# BIOREMEDIATION OF LEAD USING LEAD(II) REDUCTASE FOUND IN BACTERIA: IN SILICO MOLECULAR DOCKING

**A PROJECT REPORT**

***Submitted in partial fulfilment of the requirement for the degree of***

**MASTER OF SCIENCE**

**In**

**BIOTECHNOLOGY**

**M.Sc. (Biotechnology)**

Submitted by:


**DIVYA**

**2K22/MSCBIO/64**


Under the Supervision of

**Prof. Jai Gopal Sharma**


**DEPARTMENT OF BIOTECHNOLOGY**

**DELHI TECHNOLOGICAL UNIVERSITY**

**(Formerly Delhi College of engineering)**

**Shahabad Daulatpur, Bawana Road, Delhi-110042, India**


**JUNE 2024**

# ACKNOWLEDGEMENT

In gratitude for all your contribution to this project thesis, I wish to extend my deepest thanks to each of you.

I would like to first and foremost gratitude goes to my supervisor Prof. Jai Gopal Sharma, Professor in the Department of Biotechnology, Delhi Technological University, Delhi for supporting, encouraging, and guiding me throughout in my project. Throughout this project, their expertise, insights, and feedback played an important role.

I want to thank the Prof. Yasha Hasija, Head of Department for providing me with the department facilities and resources that I needed to complete my research effectively. I am also grateful to the faculty members of the Department of Biotechnology, Delhi Technological University, they helped me a lot with my thesis, and their opinions and advice helped me make the ideas stringer and the work better as a whole.

I am equally thankful and wish to express my wholehearted thanks to respected lab senior Ms.Megha Bansal for her support and help in the course of my thesis work. I would wish to express my gratitude and affection to my family for their constant love and support.

Finally, I would like to recognize and thank Delhi Technological University for providing me with the teaching environments, tools, and facilities I needed to learn. What I learned and did at this well-known university has had a big impact on my both academic as well as professional routes.

**Divya**

**2K22/MSCBIO/64**

**DELHI TECHNOLOGICAL UNIVERSITY**

(Formerly Delhi College of Engineering)

Shahabad Daulatpur, Main Bawana Road, Delhi-110042. India

# CANDIDATE'S DECLARATION

**I, Divya, [2K22/MSCBIO/64]**, final semester student of M.Sc. Biotechnology, hereby declare that the Project Dissertation titled **"Bioremediation of lead using lead(II) reductase found in bacteria: In Silico Molecular Docking"** in partial fulfilment of the requirements for the award of the Degree Master of Science in the Department of Biotechnology, Delhi Technological University is an authentic record of my own work carried out during the period to under the supervision of Prof. Jai Gopal Sharma.

The matter presented in this thesis has not been submitted by me for the award of any other degree of this or any other institute.

**Place: Delhi.** **DIVYA**

**Date:** **(2K22/MSCBIO/64)**

**DELHI TECHNOLOGICAL UNIVERSITY**

(Formerly Delhi College of Engineering)

Shahabad Daulatpur, Main Bawana Road, Delhi-110042. India

# CERTIFICATE BY SUPERVISOR

I, hereby certify that the Project Dissertation titled, **" Bioremediation of lead using lead(II) reductase found in bacteri: In Silico Molecular Docking "** which is submitted by, **Divya [2K22/MSCBIO/64]**, Department of Biotechnology, Delhi Technological University, Delhi in partial fulfilment of the requirement for the award of the degree of Master of Science, is a record of the project work carried out by the student under my supervision. To the best of my knowledge this work has not been submitted in part or full for any Degree or Diploma to this University or elsewhere.

| | |
|---|---|
| **Prof. Yasha Hasija** | **Prof. Jai Gopal Sharma** |
| **Head of Department** | **Supervisor** |
| **Department of Biotechnology** | **Department of Biotechnology** |
| **Delhi Technological University** | **Delhi Technological University** |

# Bioremediation of lead using lead(II) reductase found in bacteri: In Silico Molecular Docking

Divya

## ABSTRACT

Heavy metals are naturally existing components of the environment, human activity has upset their metabolic equilibrium and geochemical cycles. Increased accumulation of certain heavy metals and prolonged exposure to them can be harmful to human health and the aquatic biota. Bioremediation is one of the most cost-effective, safest, cleanest, and ecologically friendly methods for decontaminating areas contaminated with various toxins. It eliminates these harmful substances. We call this process of using biological organisms to take out hazardous material from the environment "bioremediation." Heavy metals are the toxic waste that significantly damages the environment. A comprehensive sequence analysis was carried out in order to better understand the role that Pb reductase plays in reducing Pb(II) to Pb(0). Various homology modelling techniques were then used to generate models for both wild-type and mutant Pb(II) species. The models' protein structure quality was evaluated, and the top model was chosen to undergo additional refinement through the application of an energy reduction technique. The intramolecular interactions between Pb(II) and wild-type and mutant Pb reductase were investigated using molecular docking experiments. This is a new technique for figuring out Pb reductase's three-dimensional structure and how it interacts with Pb to change it into a less dangerous form.

# LIST OF PUBLICATION

1. **Title of the Review Paper:** “Bioremediation of heavy metals in contaminated water bodies ”

**Name of Authors-** Divya , Himani Joshi, Jai Gopal Sharma*

**Name of the Journal:** African journal of biological science (AFJBS)

**Date of acceptance:** 17.05.2024

**Status of the Paper:** Published

# CONTENTS

# LIST OF TABLES



# LIST OF FIGURES

# ABBREVIATIONS

| | |
|---|---|
| Pb | Lead |
| PbR | Lead Reductase |
| HM | Heavy Metal |
| EPS | Extracellular polymeric substances |

# CHAPTER 1
# INTRODUCTION

The release of toxic compounds into the environment is known as pollution, and it affects the quality of the air, water, and soil. It results in health problems, harm to ecosystems, and climate change. It is caused by industrial operations, automobile emissions, and inappropriate waste disposal. To protect living systems from harmful metals, specific handling and transportation procedures are needed (1). Lead, mercury and arsenic are examples of heavy metals that present serious health risks. Long-term exposure damages ecosystems by contaminating water and soil, endangering wildlife, and getting into the food chain, which can result in a host of environmental and health issues. The branch of biotechnology known as **"bioremediation"** is used to reduce and treat polluted water that uses live organisms—such as bacteria and microbes—to remove poisons, pollutants, and contaminants from soil, water, and other environments. It is one of most promising technique to treat polluted water bodies and has economic benefits and no adverse environment impact. Bioremediation processes are considered more appealing than physicochemical techniques such as electrochemical treatment, ion exchange, precipitation, reverse osmosis, evaporation, and sorption at low concentrations of removed metals because the discussed processes can be more cost-effective and efficient (2).

It is well known that lead is one of the most common metals in the world and that it has contaminated the environment and led to several health issues. Children who suffer from lead poisoning frequently experience mental impairment. Lead is used extensively in industry and is extremely harmful to living things, lead contamination is a serious environmental problem. Chemical precipitation and soil excavation are two common, expensive traditional lead cleanup techniques that may have further negative effects on the environment.

Lead has an impact on the reproductive systems of both men and women. When blood lead levels surpass 40 $\mu g\ dL^{-1}$ of blood, men's sperm count is reduced by lead. Pregnant women with elevated blood lead levels have lower birth weights, preterm babies, and miscarriages (3). The use of lead-resistant bacteria in bioremediation has drawn a lot of attention as a sustainable substitute. These microbes can change, immobilize, or sequester lead from contaminated environments because they have evolved defense mechanisms to withstand high lead concentrations (4). Through bioremediation, damaged ecosystems can be detoxified and restored by utilizing the natural activities of microorganisms. Lead-resistant bacteria possess many lead resistance strategies including enzymatic conversion, adsorption and accumulation, precipitation of extracellular minerals, and expulsion from the organism. Using docking in the context of lead-resistant bacteria is one promising bioremediation strategy. The interaction which the lead ions have with specific receptor sites on bacterial surfaces for purposes to do with the transport, deposition, or alteration of lead is referred to as docking. This effectively helps in raising the bioremediation's efficiency, a process that is very important. Therefore, optimisation of the schematic lead removal via bacterial strains and environment may be conducive to gaining further insight concerning the molecular and biochemical aspects of docking that may make bioremediation more efficient. Lead-resistant bacteria display one or multiple mechanisms of lead resistance; they include enzymatic conversion, electrostatic uptake, chemical adsorption, and sequestration.

These processes often involve cellular contact points both on the cell membrane. For instance, the role of selected species of the Pseudomonas genus in the biomagnification and demanganization of heavy metals including lead is well documented and occurs through specific interactions between the bacterial cell envelope and a range of substrates. Likewise, through docking contacts, Bacillus species can carry out efficient precipitative adsorption of lead on to their cell walls thus reducing its Bioavailability in the environment (5). This thesis explores the strategies of docking in resistant bacteria, evaluates the prospect of their contribution to the bioremediation effectiveness, and discusses challenges and possibilities of docking for enhancing lead bioremediation. The aim is to provide a comprehensive depiction of how these interactions could be effectively and efficiently applied to minimize lead pollution in the community.

# CHAPTER 2
# REVIEW OF LITERATURE

## 2.1 BIOREMEDIATION

The hazardous pollutants were defined as bioremediation being an environment friendly process and an innovative biological technique that uses naturally occurring organisms for the complete disappearance of the pollutants. It therefore includes 'any action that employs microorganisms, fungi, green plants, or their enzymes with an intention of restoring the natural state of a given environment that has been affected by contaminants'. Bioremediation techniques can either be on-site or off-site depending on where they are applied. Someman bioremediation iis carried out directly at the site of contamination while in other cases, the contaminated material is transported to another location for treatment. There are several technologies that can be used in bioremediation process and some of them include; bioventing, land farming, bioreactor, compositing, bioaugmentation, rhizofiltration and lastly bio-stimulation. The microorganisms which are involved in the process of bioremediation is termed as bioremediators more specifically known as Bioaugmentation.

However, not all pollutants respond well to microorganism-based bioremediation. For example, organisms have difficulty absorbing or capturing heavy metals like lead and cadmium. Things could get worse if elements like mercury get into the food chain. By definition, bioremediation is the process of breaking down environmental pollutants into less hazardous forms by using living organisms, mainly microbes (6). Bioaugmentation is the procedure whereby microorganisms are introduced to a contaminated location in order to promote deterioration. The process by which organic weights organically breakdown under regulated conditions to a benign state is known as "bio remediation." Condition strange to levels. below the regulatory agencies' set concentration thresholds. I

n bioremediation, microorganisms must be able to secrete enzymes that bind on the contaminants and bring about a change on the contaminants to products which are harmless to human beings. Remediation or bi can be very successful and if then is omitted the process will stop abruptly. Often used regularly, this process typically involves manipulation of conditions within the environment to allow for biosustainability, and microbial breakdown to occur at an even greater efficiency. Microbial activity and growth occur only when environmental conditions are favourable. When it comes to mitigating exposure, risk for cleaning, personal exposure, or potentially widespread exposure resulting from transportation accidents, bio remediation techniques are usually more cost-effective than traditional methods like burning. Additionally, certain contaminants can be treated on site. The public views bioremediation as more acceptable than other technologies because it is predicated on natural attenuation. The majority of remediation systems operate in aerobic circumstances; but, in anaerobic conditions, microbial organisms may be able to break down otherwise resistant compounds (7).

## 2.2 Types of Bioremediation

### 2.2.1 Bioattenuation

This is how you keep an eye on the organic process of degradation to make sure that over time, the concentration of contaminants at pertinent sample sites drops. In the US, bioattenuation is a common remediation technique for sites of underground storage tanks where groundwater and soil have been contaminated by petroleum (8).

### 2.2.2 Biostimulation

The environment must be altered to promote biodegradation and speed up reaction rates in the event that natural degradation is either absent or occurs too slowly. The actions that need to be done, referred to as biostimulation, include providing the environment with electron acceptors like oxygen, nutrients like nitrogen and phosphorus, and substrates like methane, phenol, and toluene. Two well-known harmful chemical additives were utilized as substrates: toluene and phenol. Therefore, it is important to keep a close eye on these substances' concentrations during biostimulation. In Japan, small-scale field studies supported independently by the Environment Agency and by the Ministry of International Trade and Industry proved the efficacy of in situ biostimulation by methane injection into TCE-contaminated groundwater (9). Through the collection of scientific data from these kinds of field studies, in situ biostimulation is anticipated to develop into a dependable and secure remediation technique.

### 2.2.3 Bioaugmentation

Bioaugmentation, the third option in the treatment hierarchy, is a technique that involves inoculating bacteria with the required enzymatic capabilities in order to improve the biodegradative capacities of contaminated areas. When bioattenuation or biostimulation fail to operate on highly resistant compounds, this strategy is thought to be beneficial. However, because bioaugmentation's impacts on the ecosystem are uncertain, we must closely monitor its application (10). Large volumes of degradative bacteria are added to contaminated locations; therefore, it is necessary to determine beforehand how the bacteria may affect humans and the environment. Furthermore, it must be established that the injected bacteria have died off following the cleanup and are no longer a threat to the local microbial ecology. Bioaugmentation has the ability to eliminate pollutants that are resistant to removal; however, before this technique is commercialized, extensive scientific data confirming its safety must be gathered (11).

### 2.2.4 Intrinsic Bioremediation

Passive bioremediation, sometimes referred to as natural attenuation, is a natural degradation process that eliminates hazardous chemicals through the metabolism of native microorganisms alone. No artificial processes are used to accelerate the biodegradation process. Because there are no external factors involved, this method is the most economical in-situ bioremediation technique available. Still, continuous and persistent bioremediation necessitates regular monitoring. The subsequent conditions must be fulfilled for intrinsic bioremediation to function effectively: 1. A healthy population of microorganisms in the contaminated area that break down materials. 2. Ideal environmental parameters (pH, humidity, temperature, and oxygen concentration). 3. Sources of carbon and nitrogen that might encourage the growth and activity of microorganisms (12). The biological restoration of obstructed groundwater using this technology is based on a stimulation-optimization strategy driven by particle swarm optimization and machine learning (ELM-PSO). This results in less costly pumping technology and decreased operating capital needs (13). There has also been research on using intrinsic remediation to remove Pb(II) from shallow unsaturated soil. Heavy metals can interact with microorganisms through their subcellular machinery, which allows them to live in soil with high amounts of Pb(II). Heavy metal remediation can be done in situ using microbial inoculants. Through redox reactions, Pb(II) interacts with Fe (II) ions, and the reduction reaction is triggered by the release of iron in soluble forms (14).

## 2.3 Phytoremediation and it's types

Phytoremediation is an alternate technique that uses plants to remove, stabilise, or break down contaminants. Many plant species have the ability to accumulate organic contaminants or heavy metals in their tissues through processes including phytodegradation and phytoextraction.

Phytoextraction, a form of phytoremediation, includes using plant branches to exclude pollutants, and that once they have amassed the pollutant, they can be collected and separated from the region. On the other hand, phytodegradation relies on the roots or the microorganisms as is attached to to facilitate breakdown of pollutants in the soil. Phytoremediation is used frequently, to treat soil and water bodies with organic pollutants, heavy and metalloids for the restoration of polluted ecosystems (15).

Phytoremediation involves the use and employment of plants in the absorption and removal of pollutants including heavy metals from water and soil. Thus plants have unique capability of immobilizing the heavy metals from the soil through the roots, concentrating it in the plant parts which can he harvested and taken out from the site. Phytoremediation uses a variety of technologies for uptake of Heavy metals and they include phytostabilization, rhizodegradation, phytoextraction, phytodegradation,phytoaccumulation and hystovolatilization (16).

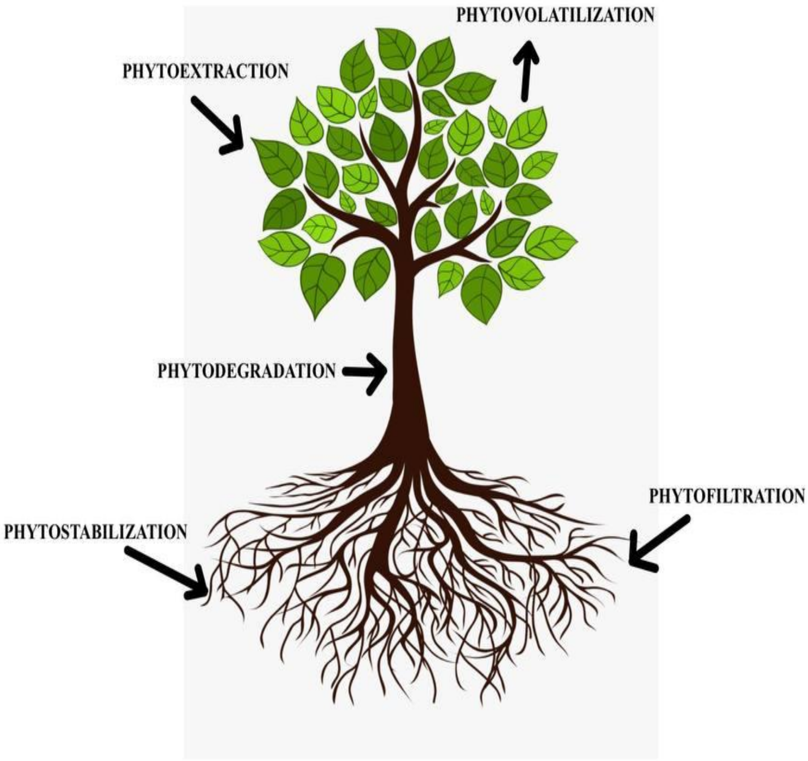



**Figure 1: Mechanisms of phytoremediation**

### 2.3.1 Phytoextraction

Phytoextraction, which, also called phytoaccumulation, phytoabsorption, and phytosequestration, is the process by which pollutants are removed from the soil or water by the roots of plants. These pollutants are transported and stored in aboveground plant organs or shoots and by the time the biomass is harvested, (17) Since, it is mostly impossible to harvest root biomass, the ability to translocate the pollutant to the shoot is a biochemical process that would be critical for phytoextraction. Phytovolatizable contaminants in plants vary over the life cycle of plants, and perennial plants that acquire high concentrations of contaminants throughout their life can be used for continuous phytoextraction (18).

### 2.3.2 Phytofiltration

The process of adsorbing or precipitating pollutants from a solution onto plant roots or absorbing them into the roots that surround the root zone is known as rhizofiltration, which is another name for phytofiltration (19). The production of particular compounds inside the roots of certain plants increases the binding capacity of contaminants, such as metal ions, by increasing the amount of phytochelatins present (20). Rhizofiltration is directly related to effluents, contaminated streams, and groundwater systems. For rhizofiltration to be implemented successfully, a complete grasp of contaminant speciation and the interactions between all contaminants and nutrients is required.

### 2.3.3 Phytostabilization

Phytostabilization, also known as phytoimmobilization, is the process of employing plants that can lower the mobility and/or bioavailability of pollutants to prevent them from seeping into ground water or entering the food chain through a variety of mechanisms, such as adsorption by roots or the formation of insoluble compounds in the root zone. Phytostabilization happens in two main ways: (1) when a pollutant is confined in the contaminated media during assimilation and aggregation by roots, adsorption onto roots, or precipitation within the plant's root region; and (2) when plants are used, along with their roots, to block the movement of pollutants by wind, water, soil drainage, and soil dispersion. The ultimate goal of phytostabilization is to stabilise pollutants rather than remove them, minimising their hazard to the environment and human health, in the hopes that plants will act similarly to soil amendments. Unfortunately, phytostabilization does not reduce the concentration of contaminants; instead, it only lowers the contamination of nearby media and places (21). This means that phytostabilization is not a permanent solution for contamination.

### 2.3.4 Phytovolatilization

Phytovolatilization is another phytoremediation technique that uses the uptake of pollutants by plants, which then converts them into volatile chemicals. These compounds are eventually released into the atmosphere in their original form or in a modified form because of the plants' metabolic and transpiration pull (22). Water vapor from leaf surfaces evaporates into the atmosphere through stomata during transpiration.Certain plant species have deep rootstocks which effectively facilitate uptake and degradation of pollutants within plant species as a result of certain enzymes or genes. Pesticides are taken up from the soil and water during phytovolatilization and redistribute into less hazardous gaseous form before being released through plant transpiration (23).

### 2.3.5 Phytodegradation

Phytodegradation, also referred to as phytotransformation, is a procedure that ‘pulls’ pollutants and nutrients from water, sediment or soil and brings about the chemical alteration of these substances in and for the plants themselves. It often leads to the neutralization or even elimination, degradation, or fixating of pollutants in the plant roots and/or shoots (24).

## 2.4 Bioremediation using microorganisms

Microbial mediated bioremediation involves the use of microorganisms including bacteria fungi and algae to help in the sequestration or elimination of the heavy metal in water. In another process, microorganisms can remove or immobolise metals in more than one way; two of these are biosorption and bioaccumulation, the third is biotransformation. Bioprocessing transformations; Bioremediation reactions detoxify heavy metal with help of enzyme in such a way that, they become sometime less toxic or less mobile. Microbial bioremediation is effective, affordable, eco-friendly, thus making the procedure essential for healing heavy metal polluted water. Bacteria are known to have multiple enzymes that can degrade or transform numerous pollutants found within the environment (25). Depending on the type of contamination and the surrounding conditions, microbial bioremediation can occur in either anaerobic or aerobic environment. By giving microbial populations the right circumstances and nutrition, one can increase breakdown rates and cleanup efficiency.

Surface complexation is the process by which molecules of pesticides or heavy metal ions bind to functional groups on the surface of microorganisms. Depending on the kinetic equilibrium and metal composition at the cell surface, microbial cells can interact to adsorb or absorb heavy metals onto binding sites on the cell surface. Electrostatic interactions, redox reactions, ion exchange, precipitation, and surface complexation are thus involved in biosorption. By passive absorption via surface complexation, dead or living biomass, including cellular fragments, can biosorb onto the cell wall and other exterior layers. Cell metabolism has no effect on this process. By successfully immobilising pollutants on the surface of microbial cells, surface complexation improves the removal of contaminants from water (26).

The process of exchanging heavy metal ions with other ions in the surrounding solution is known as ion exchange. Following the active uptake of heavy metal, the ions enter the cytoplasm through the plasma membrane (bioaccumulation method), where they are then subjected to intracellular and extracellular chemical, biological, physical, and other processes in the cell. The key components of biosorption are discovered to be processes including ion exchange, adsorption, and covalent bonding that happen as a result of the chemiosmotic gradient across the cell without the need of ATP. Ion exchange lowers the concentration of heavy metals in the aqueous phase by facilitating their uptake and concentration by microbial cells. The two pillars around which biosorption mechanisms are built are the cell's metabolism and the location within the cell where the metal is removed. The microbe after uptake of metal, transforms the metal changing it from toxic to harmless form, while this transformation of metal the microbe tolerates the high concentration of metal or a combination of metals. The way that bacteria interact with their surroundings is greatly influenced by their surface features (27).

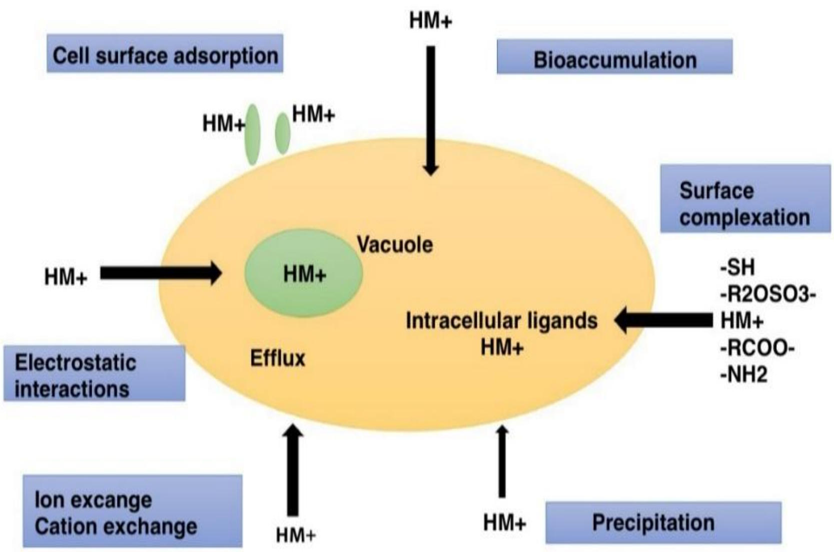



**Figure 2: Mechanisms of heavy metal uptake by microorganism**

## 2.5 Biochemical and molecular mechanisms of lead resistance in bacteria

### 2.5.1 Biosorption of lead on cell surface

As a result of the different macromolecules in the cell wall's functional groups participating in metal binding, the cell wall serves as a natural defence against dangerous metals (28). Naturally occurring negative charges in the cell walls of both Gram-positive and Gram-negative bacteria bind to metal cations to regulate the movement of metals across membranes. While phosphate groups are important for metal binding in Gram-negative bacteria, carboxyl groups are the main sites where metal cations attach to the cell walls of Gram-positive bacteria.It is known that substances with amide, amino, hydroxyl, or carboxyl groups have a role in $Pb2+$ binding Synecochoccus sp. cell wall, while amino groups, phosphate, carbonyl, and hydroxyl groups in Pseudomonas aeruginosa ASU6a cell wall (30) are known to have a role in $Pb2+$ binding. It's noteworthy to notice that in E. coli, nearly all of the $Pb2+$ is bonded in the cell membrane, while almost none of it is detected in peptidoglycan. The capacity of $Pb2+$ to adsorb on the cell surface is significantly influenced by pH and initial lead concentration. The effects of pH on metal removal (e.g., lead) were investigated by Pseudomonas pseudoalcaligenes and Micrococcus luteus. The findings demonstrated that metal biosorption increased as pH rose from 2 to 6, with a pH 5 maximal absorption capability and a 100 mg $L-1$ starting metal concentration (30).

### 2.5.2 Biosorption of lead

Many bacteria that are resistant to metals also have a method for absorbing metals through the secretion of extracellular polymeric substances (EPS). Due to their role in the flocculation process and the binding of metal ions from solutions, these EPS are especially relevant to the bioremediation process. EPS are a complex mixture of high molecular weight polyanionic polymers that bind cationic metals with varying degrees of selectivity and affinity. Examples of these polymers include proteins, humic acids, polysaccharides, and nucleic acids. It has been discovered that a number of lead-resistant bacteria can absorb lead in monoand mixed metal solutions, including Serratia sp. L30, Raoultella planticola R3, Providencia rettgeri L2, and Klebsiella michiganensis R19 (31). The growth rate of these bacterial strains is 1.25 or 1.5 g $L-1$ of Pb $(NO3)2$.

K. michiganensis R19 exhibits the maximum $Pb^{2+}$ removal in mono-metal (lead alone) solutions. As, Pb, Cu, Mn, Zn, Cd, Cr, and Ni are the eight metals found in the many metal solutions. The highest $Pb^{2+}$ removal (297 mg Pb $g^{-1}$) is shown by K. michiganensis, which is followed by R. planticola R3 (102 mg Pb $g^{-1}$) and P. rettgeri L2 (0.11 mg Pb $g^{-1}$). Because of its extremely high specific affinity for $Pb^{2+}$, strain K. michiganensis R19 can be used to recover $Pb^{2+}$ from solutions containing several metals. The binding process immobilises the metal and prevents hazardous cationic metals from entering the cell. It has been documented that certain lead-resistant bacteria exhibit EPS binding of $Pb^{2+}$. EPS metal sorption is influenced by a number of factors, including pH, sodium chloride concentrations, and lead ($Pb^{2+}$) binding concentrations.

Upon treatment with 1.6 mM lead nitrate in Tris buffered minimal media (pH 7.2), Enterobacter cloacae P2B cells exhibit a significant uptick in EPS synthesis (108 mg $l^{-1}$ dry weight). (32) underlined that pH is the only factor affecting $Pb^{2+}$ immobilisation in the EPS. Paenibacillus jamilae has the ability to bind lead at its maximal capacity of 303 mg $g^{-1}$ EPS at pH 6, as per their research. Marinobacter sp. prefers an acidic pH over a near-neutral pH for the sorbed $Cu^{2+}$ and $Pb^{2+}$ ions. The maximum calculated binding ability of the EPS is 182 nmol of copper and 13 nmol of lead $mg^{-1}$. However, the sorption of these metals reduced as the concentration of sodium chloride increased (33).

### 2.5.3 Bioaccumulation of lead

The active metabolic process of bioaccumulation requires energy. Bioaccumulation binds metals intracellularly, as opposed to the biosorption mechanism discussed above (34). Perhaps the most well-known process is the one that entails metal-binding with metallothioneins. Low molecular weight, cysteinerich proteins called metallothioneins aid in the sequestration or accumulation of dangerous metals inside of cells. Because this resistance mechanism is often plasmid-borne, cell-to-cell transmission is facilitated. Bacteria that are exposed to additional metals generate metallothioneins. It has been demonstrated that the following bacteria, when exposed to $Pb^{2+}$: Bacillus cereus, Bacillus megaterium, Proteus penneri GM-10, Providencia vermicola strain SJ2A, Pseudomonas aeruginosa strain WI-1, Salmonella choleraesuis, and Streptomyces sp., 4A (35).

### 2.5.4 Precipitation of lead

Precipitation is another technique used by certain bacteria to convert the concentration of free metals to insoluble complexes, hence lowering their toxicity and bioavailability. Pb2+ is known to react with a wide range of anions, such as carbonates, phosphates, sulphides, hydroxyl ions, and chlorides, to produce insoluble precipitates. Within the cell, the precipitation process occurs either extracellularly or intracellularly (36). It has been demonstrated that the bacteria Alcalifaciencia Providencia 2EA, ATCC13525 Pseudomonas fluorescens, Staphylococcus aureus, Vibrio harveyi, and Bacillus thuringiensis 016 precipitate lead into lead phosphate. It is shown that the phosphate-solubilizing bacteria E. cloacae immobilises lead as an insoluble lead phosphate called pyromorphite, which is how it resists lead. The lead-resistant Bacillus iodinium GP13, Bacillus pumilus S3, and Klebsiella aerogenes NCTC418 precipitate lead into lead sulphide (37). Recently, the bacteria Pararhodobacter sp. and the technique of microbially induced calcium carbonate precipitation (MICP) have been used to bioremediate lead-contaminated mining wastes. Laboratory scale experiments showed that 1036 mg L−1 of lead had been completely eliminated and that lead and calcium carbonate coexisted. Complete removal of Pb2+ from soils is possible with Pararhodobacter sp. This result is comparable to other ureolytic bacteria that eliminated lead 90–100% of the way, such as 100% for Terrabacter tumescens, 90.31% for Rhodobacter spharoides, and Sporosarcina pasteurii (38). used the calcite-precipitating bacteria Kocuria flava to study the effects of Pb contamination on soil bioavailability. These findings showed that lead-contaminated soil can be cleaned up by lead precipitation using MICP. What enables ureolytic bacteria to completely remove lead is their ability to efficiently hydrolyze urea to create carbonate ions and raise pH to alkaline levels (8.0–9.1), which encourages the precipitation of lead and calcium carbonate. The effects of pH on precipitating metals are well documented. For example, with pH values of 6.6 and above, various Pb phosphates (in noncalcareous soils) and PbCO3 may precipitate. Increased acidity in the surroundings also appears to lessen metal complexation.These findings thus validate the hypothesis that pH changes must be taken into account when using the precipitation approach for metal bioremediation.

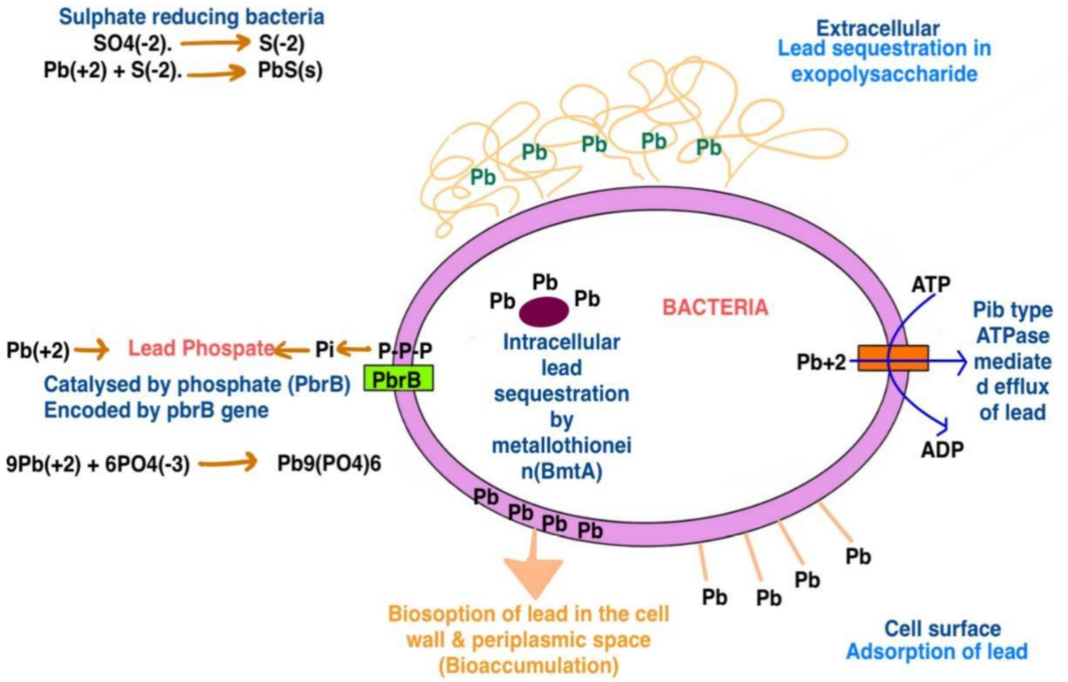



**Figure 3. Lead resistance mechanism in bacteria**

## 2.6 SOURCES OF LEAD

Lead pollution stems from various origins, comprising natural occurrences and anthropogenic activities. Naturally, lead is ubiquitous in the Earth's crust, present in soil, rocks, and minerals, with the potential to leach into groundwater. Anthropogenic sources include industrial processes like lead smelting and mining, which release lead emissions into the environment. Historically, the combustion of leaded gasoline in vehicles was a significant source of atmospheric lead contamination, although regulations have curtailed this practice in many regions. Consumer products have also contributed to lead exposure, with lead-based paints, ceramics, and plumbing materials being notable examples. Water and air pollution pathways further exacerbate lead exposure, with lead leaching from soil into groundwater and lead particles emitted into the atmosphere from industrial activities and combustion processes. Addressing lead pollution necessitates comprehensive strategies targeting its diverse sources and pathways, alongside regulation of lead containing products and remediation of contaminated environments (39).

## 2.7 REDUCTION OF LEAD

Lead (II) reductase is an enzyme that catalyzes the reduction of Pb(II) to metallic lead. This process can be important in the context of bioremediation, where microorganisms use such enzymes to detoxify heavy metals in contaminated environments. Below is an explanation of the reduction of lead by lead (II) reductase, along with some references to scientific literature.

**Mechanism of Action**: Lead (II) reductase typically reduces Pb(II) ions to Pb(0) (metallic lead) through a series of redox reactions. The enzyme forms a complex with Pb(II) and then, with the help of electrons as from elemental cofactors such as NADH or NADPH, the enzyme converts lead to the first lowest oxidation state as a lead form. This process occasionally includes changing of the oxidation state of the reduced form of the cofactor by donating electrons to the metal ion.

**Microbial Role:** It has also been found that certain bacteria contain organelles called lead (II) reductase enzymes that can reduce lead as a means of metabolism. Being able to live in heavy metal-contaminated environments and in doing bio leaching of toxic Pb(II) to insoluble and non bioavailable Pb(0).

**Bioremediation Potential:** Microorganisms possessing lead (II) reductase activity are considered to be efficient bioremediation tools to eliminate contaminants containing heavy metals, particularly lead. They have the advantage of preventing lead from coming into contact with the environment in any uncontrolled way: The process ‘fixes’ lead in its metallic form and so the bioavailability of that lead is virtually nil (40).

**TABLE 1. PHYSIO-CHEMICAL PROPERTIES OF LEAD(II) REDUCTASE**

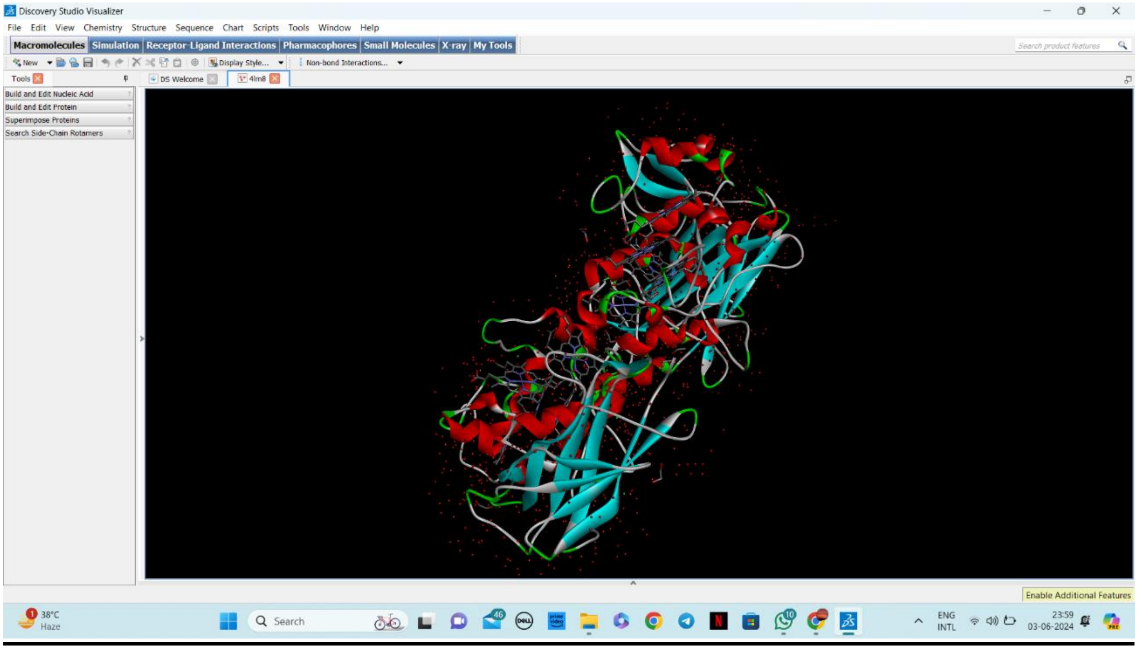
| PARAMETER | MEASURES |
|---|---|
| Molecular Weight | 20-200 kDa |
| Isoelectric Point (pI) | 7.64 |
| Optimal pH | 6-7.5 |
| Optimal Temperature | 25°C to 37°C |
| No. of amino acids | 320 |
| Aliphatic index | 85.67 |
| Instability index (II) | 32.15 |

# CHAPTER 3
# METHODOLOGY

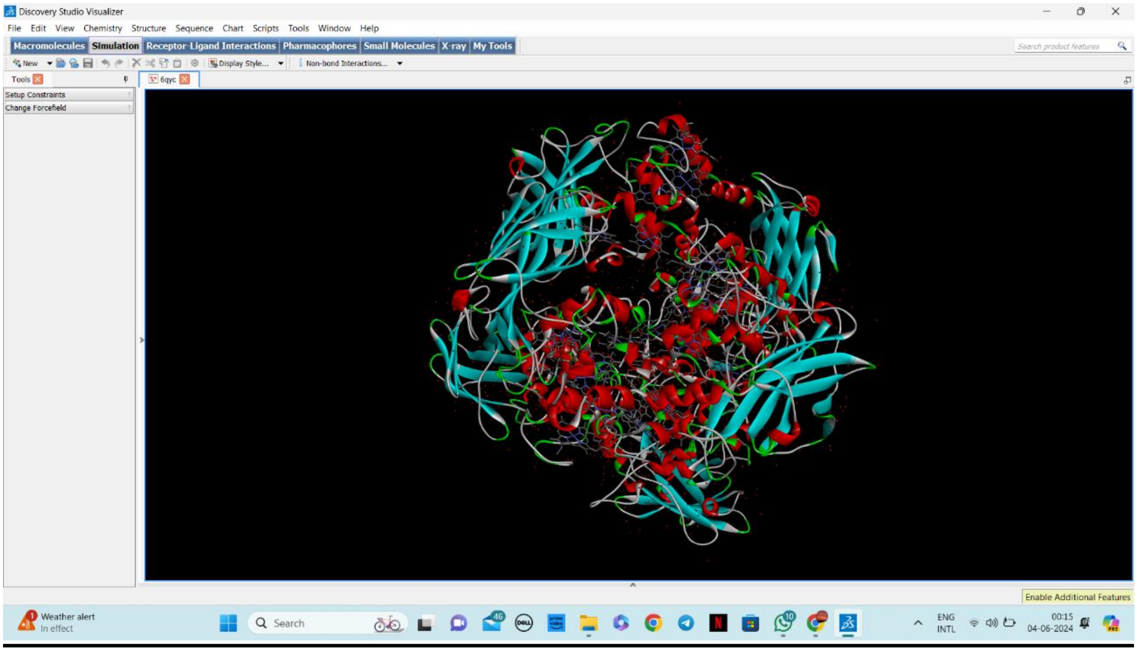
PyRx is the software in use for the docking process. A software known as PyRx, or Python Prescription, is employed in computational chemistry and bioinformatics to perform tasks as molecular docking and virtual screening. In essence, anyone who wants to comprehend which direction a ligand (or any other molecule) would favour binding itself to a receptor (or any other molecule) so as to form a stable complex can use it. It enables virtual screening, a form of computational method involving the process of running through a large database of the molecular substances in the search of the efficient therapeutic target. This is done through ligand docking that tries to arrange different molecules against a particular receptor and thereby, ranks the compounds that could form high binding affinity for further experimental validation.

## 3.1 LIGAND PREPARATION

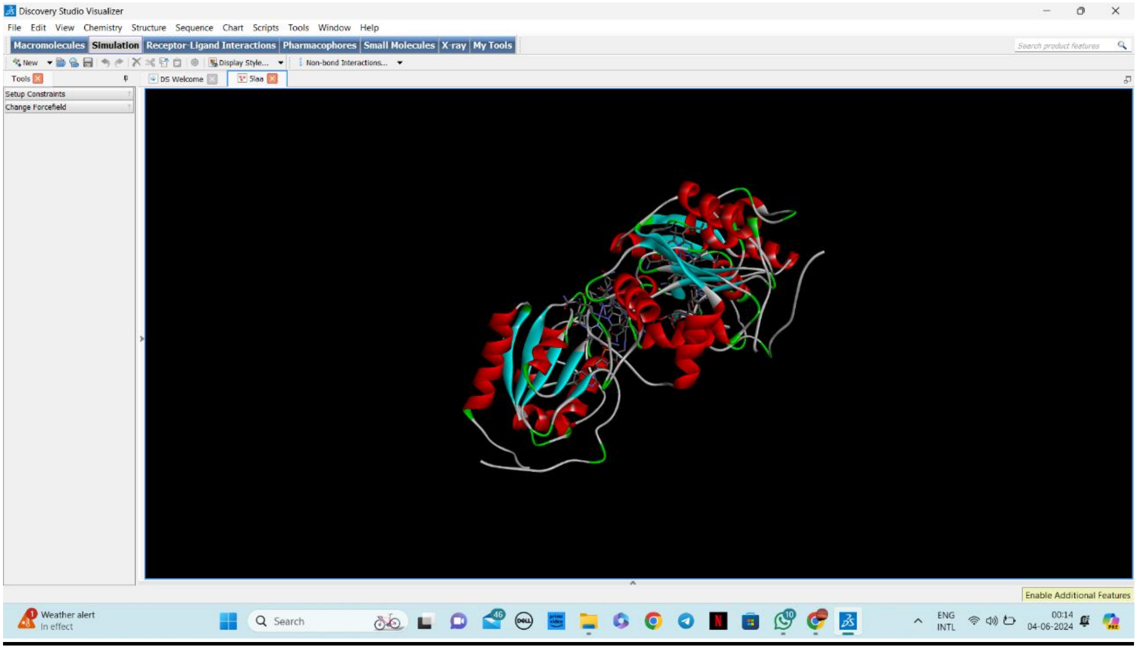
For the ligand, a 3D structure in PDB format is retrieved from PubChem before preparing it for the subsequent docking stage. PubChem is a large chemical substance information data base managed by the National Centre for Biotechnology Information or NCBI, which is an arm of the National Institutes for Health or NIH. It is therefore a very useful way for scientists, researchers and anybody in the general public who wants to know more about the biological action of small molecules to learn from for free. PubChem contains information regarding chemical compounds; empirical details, properties, and structure of each compound. It encompasses a wide range of carbon containing products – small molecules, synthetic products, medicines, organic compounds, and inorganic compounds as well as natural products. This is becauseannis is free for everyone and everyone is specially encourage to participate in scientific collaboration and sharing of data. Ligands are available in JSON, SDF, and XML formats. Docking is performed using these conformations as the initial conformation. Lead(II)Reductase was obtained from different bacteria like Shewanella oneidensis, Rhodopseudomonas palustris and Pseudomonas strain in pdb format and water molecules and ions were removed for better understanding of interaction.




**SCREENSHOT OF LEAD(II) REDUCTASE FROM Shewanella oneidensis**




**SCREENSHOT OF LEAD(II) REDUCTASE FROM Rhodopseudomonas palustris**




**SCREENSHOT OF LEAD(II) REDUCTASE FROM Pseudomonas strain**

## 3.2 TARGET PREPARATION

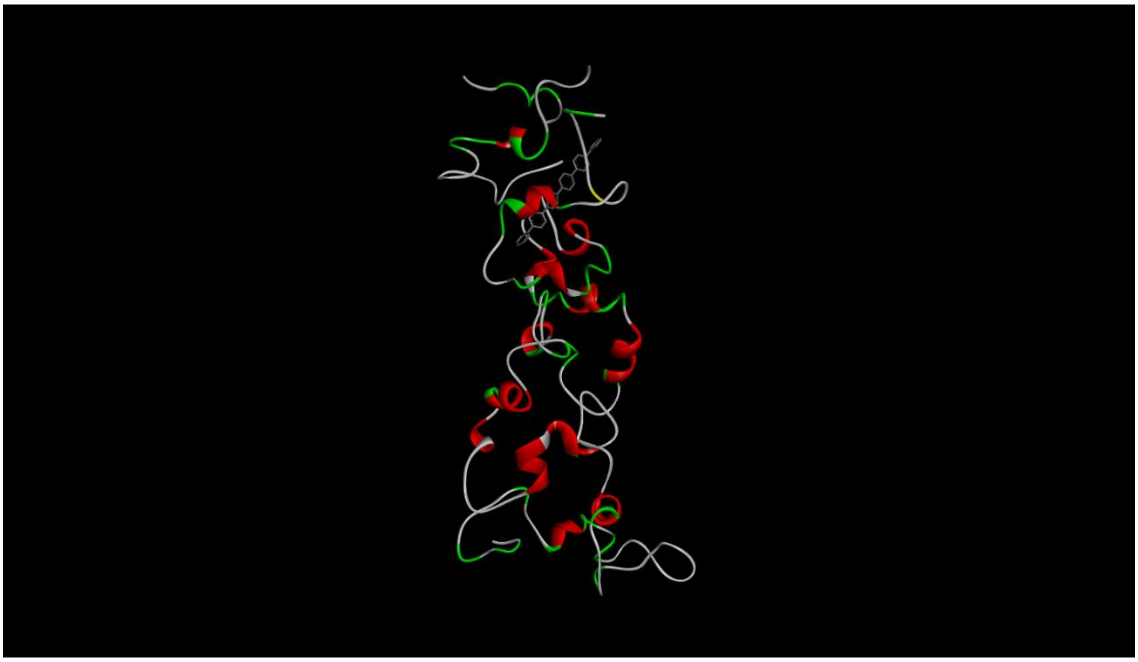
Following the identification of suitable targets, secondary research using international journals, articles, and publications was used to select the most efficient and relevant target.

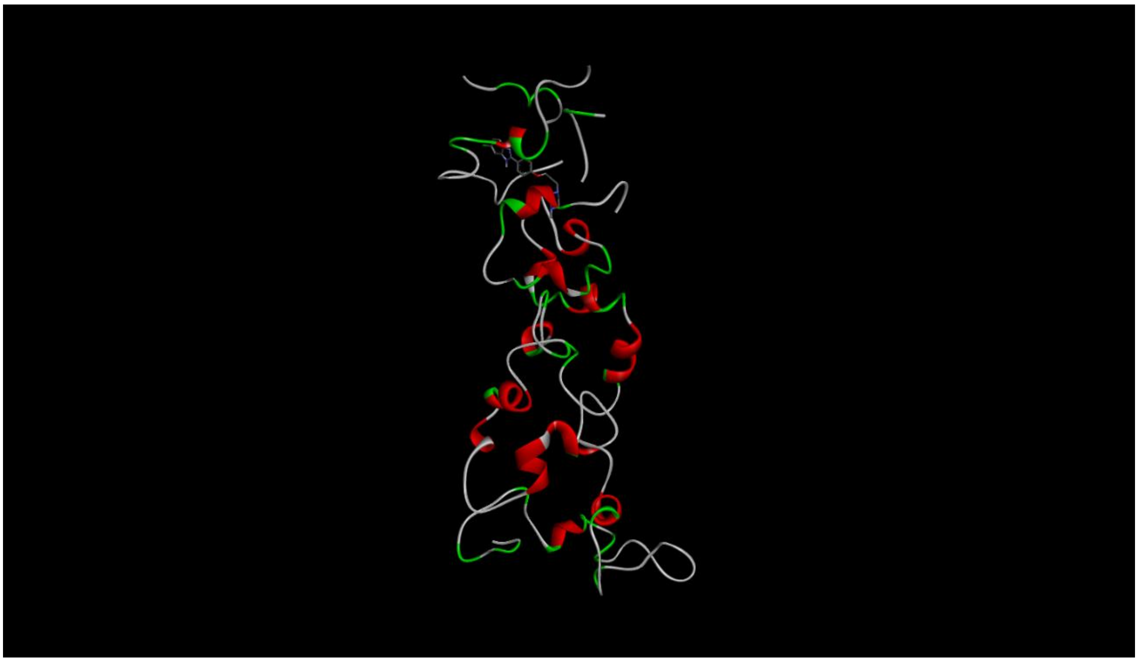
The Protein Data Bank provided the X-ray structures in PDB format. The targets were downloaded in a manner that prevented the presence of water, ions, or complexes with other bioactive compounds.

**3D Structure of Lead**

# CHAPTER 4
# RESULTS AND DISCUSSION

Using the PyRx tool, the docking was done with 3 distinct bacterial species that produced the lead(II) reductases. The lead metal and enzyme docked molecule with the lowest energy was selected and considered. Lead reductase, Rhodopseudomonas palustris, is an FMN-containing organism whose structure varies in reaction to lead anion and NADH interaction. This rearrangement allows both species to bind simultaneously, which is necessary for practical enzyme cycling because otherwise the binding site would be occupied by the electron donor.





# CHAPTER 5
# CONCLUSION

Bioremediation strategies have been developed in response to the challenging task of eliminating lead contamination from the environment. One such strategy is the use of microorganism-based reduction procedures, in which four distinct bacterial species are coupled against chromium metal. These bacteria possess a strong metabolism, resilience to oxidative stress and toxic compounds, and flexibility in genetic alteration, which enable them to change into cell factories that generate natural products for a range of biological applications. A validated three-dimensional structure of lead reductase, determined from bacterial strains and mutant models, is shown in this paper. This structure stabilises the system and allows for improved interaction with Pb(II) and reduction to Pb(0). One possible bioremediation technology is biostimulation, one of the bioremediation strategies. The alteration improves bioremediation; nevertheless, an excess of biomass may clog subsurface pores, reducing the treatment's efficacy. Therefore, biostimulation is useless in these areas. Chromate cleanup is made worse by toxic intermediates produced during chromate reduction, which harm remediation microorganisms. Techniques in genetic and protein engineering may be able to address some of these issues. Certain promoters can be used to maximise the production of desirable genes in slow-growing bacteria, hence decreasing the accumulation of biomass. Bacterial chromate reductases can also be engineered to produce improved enzymes that reduce chromate more efficiently, are less harmful to the bacteria carrying out the cleanup, and can continue to function in the presence of more contaminants.

# REFRENCES

1. Rehman, A., Zahoor, A., Muneer, B. and Hasnain, S. (2008) Chromium tolerance and reduction potential of a Bacillus sp.ev3 isolated from metal contaminated wastewater. Bulletin of Environmental and Contamination Toxicology, 81, 25-29.

2. Bogdanova, E.S., Mindlin, S.Z., Pakrova, E., Kocur, M. and Rouch, D.A. (1992) Mercuric reductase in environmental Gram-positive bacteria sensitive to mercury. FEMS Microbiology Letters, 97, 95-100.

3. Cleveland LM, Minter ML, Cobb KA, Scott AA, German VF (2008) Lead hazards for pregnant women and children. Am J Nurs 108: 40–49.

4. Gadd GM (2009) Biosorption: critical review of scientific rationale, environmental importance and significance for pollution treatment. J Chem Technol Biotechnol 84:13–28.

5. Abou-Shanab, R.A., Ghanem, K., Ghanem, N., & Al-Kolaibe, A. (2007). The role of bacteria on heavy-metal extraction and uptake by plants growing on multi-metal-contaminated soils. World Journal of Microbiology and Biotechnology, 23(4), 619-625.

6. Haferburg, G., & Kothe, E. (2007). Microbes and metals: interactions in the environment. Journal of, 47(6), 453-467.

7. Kensa, V. M. (2011). Bioremediation-an overview. Journal of Industrial Pollution Control, 27(2), 161-168.

8. 7. Ryan, J. R., Loehr, R. C., and Rucker, E.: Bioremediation of organic contaminated soils. J. Hazard. Materials, 28, 159-169 (1991).

9. Belser, L. W.: Population ecology of nitrifying bacteria. Ann. Rev. Microbial., 33, 309-333 (1979).

10. Wolin, M. J. and Miller, T. L.: Bioconversion of organic carbon to CH. and CO,. Geomicrobiol. J. 5.239-260 (1987).

11. Lovlei, D. R./ Dissimilatory Fe' (iI1) and bin (Iv) reduction. Microbial. Rev., 55,259-287 (1991).

12. lsen, R. A. and Bakken, L. R.: Viability of soil bacteria, optimization of plate-counting technique and comparison between total counts and plate counts within different size groups. Microb. Ecol., 13,59-74 (1987).

13. Sales da Silva IG, Gomes de Almeida FC, Padilha da Rocha e Silva NM, Casazza AA, Converti A, AsforaSarubbo L. Soil bioremediation: Overview of technologies and trends. Energies. 2020 Sep 8;13(18):4664.

**14.** Bala S, Garg D, Thirumalesh BV, Sharma M, Sridhar K, Inbaraj BS, Tripathi M. Recent strategies for bioremediation of emerging pollutants: a review for a green and sustainable environment. Toxics. 2022 Aug 19;10(8):484.

**15.** Roe RA, MacFarlane GR. The potential of saltmarsh halophytes for phytoremediation of metals and persistent organic pollutants: An Australian perspective. Marine Pollution Bulletin. 2022 July.

**16.** Sabreena, Hassan S, Bhat SA, Kumar V, Ganai BA, Ameen F. Phytoremediation of heavy metals: An indispensable contrivance in green remediation technology. Plants. 2022 May 6;11(9):1255.

**17.** Ghosh, M., & Singh, S. P. (2005). A review on phytoremediation of heavy metals and utilization of it's by products. Asian J Energy Environ, 6(4), 18.

**18.** Sarwar, N., Imran, M., Shaheen, M. R., Ishaque, W., Kamran, M. A., Matloob, A., ... & Hussain, S. (2017). Phytoremediation strategies for soils contaminated with l heavy metals: modifications and future perspectives. Chemosphere, 171, 710-721.

**19.** Khan, I., Iqbal, M., & Shafiq, F. (2019). Phytomanagement of lead-contaminated soils: critical review of new trends and future prospects. International Journal of Environmental Science and Technology, 16, 6473-6488.

**20.** Singh, N. P., & Santal, A. R. (2015). Phytoremediation of heavy metals: the use of green approaches to clean the environment. Phytoremediation: Management of Environmental Contaminants, Volume 2, 115-129.

**21.** Bolan, N. S., Park, J. H., Robinson, B., Naidu, R., & Huh, K. Y. (2011). Phytostabilization: a green approach to contaminant containment. Advances in agronomy, 112, 145-204.

**22.** Kumar, S. S., Kadier, A., Malyan, S. K., Ahmad, A., & Bishnoi, N. R. (2017). Phytoremediation and rhizoremediation: uptake, mobilization and sequestration of heavy metals by plants. Plant-microbe interactions in agro-ecological perspectives: volume 2: microbial interactions and agro-ecological impacts, 367-394.

**23.** Khalid, S., Shahid, M., Niazi, N. K., Murtaza, B., Bibi, I., & Dumat, C. (2017). A comparison of technologies for remediation of heavy metal contaminated soils. Journal of geochemical exploration, 182, 247-268.

**24.** Bulak, P., Walkiewicz, A., & Brzezińska, M. (2014). Plant growth regulators-assisted phytoextraction. Biologia plantarum, 58, 1-8.

**25.** Qurbani K, Hamzah H. Intimate communication between Comamonas aquatica and Fusarium solani in remediation of heavy metal-polluted environments. Archives of microbiology. 2020 Aug;202:1397-406.

**26.** Motteran F, Varesche MB, Lara-Martin PA. Assessment of the aerobic and anaerobic biodegradation of contaminants of emerging concern in sludge using batch reactors. Environmental Science and Pollution Research. 2022 Dec;29(56):84946-61.

27. Wang J, Zhang Y, Liu T, Shi Y, Ding Y, Zhang Y, Xu W, Zhang X, Wang Y, Li D. A biodegradable chitosan-based polymer for sustained nutrient release to stimulate groundwater hydrocarbon-degrading microflora. Chemosphere. 2023 Dec 1;344:140346.
28. Fomina M, Gadd GM (2014) Biosorption: current perspectives on con- cept, definition and application. Bioresour Technol 160:3– 14.
29. Gabr RM, Hassan SHA, Shoreit AAM (2008) Biosorption of lead and nickel by living and non-living cells of Pseudomonas aeruginosa ASU 6a. Int Biodeterior Biodegrad 62:195–203.
30. Leung WC, Wong MF, Chua H, Lo W, Yu PHF, Leung CK (2000) Removal and recovery of heavy metals by bacteria isolated from activated sludge treating industrial effluents and municipal waste- water. Water Sci Technol 41:233–240.
31. Bowman N, Patel D, Sanchez A, Xu W, Alsaffar A, Tiquia-Arashiro SM (2018) Lead-resistant bacteria from Saint Clair River sediments and Pb removal in aqueous solutions. Appl Microbiol Biotechnol 102: 2391–2398.
32. Perez MPJA, Garcıa-Ribera R, Quesada T, Aguilera M, Ramos- Cormenzana A, Monteoliva-Sanchez M (2008) Biosorption of heavy metals by the exopolysaccharide produced by Paenibacillus jamilae. World J Microbiol Biotechnol 24:2699–2704.
33. Bhaskar PV, Bhosle NB (2006) Bacterial extracellular polymeric sub- stances (EPS) a carrier of heavy metals in the marine foodchain. Environ Int 32:192–198.
34. Das S, Dash HR, Chakraborty J (2016) Genetic basis and importance of metal resistant genes in bacteria for bioremediation of contaminated environments with toxic metal pollutants. Appl Microbiol Biotechnol 100:2967–2984.
35. Joutey NT, Sayel H, Bahafid W, El Ghachtouli N (2015) Mechanisms of hexavalent chromium resistance and removal by microorganisms. Rev Environ Contam Toxicol 233:45–69.
36. Levinson HS, Mahler I, Blackwelder P, Hood T (1996) Lead resistance and sensitivity in Staphylococcus aureus. FEMS Microbiol Lett 145:421–425.
37. Aiking H, Govers H, van't Riet J (1985) Detoxification of mercury, cad- mium, and lead in Klebsiella aerogenes NCTC 418 growing in continuous culture. Appl Environ Microbiol 50:1262–1267.
38. Li M, Cheng X, Guo H, Yang Z (2015) Biomineralization of carbonate by Terrabacter tumescens for heavy metal removal and biogrouting applications. J Environ Eng.
39. Plumlee, G. S., & Morman, S. A. (2011). The environmental geochemistry of lead. In Environmental geochemistry: Site characterization, data analysis and case histories (pp. 215-245).
40. Jiang, W., & Fan, W. H. (2008). Bioreduction of Lead(II) to Lead(0) Nanoparticles by Shewanella oneidensis MR-1.

# PROOF/LIST OF PUBLICATION

**Title of the Review Paper:** "Bioremediation of heavy metals in contaminated water bodies"

**Name of the Authors:** Divya, Himani Joshi, Jai Gopal Sharma*

AFJBS
African Journal of Biological Sciences


**Bioremediation of heavy metals in contaminated water bodies**

**Divya[1], Himani Joshi[1] & Jai Gopal Sharma[1*]**

[1]Department of Biotechnology, Delhi Technological University, New Delhi-110042, India
Corresponding Author: sharmajaigopal@dce.ac.in

Article History
Volume 6, Issue 5, 2024
Received: 09 May 2024
Accepted: 17 May 2024
doi: *10.33472/AFJBS.6.5.2024. 5045-5070*

**Abstract**

Heavy metals are naturally occurring elements of the environment, but their geochemical cycles and biochemical balance have been disrupted by human use. Heavy metals including cadmium, copper, lead and others are consequently released in excess into natural resources like soil and aquatic ecosystems. Long-term exposure to and increased buildup of these heavy metals can have detrimental impacts on aquatic biota and human health. In order to get rid of these toxic contaminants one of the most environmentally friendly, economical, safer, and cleanest technologies for decontaminating places contaminated with a variety of contaminants is bioremediation. The technique of employing biological agents to remove harmful waste from the environment is referred to as "bioremediation". The hazardous waste that greatly harms the environment is heavy metals in wastewater. This review article provides a comprehensive analysis of bioremediation, including its types, effects of heavy metals on human health, bioremediation of heavy metals in polluted water, microbe-mediated bioremediation of heavy metals. There are also reports on a few possible species of plants and microbes that are frequently employed to remove heavy metals. The phytoremediation of heavy metals, phytoremediation methods, difficulties in microbial-mediated bioremediation, and recommendations for further bioremediation research are also examined in this article.

**Keywords:** Heavy metal; Decontaminating; Contaminants; Bioremediation; Phytoremediation

# PLAGIARISM REPORT

**Similarity Report**

PAPER NAME

THESIS MSCBIO-64.docx

| WORD COUNT | CHARACTER COUNT |
|---|---|
| 5324 Words | 31669 Characters |
| PAGE COUNT | FILE SIZE |
| 19 Pages | 1.7MB |
| SUBMISSION DATE | REPORT DATE |
| Jun 4, 2024 9:49 PM GMT+5:30 | Jun 4, 2024 9:50 PM GMT+5:30 |

## 11% Overall Similarity

The combined total of all matches, including overlapping sources, for each database.

- 7% Internet database
- 10% Publications database
- Crossref database
- Crossref Posted Content database

## Excluded from Similarity Report

- Submitted Works database
- Bibliographic material
- Quoted material
- Cited material
- Small Matches (Less then 8 words)

# AI REPORT

## THESIS MSCBIO-64.docx

Delhi Technological University

### Document Details

Submission ID
**trn:oid:::27535:60711697**

Submission Date
**Jun 4, 2024, 9:49 PM GMT+5:30**

Download Date
**Jun 4, 2024, 9:51 PM GMT+5:30**

File Name
**THESIS MSCBIO-64.docx**

File Size
**1.7 MB**

**Pages**

**Words**

**Characters**

turnitin Page 1 of 21 - Cover Page Submission ID trn:oid:::27535:60711697

turnitin Page 2 of 21 - AI Writing Overview Submission ID trn:oid:::27535:60711697

**How much of this submission has been generated by AI?**

**0%**

of qualifying text in this submission has been determined to be generated by AI.

**Caution: Percentage may not indicate academic misconduct. Review required.**

It is essential to understand the limitations of AI detection before making decisions about a student's work. We encourage you to learn more about Turnitin's AI detection capabilities before using the tool.

# CV

## DIVYA VAID

+91 9350609564
divyavaid2000@gmail.com

**EDUCATION**

| | | | |
|---|---|---|---|
| B.sc (Hons.) Zoology | 2019-2022 | Kalindi College, Delhi university | 7.784 CGPA |
| CBSE XII | 2018 | OSDAV, Public School, Kaithal, HARYANA | 77% |
| CBSE X | 2016 | LITTLE FLOWER SER. SEC. CONVENT SCHOOL HARYANA | 9.6 CGPA |

**ACADEMIC PROJECT**

**Project- Engineering the Microbiome for Therapeutic Applications**

- Project proposal for the creation of Microbiome for Therapeutic Applications
- Potential Applications of Microbiome Engineering- Therapeutic Interventions, Nutrition and Metabolic Health, Agriculture and Crop Health, Environmental Remediation, Probiotics and Functional Foods.

**Project- c-DNA Library**

- Studied the construction of c-DNA libraries for genomic studies
- Learned the advantages and disadvantages of c-DNA libraries and their applications in genomic studies

**SKILLS**

| | |
|---|---|
| Laboratory Techniques: DNA extraction, PCR, gel electrophoresis, microscopy, cell culture, protein purification, and spectroscopy etc. | MS Office, MS PowerPoint, MS excel, and MS Outlook |

**EXTRA-CURRICULAR ACTIVITIES AND ACHIEVEMENTS**

- Certification course in Digital Marketing
- Certification course in Fundamentals of Digital Marketing from GOOGLE DIGITAL UNLOCKED
- Certification course in Content Writing & Blogging