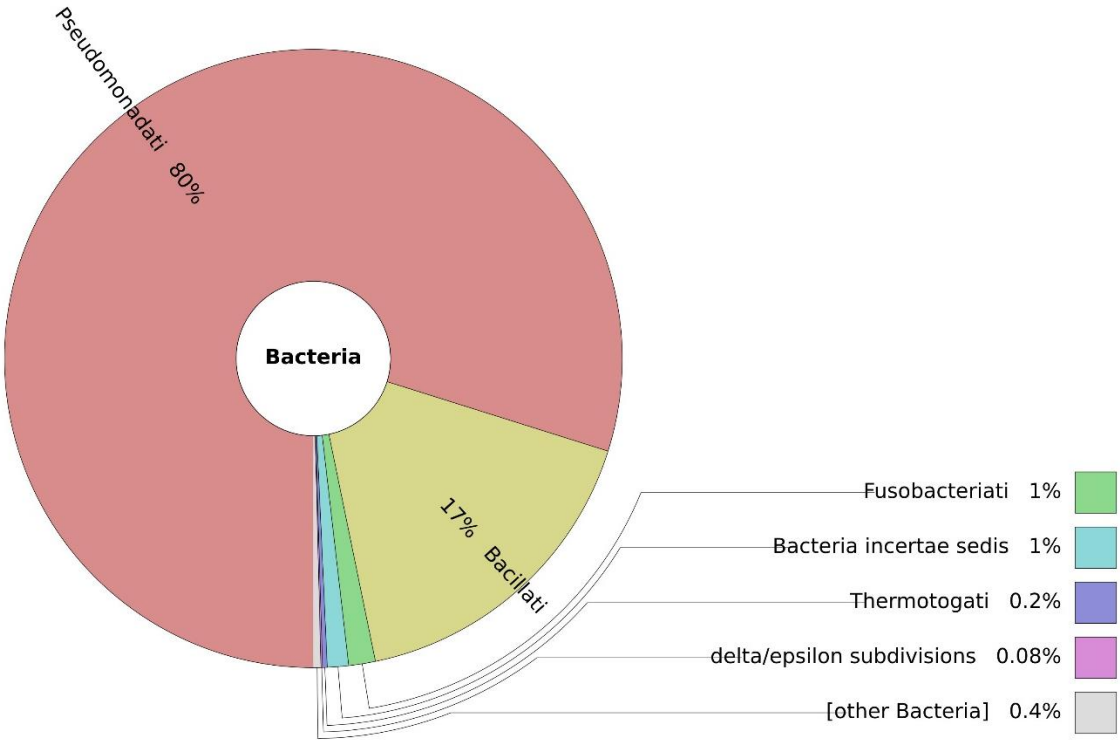



**Figure-2:** Krona Pie Chart showing bacterial population in dataset 3 (from soil sample of Comilla) after metagenomic analysis

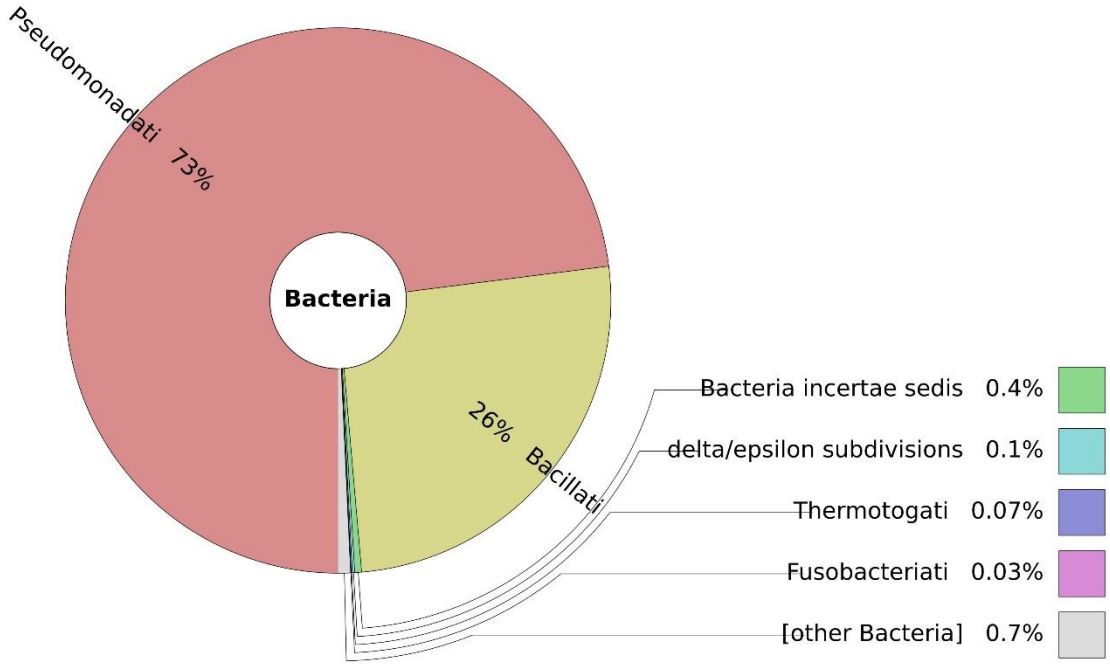



**Figure-3:** Krona Pie Chart showing bacterial population in dataset 4 (from soil sample of Mohakhali, Dhaka) after metagenomic analysis

## 3.3. Bacterial Isolation:

After confirming that the collected samples contained free lead exposure, the bacteria were selectively isolated. After conducting the first spread plate technique, several colony-forming units (CFUs) were found that were seen as prominent white dots. After the incubation of the subcultures of those colonies was done, bacterial growth was found in the lead nitrate-supplemented broth, revealing the fact that the bacteria were found to be able to grow even in lead-mediated stress and in liquid medium. To confirm the bioremediating property of the bacteria under a liquid state environment, bacterial AAS was performed later.

## 3.4. Atomic Absorption Spectroscopy of Bacterial Samples:

Bacterial cell-free supernatants of L1 and L2, along with the positive and negative controls, were examined with the Atomic Absorption Spectroscopy (AAS) technique. After the analysis, it was found that the strains could successfully reduce the amount of lead suspended in the nutrient broth by comparing with their control. After measuring the amount of the provided amount of lead in the positive control and the amount of lead remaining in the cell-free supernatants after 24 hours of incubation and comparing the controls and experimental samples (L1 and L2), it was confirmed that the strains removed approximately 97% and 95% of lead, respectively, from 100 ppm lead solutions.

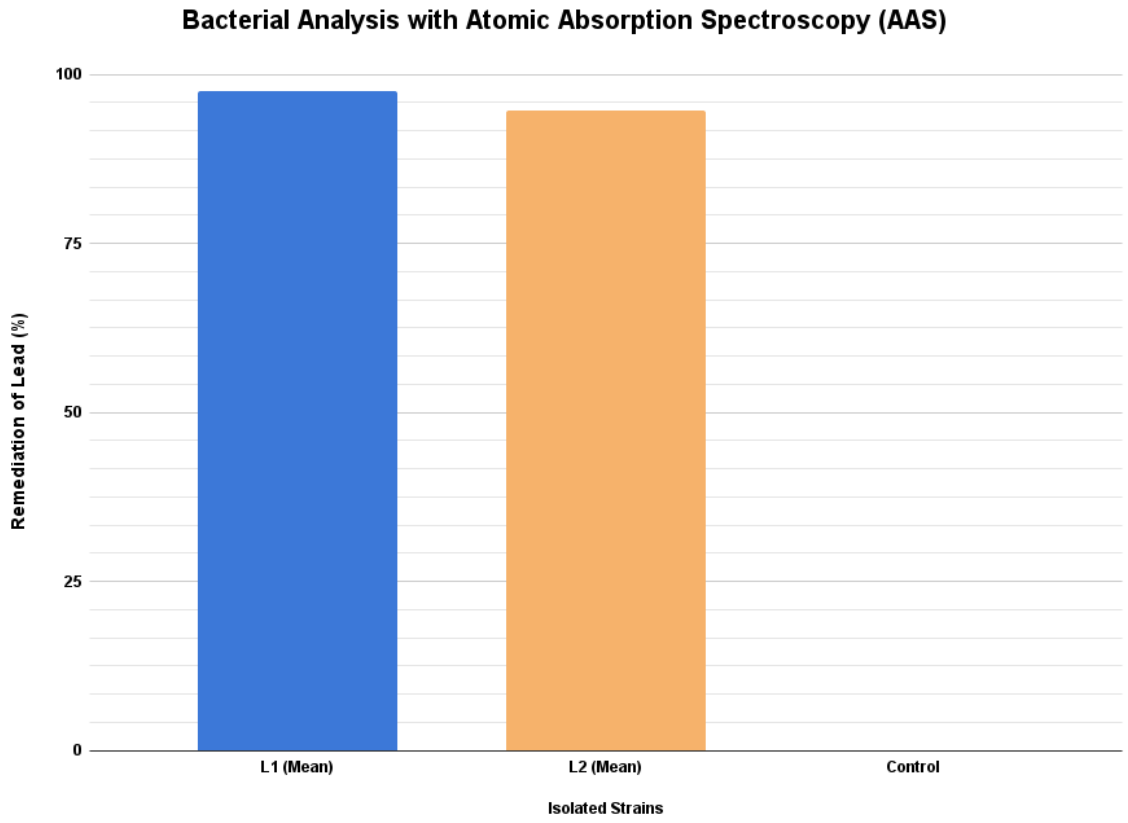
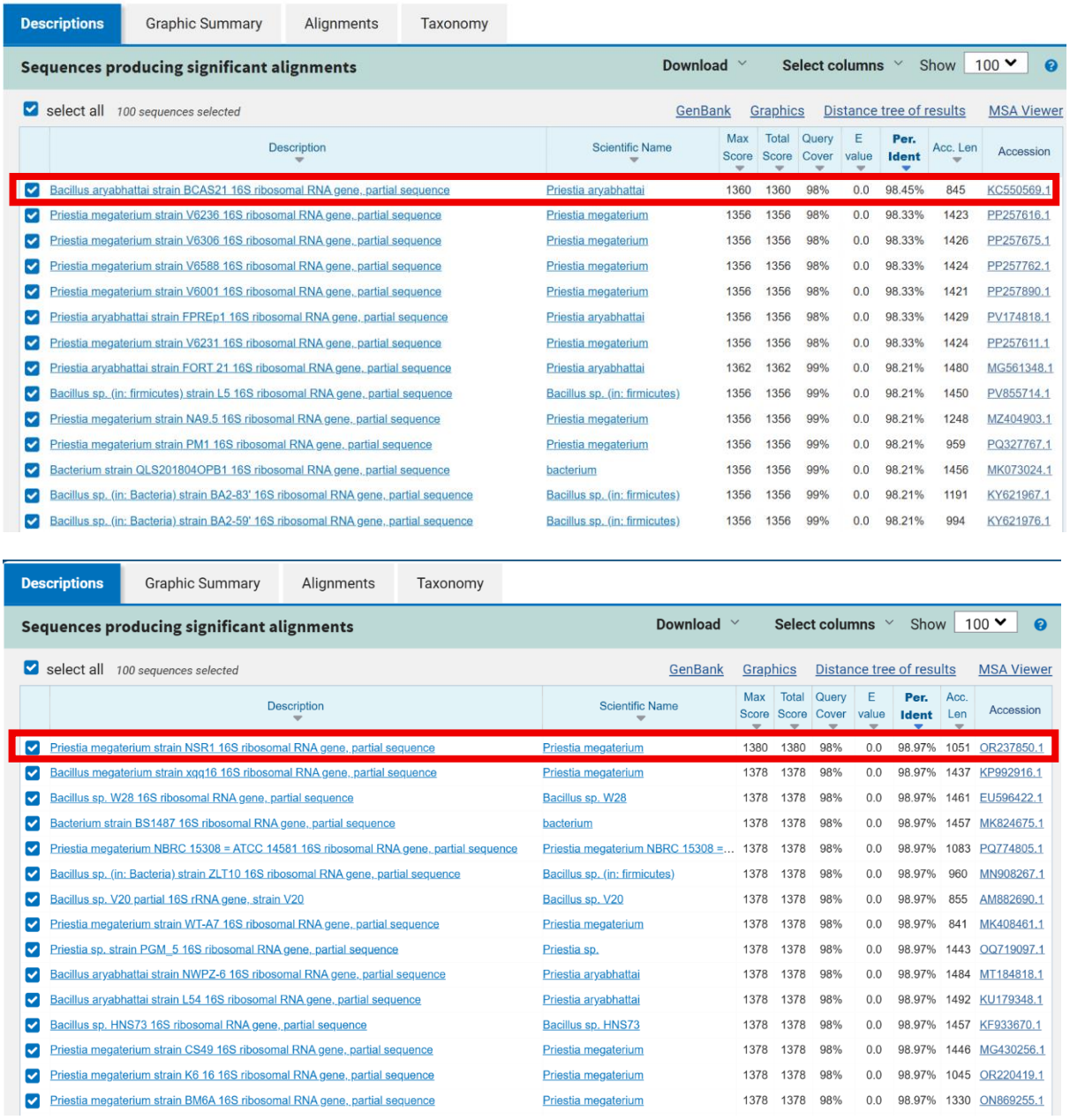



**Figure-4:** Bacterial Analysis of L1 and L2 strains (isolated from Comilla soil samples) with Atomic Absorption Spectroscopy

## 3.5. Identification of Microbes by 16S rDNA Sequencing:

The data of 16S rDNA sequencing were obtained from DNA Solutions Ltd as a FASTA file format. After running BLASTn, it was found that the L1 and L2 isolates - both of them had 98.45% and 98.97% of percent identity with *Priestia* spp. uniquely.



Descriptions | Graphic Summary | Alignments | Taxonomy

Sequences producing significant alignments — Download — Select columns — Show 100

select all 100 sequences selected — GenBank | Graphics | Distance tree of results | MSA Viewer

| Description | Scientific Name | Max Score | Total Score | Query Cover | E value | Per. Ident | Acc. Len | Accession |
|---|---|---|---|---|---|---|---|---|
| Bacillus aryabhattai strain BCAS21 16S ribosomal RNA gene, partial sequence | Priestia aryabhattai | 1360 | 1360 | 98% | 0.0 | 98.45% | 845 | KC550569.1 |
| Priestia megaterium strain V6236 16S ribosomal RNA gene, partial sequence | Priestia megaterium | 1356 | 1356 | 98% | 0.0 | 98.33% | 1423 | PP257616.1 |
| Priestia megaterium strain V6306 16S ribosomal RNA gene, partial sequence | Priestia megaterium | 1356 | 1356 | 98% | 0.0 | 98.33% | 1426 | PP257675.1 |
| Priestia megaterium strain V6588 16S ribosomal RNA gene, partial sequence | Priestia megaterium | 1356 | 1356 | 98% | 0.0 | 98.33% | 1424 | PP257762.1 |
| Priestia megaterium strain V6001 16S ribosomal RNA gene, partial sequence | Priestia megaterium | 1356 | 1356 | 98% | 0.0 | 98.33% | 1421 | PP257890.1 |
| Priestia aryabhattai strain FPREp1 16S ribosomal RNA gene, partial sequence | Priestia aryabhattai | 1356 | 1356 | 98% | 0.0 | 98.33% | 1429 | PV174818.1 |
| Priestia megaterium strain V6231 16S ribosomal RNA gene, partial sequence | Priestia megaterium | 1356 | 1356 | 98% | 0.0 | 98.33% | 1424 | PP257611.1 |
| Priestia aryabhattai strain FORT 21 16S ribosomal RNA gene, partial sequence | Priestia aryabhattai | 1362 | 1362 | 99% | 0.0 | 98.21% | 1480 | MG561348.1 |
| Bacillus sp. (in: firmicutes) strain L5 16S ribosomal RNA gene, partial sequence | Bacillus sp. (in: firmicutes) | 1356 | 1356 | 99% | 0.0 | 98.21% | 1450 | PV855714.1 |
| Priestia megaterium strain NA9.5 16S ribosomal RNA gene, partial sequence | Priestia megaterium | 1356 | 1356 | 99% | 0.0 | 98.21% | 1248 | MZ404903.1 |
| Priestia megaterium strain PM1 16S ribosomal RNA gene, partial sequence | Priestia megaterium | 1356 | 1356 | 99% | 0.0 | 98.21% | 959 | PQ327767.1 |
| Bacterium strain QLS201804OPB1 16S ribosomal RNA gene, partial sequence | bacterium | 1356 | 1356 | 99% | 0.0 | 98.21% | 1456 | MK073024.1 |
| Bacillus sp. (in: Bacteria) strain BA2-83' 16S ribosomal RNA gene, partial sequence | Bacillus sp. (in: firmicutes) | 1356 | 1356 | 99% | 0.0 | 98.21% | 1191 | KY621967.1 |
| Bacillus sp. (in: Bacteria) strain BA2-59' 16S ribosomal RNA gene, partial sequence | Bacillus sp. (in: firmicutes) | 1356 | 1356 | 99% | 0.0 | 98.21% | 994 | KY621976.1 |

Descriptions | Graphic Summary | Alignments | Taxonomy

Sequences producing significant alignments — Download — Select columns — Show 100

select all 100 sequences selected — GenBank | Graphics | Distance tree of results | MSA Viewer

| Description | Scientific Name | Max Score | Total Score | Query Cover | E value | Per. Ident | Acc. Len | Accession |
|---|---|---|---|---|---|---|---|---|
| Priestia megaterium strain NSR1 16S ribosomal RNA gene, partial sequence | Priestia megaterium | 1380 | 1380 | 98% | 0.0 | 98.97% | 1051 | OR237850.1 |
| Bacillus megaterium strain xqq16 16S ribosomal RNA gene, partial sequence | Priestia megaterium | 1378 | 1378 | 98% | 0.0 | 98.97% | 1437 | KP992916.1 |
| Bacillus sp. W28 16S ribosomal RNA gene, partial sequence | Bacillus sp. W28 | 1378 | 1378 | 98% | 0.0 | 98.97% | 1461 | EU596422.1 |
| Bacterium strain BS1487 16S ribosomal RNA gene, partial sequence | bacterium | 1378 | 1378 | 98% | 0.0 | 98.97% | 1457 | MK824675.1 |
| Priestia megaterium NBRC 15308 = ATCC 14581 16S ribosomal RNA gene, partial sequence | Priestia megaterium NBRC 15308 =... | 1378 | 1378 | 98% | 0.0 | 98.97% | 1083 | PQ774805.1 |
| Bacillus sp. (in: Bacteria) strain ZLT10 16S ribosomal RNA gene, partial sequence | Bacillus sp. (in: firmicutes) | 1378 | 1378 | 98% | 0.0 | 98.97% | 960 | MN908267.1 |
| Bacillus sp. V20 partial 16S rRNA gene, strain V20 | Bacillus sp. V20 | 1378 | 1378 | 98% | 0.0 | 98.97% | 855 | AM882690.1 |
| Priestia megaterium strain WT-A7 16S ribosomal RNA gene, partial sequence | Priestia megaterium | 1378 | 1378 | 98% | 0.0 | 98.97% | 841 | MK408461.1 |
| Priestia sp. strain PGM_5 16S ribosomal RNA gene, partial sequence | Priestia sp. | 1378 | 1378 | 98% | 0.0 | 98.97% | 1443 | OQ719097.1 |
| Bacillus aryabhattai strain NWPZ-6 16S ribosomal RNA gene, partial sequence | Priestia aryabhattai | 1378 | 1378 | 98% | 0.0 | 98.97% | 1484 | MT184818.1 |
| Bacillus aryabhattai strain L54 16S ribosomal RNA gene, partial sequence | Priestia aryabhattai | 1378 | 1378 | 98% | 0.0 | 98.97% | 1492 | KU179348.1 |
| Bacillus sp. HNS73 16S ribosomal RNA gene, partial sequence | Bacillus sp. HNS73 | 1378 | 1378 | 98% | 0.0 | 98.97% | 1457 | KF933670.1 |
| Priestia megaterium strain CS49 16S ribosomal RNA gene, partial sequence | Priestia megaterium | 1378 | 1378 | 98% | 0.0 | 98.97% | 1446 | MG430256.1 |
| Priestia megaterium strain K6 16 16S ribosomal RNA gene, partial sequence | Priestia megaterium | 1378 | 1378 | 98% | 0.0 | 98.97% | 1045 | OR220419.1 |
| Priestia megaterium strain BM6A 16S ribosomal RNA gene, partial sequence | Priestia megaterium | 1378 | 1378 | 98% | 0.0 | 98.97% | 1330 | ON869255.1 |

**Figure-5:** Snapshots of BLASTn results of L1 (i) and L2 (ii) isolates in NCBI

# Chapter 4: Discussion

## 4.1. Soil Sample Analysis:

The objective of this research is to bioremediate industrial wastewater but the bacterial strains were isolated from soil samples. First of all, there was a higher probability of Pb accumulation in soil rather than water. Bangladesh has a very poor record regarding urban planning. The land that has houses for people can also have industrial factories such as tanneries, battery factories, or sometimes landfills. Most of these factories do not have proper waste management and treatment systems, and they dump their waste in the surrounding areas. As a result, lead contamination has been increasing in Bangladesh for years. The same situation cannot be expected in water. Lead levels in agricultural soils were 84 to 574 ppm (Islam et al., 2014). Soil is acting as a constant sink for lead. Since soil has persistent exposure to lead with an enclosed surface area, the microbial communities in soil also have constant exposure, leading to swift evolution. The bacteria are forced to find survival initiatives, which leads to adaptability in stressful conditions (Santos et al., 2025). In water, pollutants can get diluted or dispersed, but soil can retain these pollutants because of its complex binding capacity and matrix structure. A stable niche for microbial communities is formed (Santos et al., 2025). In addition, soil also provides solid-phase interfaces for bacteria, which helps to initiate transformation or immobilization (Santos et al., 2025). On the contrary, water is always in a floating state. Using soil samples indicates that in the future, it can provide synergistic advantages (Santos et al., 2025). For example, if bacteria from agricultural soils are capable of bioremediating heavy metals, there is a high probability that they would support plant-microbe interactions. But this cannot happen for water because of its transient nature. To find out the concentration of heavy metals in the soil samples, atomic absorption spectroscopy was done.

The first soil sample was collected from the battery factories near the offices of Bangladesh Small and Cottage Industries Corporation (BSCIC), Comilla. When the soil sample from Comilla was also analyzed using AAS, it showed that the Pb concentration was 42.6 ppm, well above the average urban soil Pb levels. Therefore, the same conditions were investigated for both sites, as they fulfilled all the theoretical requirements. Mohakhali automobile workshop also had painting services, but no environmental regulations were followed. The soils were collected from multiple zones of the automobile workshop, which was perceived to be the perfect sample for finding the required bacteria needed for lead bioremediation. Another unique aspect of the site is that the vehicle battery parts were dumped near the workshop. Hence, different types of garbage had been stored on that site for years. The probability of the bacterial evolution required for bioremediation of heavy metals was high, and lead was one of them. Bacteria being continuously exposed to high concentrations of lead (Pb) leads to the development of resistance mechanisms for survival in the toxic environment (Shan et al., 2022). In this way, lead is creating persistent selective pressure that can act as a powerful selective agent, which only favors those bacteria that have developed detoxification abilities. The automobile workshop at Mohakhali had continuous exposure to Pb, which is why that site was selected. When the soil sample from Mohakhali was analyzed using AAS, 61.5 ppm of lead concentration was determined. Hence, the concentration rate indicated that the Pb level exceeded the average urban soil Pb levels (Akter, 2024). As a result, a high concentration of lead would trigger the pH variability (Shan et al., 2022). For survival, bacteria would trigger their detoxification pathways to make their metabolism unaffected. In addition, lead would induce reactive oxygen species and cause nutrient limitations. The AAS has higher sensitivity and accuracy than most biochemical tests, such as colorimetric assays for heavy metal concentration detection (James, 2023). Contrarily, biochemical tests cannot provide the required resolutions, whereas AAS can aid in detecting heavy metals at parts per billion (ppb)

or even at parts per trillion (ppt). In addition to it, AAS uses component-specific wavelengths, which ensure the correct quantifications even when the compounds are in a complex structure. Therefore, this is an isolation process that is free from environmental influences, unlike the biochemical tests (James, 2023). AAS also has specific calibration protocols that inhibit the interference of samples that have a complex matrix structure, such as soil. In a nutshell, AAS was the perfect tool to determine the concentration of heavy metals accurately. After confirming the Pb concentration levels from the samples, they were diluted and spread onto nutrient agar (NA) supplemented with 100 ppm lead (as lead nitrate) to isolate resistant strains. Nutrient agar, as the solid medium, was used because nutrient agar provides a solid surface and also the essential nutrients needed for colony formation. Moreover, nutrient agar was supplemented with Pb to ensure the growth of only the isolates capable of surviving at high levels of Pb concentration. Hence, NA, being supplemented with lead nitrate, will ensure the growth of bacterial colonies capable of tolerance or detoxification (Sridevi et al., 2018). Furthermore, it will provide pure strains. 100 ppm of Pb was chosen as the threshold based on the assessments of the soil samples. Both of the samples had Pb concentrations ranging from 50 to 70 ppm. Based on this environmental assessment, 100 ppm was selected as the perfect threshold to determine the actual resistance level of bacteria. Later on, selected colonies were transferred to nutrient broth (NB) with various lead concentrations. NB supplemented with lead nitrate mimics the industrial wastewater environment. Hence, using NB supplemented with lead nitrate will ensure uniform exposure of Pb at various concentrations to the bacteria. Throughout the whole research, mostly liquid medium supplemented with Pb was used to ensure accurate quantitative analysis and also to create environmental conditions that match the research goals. In this research, lead nitrate was used because lead nitrate dissolves easily in water (Sridevi et al., 2018). The iconic form of Pb ($Pb^{2+}$) causes toxicity by entering the cells, which halts the metabolic activity of bacteria. Therefore, lead nitrate in NB would get

dissociated into $Pb^{2+}$ and $NO_3^-$ ions. Nitrate ions do not interfere with the growth of bacteria, which makes them an ideal opposite ion. Other forms, such as lead chloride, were not used because it has low solubility. It can precipitate and react with other components of NB. Lead acetate was not used since acetate can work as a carbon source, leading to problematic results.

## 4.2.Bacterial Analysis:

Since this research was totally focused on bacteria, the standard database was used, which only gave a taxonomic profile of bacteria while performing the metagenomic analysis by 16S rDNA sequencing. The Krona report of the metagenomic analysis on the soil sample at the Mohakhali automobile workshop provided some unique insights into the microbial community. The root of the Krona pie chart indicated that all the full taxonomic profiling is on bacteria, and no hits were 1%. Overall, the dominant groups are the Pseudomonadati kingdom, the most abundant group with potential metal resistance. In the whole pie chart, they are 73% of the total reads. The second dominant group is Planctomycetia, with 15% of the total reads. The last dominant group belongs to the Verrucomicrobia phylum. There are also unassigned bacteria; lower-abundant groups such as Actinobacteriota and Firmicutes are observed. In the Pseudomonadati kingdom, two groups are also observed, which are the FCB group (Fibrobacterota, Chlorobiota, and Bacteroidota) and the PVC group (Planctomycetota, Verrucomicrobiota, and Chlamydiota). The microbial composition suggests that the soil sample had a typical soil microbiome. The groups such as Planctomycetota and Verrucomicrobiota were relatively abundant, indicating the microbial community was adapting to stress conditions. Pathogenic species are also present but at lower rates, such as *Klebsiella pneumoniae, Salmonella enterica,* etc., which can be found in the deeper sections of the pie chart. Overall, the soil sample from Mohakhali, Dhaka, had a healthy environment, but

dysbiosis and the presence of pathogenic taxa were found too. Their presence indicates a high level of harsh conditions, pollution, and exposure to waste disposal. So, the overall environmental status indicates Pb contamination, harsh conditions, and selective pressure, which is favoring the survivability of the bacteria that are capable of adapting to these problems **(Abba et al., 2024)**. Hence, the soil sample collection method was correct because in both cases, the dominant groups are mostly similar. The pie chart was zoomed into the family Bacillaceae because that is where the desired bacteria *Priestia* spp. belongs. The overall genus composition is 30% Bacillus, 17% Paenibacillus, 7% Virgibacillus, 6% Brevibacillus, 5% Lysinibacillus, 5% Aneurinibacillus, 4% Fictibacillus, 4% Alkalihalobacillus, 3% Priestia, 3% Sutcliffiella, 2% Metabacillus, 2% Cytobacillus, 2% Peribacillus, and 10% others combined. Their presence indicates that the environment was stress-induced because most of them are spore-forming bacteria **(George & Wan, 2019)**.

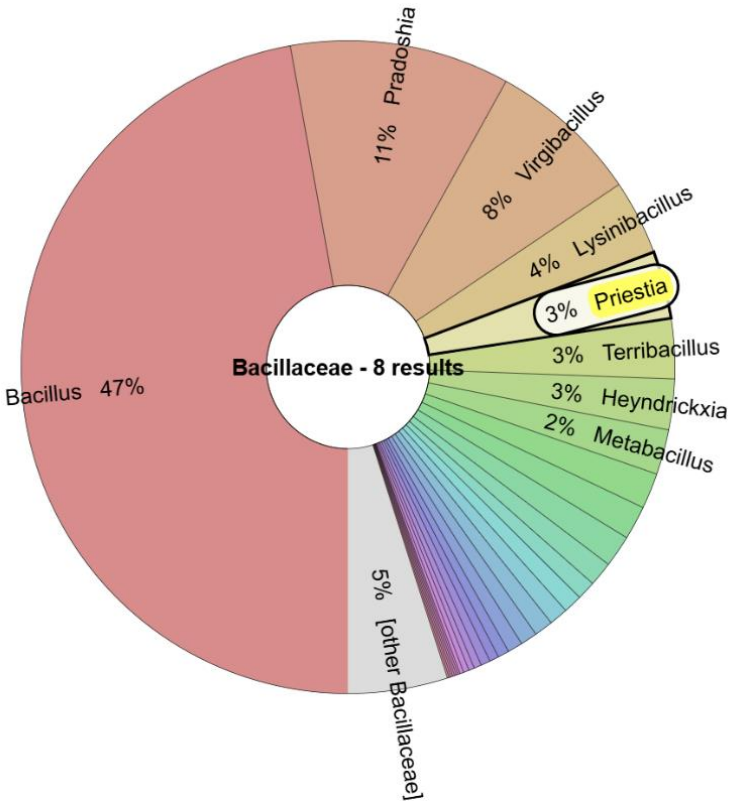



**Figure 6:** Krona Pie Chart showing the presence of *Priestia* as 3% population of Bacillaceae family in dataset-4 (from the soil sample of Mohakhali, Dhaka) after metagenomic analysis

The overall composition of the genus suggests that the microbiome has adapted to the stressed environment caused by lead (Pb) pollution. The soil sample was enriched in the NA media, followed by subculturing in the NA media. AAS results suggest 42.6 ppm. Although stress-inducing Pb is available in the environment, the microbial community was successful in generating its tolerance. As a result, one of the dominant groups was *Bacillaceae.* As the pie chart was further analyzed, it indicated that in the family *Bacillaceae,* an abundant bacterial group was *Bacillus cereus* (26%). They are pathogenic but also can be found at industrial sites. *Bacillus cereus* is known for showing resilience against Pb pollution through mechanisms such as bioaccumulation and biosorption (Harun et al., 2023). The presence of *Bacillus subtilis* (11%) is observed. They are also known for heavy-metal tolerance (Li et al., 2021). Other taxa groups were mostly moderate, such as *Terribacillus aidingensis* (3%) and *Priestia megaterium* (3%). *Priestia*, which was formerly classified under *Bacillus,* was 3% of the overall community, with *Priestia megaterium* representing 75% of the total *Priestia* population. *Priestia megaterium* can precipitate Pb into an insoluble form (Pouchucq et al., 2025). Using this method, they help promote the growth factor of plants. So, after the induced stress of up to 100 ppm, only *Priestia spp.* survived, making them the sole survivors at high Pb concentrations. The survival of *Priestia spp.* indicates the factory area in Comilla is heavily polluted, suggesting constant Pb exposure, such as water leaching. It also shows the deadly effects of Pb pollution because, before that, a rich microbial community was present. After 100 ppm, only *Priestia spp.* survives. The chart also has some bacterial species with low counts, such as *Oceanobacillus mesonae* (1%), *Peribacillus* (1%), and even smaller ones like *Mesobacillus* (0.9%), *Rossellomorea* (0.9%), *Lentibacillus* (0.9%), *Cytobacillus* (0.9%), and *Litchfieldia suaedae* (0.7%). They may have minor roles in the environment. *Oceanobacillus* has a very minor capacity to help heavy-metal-induced stress conditions, but that is not effective for Pb pollution. Among *Priestia* spp., the presence of *Priestia aryabhattai* is 11%.

In a nutshell, for the soil samples from Mohakhali, Dhaka, and Comilla, the Krona reports are relatively similar. In the report of dataset-3 (from Comilla), the dominant groups are Pseudomonadati (80%) and Bacillati (17%). The overall report is a high abundance of Firmicutes, Proteobacteria, and Actinobacteria. The soil sample was from a battery factory in Comilla, Bangladesh. In battery factories, heavy metals such as Mn, Cu, Zn, Pb, etc. are often used and released in the environment through wastewater, non-functional waste disposal, etc. Eventually, the Pb levels in the soil of Bangladesh are rapidly increasing, leading to the creation of an acidic, metal-rich environment.

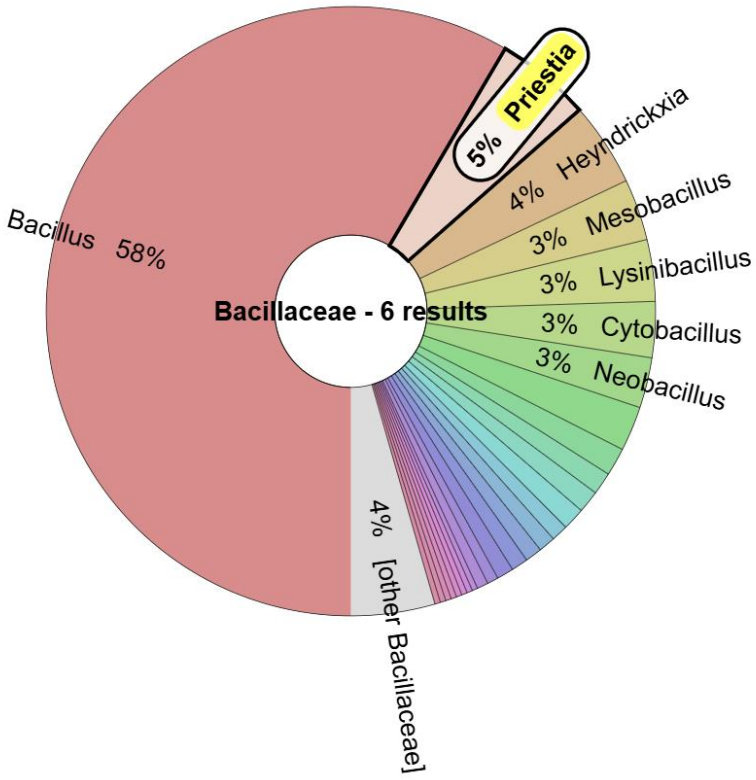



**Figure 7:** Krona Pie Chart showing the presence of *Priestia* as 5% population of Bacillaceae family in dataset-3 (from soil sample of Comilla) after metagenomic analysis

In both cases, most bacterial species have remediation potential, spore-forming capability, etc. However, the Krona report of dataset-3 suggests the soil sample from Comilla was more contaminated than the soil sample from Mohakhali. If the Krona report on Comilla is observed, the taxa are skewed. Additionally, it has a higher number of bacteria with spore-forming capacity than bacteria in the soil sample from Mohakhali. More skewed taxa indicate the presence of continuous selective pressure in the soil sample of Comilla. Although the lead (Pb) level in the soil sample of Mohakhali is 61.5 ppm, higher than the lead level in the Comilla soil sample, the Comilla soil sample seemed more contaminated because it has chronic Pb exposure from the battery factories. In Mohakhali, the automobile shop has high exposure but not as chronic as in Comilla. Also, the soil matrix is involved. Mohakhali soil was more clay-like with exposure to other wastes such as plastics, medical wastes, etc. Again, the similarity is observed. In the *Bacillati* kingdom, *Bacillus cereus* (33%) and *Bacillus subtilis* (15%) are mostly present. Both of them are well documented for their ability to be resistant to Pb. Then, *Heyndrickxia oleronia* (4%) is observed, which was recently reclassified from *Bacillus.* They also have Pb-resistant capacity, as they produce EPS, which has high biosorption capacity. Another similarity here is in both soil samples, the presence of *Priestia* is moderate. *Priestia megaterium* is dominant with an 82% reading, but *the Priestia aryabhattai* reading is 12%. After all the experiments at the lab, two of the isolates showed promising results. Those were subcultured in NA supplemented with 100 ppm lead nitrate. In addition, the isolates L1 and L2 were again grown in the NB broth supplemented with various ranges of concentrations of lead nitrate, starting from 50 ppm. For both cases, prominent growth in the liquid medium was observed at 100 ppm, but as the concentration increased, the growth rate decreased too. Moreover, both of the isolates were at their highest cellular growth at 100 ppm, which meant both isolates were prominently resistant to Pb at 100 ppm. Both of the isolates were grown in the liquid broth again, but this time supplemented with lead ranging from 50 to 100 ppm. In

addition, there was a negative control of lead, which had only NB but no Pb. The positive control had NB, Pb, and no bacteria. After 24 hours of incubation, they centrifuged at 1000 rpm for 10 minutes. Then the pellets were discarded, and the cell-free supernatants were collected. The initial concentration was 100 ppm for Pb. When the negative control was analyzed, it was observed that 80 ppm was accessible. The possible reason might have been the sedimentation of the lead occupied by other components of the nutrient broth (such as amino acids, minerals, and so on). Therefore, the positive control and the other cultures must have Pb less than 80 ppm to prove that selected isolates were Pb-resistant, even if they are not bioremediating Pb. When the cell-free supernatants were analyzed using AAS, it was observed that only 2 ppm was in the supernatants. So, 78 ppm Pb was taken by both of the isolates. Both L1 and L2 showed high Pb removal efficiency, 97% and 95% of lead, respectively. 16S rDNA sequencing indicated that *Priestia spp*. is our desired genus as lead-resistant bacteria, obtained by PCR and capillary electrophoresis outcomes.

# Conclusion

The rising levels of lead (Pb) contamination in Bangladesh, which come from industrial sources such as the production of batteries and cars, pose a serious and ongoing risk to the environment and public health. There are numerous drawbacks to conventional lead-laden wastewater treatment techniques, such as membrane separation, ion exchange, and coagulation-flocculation. These disadvantages, which include high operating costs, the production of hazardous sludge, membrane fouling, and an inability to continuously satisfy regulatory criteria, highlight how urgently sustainable and affordable alternatives are needed. Microbial bioremediation stands out as a very promising measure in this regard. Bacteria use a wide range of complex natural processes, and their practical application is increased by their capacity to use both living and dead biomass. In constructed systems like bioreactors, bioremediation techniques can provide more control and quicker outcomes. The isolates of this scientific study belong to *Priestia* spp., which can efficiently remediate 95-97% of the given lead in liquid solution, which also infers that this bacterium can provide us with the prospect of treating industrial wastewater contaminated by lead with its lead-resistant capacity. In this context, the whole genome sequence of the isolated bioremediating bacteria could help us find out the specific bioremediation pathways with their related genes and expressed enzymes. Further research must be encouraged in exploring the lead-resistant bacteria, and the whole blueprint of bioreactors must be constructed for treating industrial wastewater practically and developing a revolutionary technology under the realm of environmental biotechnology.

## References:


Abbas, S., Ahmed, I., Iida, T., Lee, Y.-J., Busse, H.-J., Fujiwara, T., & Ohkuma, M. (2015). A heavy-metal tolerant novel bacterium, Alcaligenes pakistanensis sp. nov., isolated from industrial effluent in Pakistan. *Antonie van Leeuwenhoek*, *108*(4), 859–870. https://doi.org/10.1007/s10482-015-0540-1

Abba, M., Shafiu, S., Ibrahim, B., Aisha, M., Aliyu, H., Jamilu, Y., & Umar, Y. M. (2024). Understanding the Impact of Lead Contamination on Microbial Diversity: A Comprehensive Review. International Journal of Research Publication and Reviews, 5(7), 1119–1130. https://doi.org/10.55248/gengpi.5.0724.1721

Beaty, R., & Kerber, J. (1993). Concepts, *Instrumentation and Techniques in Atomic Absorption Spectrophotometry Second Edition THE PERKIN-ELMER CORPORATION*. https://www.iitk.ac.in/che/PG_research_lab/pdf/resources/AAS-GTA-reading-material.pdf

Chowdhury, I. R., Chowdhury, S., Mazumder, M. A. J., & Al-Ahmed, A. (2022). Removal of lead ions (Pb2+) from water and wastewater: a review on the low-cost adsorbents. *Applied Water Science*, *12*(8). https://doi.org/10.1007/s13201-022-01703-6

George, S. E., & Wan, Y. (2019). Advances in characterizing microbial community change and resistance upon exposure to lead contamination: Implications for ecological risk assessment. *Critical Reviews in Environmental Science and Technology*, *50*(21), 2223–2270. https://doi.org/10.1080/10643389.2019.1698260

Harun, F. A., Yusuf, M. R., Usman, S., Shehu, D., Babagana, K., Sufyanu, A. J., Jibril, M. M., Bello, A. M., Musa, K. A., Jagaba, A. H., Shukor, M. Y., & Yakasai, H. M. (2023). Bioremediation of lead contaminated environment by Bacillus cereus strain BUK_BCH_BTE2: Isolation and characterization of the bacterium. *Case Studies in*

*Chemical and Environmental Engineering*, *8*, 100540. https://doi.org/10.1016/j.cscee.2023.100540

Hložková, K., Šuman, J., Strnad, H., Ruml, T., Paces, V., & Kotrba, P. (2013). Characterization of pbt genes conferring increased Pb2+ and Cd2+ tolerance upon Achromobacter xylosoxidans A8. *Research in Microbiology*, *164*(10), 1009–1018. https://doi.org/10.1016/j.resmic.2013.10.002

Hu, C., Yang, Z., Chen, Y., Tang, J., Zeng, L., Peng, C., Chen, L., & Wang, J. (2024). Unlocking soil revival: the role of sulfate-reducing bacteria in mitigating heavy metal contamination. *Environmental Geochemistry and Health*, *46*(10). https://doi.org/10.1007/s10653-024-02190-1

Li, Y., Ali, A., Jeyasundar, P. G. S. A., Azeem, M., Tabassum, A., Guo, D., Li, R., Mian, I. A., & Zhang, Z. (2021). Bacillus subtilis and saponin shifted the availability of heavy metals, health indicators of smelter contaminated soil, and the physiological indicators of Symphytum officinale. *Chemosphere*, *285*, 131454. https://doi.org/10.1016/j.chemosphere.2021.131454

Naik, M. M., & Dubey, S. K. (2013). Lead resistant bacteria: Lead resistance mechanisms, their applications in lead bioremediation and biomonitoring. *Ecotoxicology and Environmental Safety*, *98*, 1–7. https://doi.org/10.1016/j.ecoenv.2013.09.039

Naik, M., Dubey, S. K., Khanolkar, D. S., & D'Costa, B. (2013). P-type ATPase and MdrL efflux pump-mediated lead and multi-drug resistance in estuarine bacterial isolates. *Current Science*, *105*(10), 1366–1372. https://www.researchgate.net/publication/285715434_P-type_ATPase_and_MdrL_efflux_pump-mediated_lead_and_multi-drug_resistance_in_estuarine_bacterial_isolates

Naik, M. M., Shamim, K., & Dubey, S. K. (2012). Biological characterization of lead resistant bacteria to explore role of bacterial metallothionein in lead resistance. *Current Science*, *103*(23), 1113–1130. https://www.researchgate.net/publication/231705552_Biological_characterization_of_lead_resistant_bacteria_to_explore_role_of_bacterial_metallothionein_in_lead_resistance

Navgire, G. S., Goel, N., Sawhney, G., Sharma, M., Kaushik, P., Mohanta, Y. K., Mohanta, T. K., & Al-Harrasi, A. (2022). Analysis and Interpretation of metagenomics data: an approach. *Biological Procedures Online*, *24*(1). https://doi.org/10.1186/s12575-022-00179-7

Park, J. H., Bolan, N., Megharaj, M., & Naidu, R. (2011). Isolation of phosphate solubilizing bacteria and their potential for lead immobilization in soil. *Journal of Hazardous Materials*, *185*(2-3), 829–836. https://doi.org/10.1016/j.jhazmat.2010.09.095

Pouchucq, L., Marcoleta, A. E., Rojas, C., Arce, H., Becerra, C., Bahamondes, C., & Lobos-Ruiz, P. (2025). Genomic and Functional Characterization of*Priestia megaterium* MOD5IV isolated from the rhizosphere of*Caesalina spinosa*(Mol.): Enhancing Phytoremediation Potential in Multi-Metal Contaminated Soils. *BioRxiv (Cold Spring Harbor Laboratory)*. https://doi.org/10.1101/2025.04.15.648928

Raj, K., & Das, A. P. (2023). Lead pollution: Impact on Environment and Human Health and Approach for a Sustainable Solution. *Environmental Chemistry and Ecotoxicology*, *5*, 79–85. https://doi.org/10.1016/j.enceco.2023.02.001

Volaric, A., Svircev, Z., Tamindzija, D., & Radnovic, D. (2021). Microbial bioremediation of heavy metals. *Chemical Industry*, *75*(2), 103–115. https://doi.org/10.2298/hemind200915010v

Wood, D. E., & Salzberg, S. L. (2014). Kraken: Ultrafast metagenomic sequence classification using exact alignments. *Genome Biology*, *15*(3), R46. https://doi.org/10.1186/gb-2014-15-3-r46

Zhang, Y., Peng, J., Wang, Z., Zhou, F., Yu, J., Chi, R., & Xiao, C. (2025). Metagenomic analysis revealed the bioremediation mechanism of lead and cadmium contamination by modified biochar synergized with Bacillus cereus PSB-2 in phosphate mining wasteland. *Frontiers in Microbiology*, *16*. https://doi.org/10.3389/fmicb.2025.1529784