IN SILICO ANALYSIS OF HEAVY METAL ACCUMULATION AND BINDING AFFINITY IN *POPULUS DELTOIDES*: IMPLICATIONS FOR PHYTOREMEDIATION ENHANCEMENT

A Dissertation

Submitted in partial fulfilment of the requirement for the degree of

MASTERS OF SCIENCE

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BIOTECHNOLOGY

by

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CANDIDATE'S DECLARATION

I, **Abhilipsa Bhoi**, hereby certify that the work is being presented as the Major Project in the thesis entitled **" *In Silico* Analysis of Heavy Metal Accumulation and Binding Affinity in Populus deltoides: Implications for Phytoremediation Enhancement "** in partial fulfilment of the requirement for the award of the Degree of Master of Science in Biotechnology and submitted to the Department of Biotechnology, Delhi Technological University, Delhi is an authentic record of my work, carried out during the period from January 2025 to May 2025 under the supervision of **Prof. Jai Gopal Sharma**.

I have not submitted the matter presented in the report for the award of any other degree of this or any other institute/University.

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CERTIFICATE

1 hereby certify that the Project Dissertation titled "***In Silico* Analysis of Heavy Metal Accumulation and Binding Affinity in *Populus deltoides*: Implications for Phytoremediation Enhancement**" which is submitted by Abhilipsa bhoi, 2k23/MSCBIO/02, Department of Biotechnology, Delhi Technological University, Delhi in partial fulfilment of the requirement for the award of the degree of Masters of Science, is a record of the project work carried out by her 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.

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ABSTRACT

Heavy metal contamination in soil and water is a major hazard for the environment and public health. The study used an in-silico method to determine the potential of *Populus deltoides*, a fast-growing and metal-tolerant plant species for phytoremediation of soil and water contaminated by heavy metals. 93 metal-uptake related genes from several hyperaccumulating species were identified in an integrative in silico way by literature mining. Homologous genes in Populus deltoides were deduced through Blast analysis, followed by protein structural prediction using AlphaFold and SWISS-MODEL 3D algorithms. Using PyRx to dock $CdCl_2$, $ZnCl_2$, MnS, and CdS at the molecular level was done in order to determine whether there existed high-affinity binding sites for heavy metal chelates. Altogether, we detected at least three distinct lead-interacting complexes comprising two or more different cherry proteins. At the very least, these interactions argue that the *Populus deltoides* proteins do indeed possess powerful metal binding capabilities, making them an excellent candidate for phytoremediation. Combining plant breeding with genetic engineering, this study not only reveals the genetic and molecular basis of heavy metal detoxification in *populus deltoides* but also offers new theoretical tools for improving phytoremediation efforts. We anticipate that the results of this study will facilitate the subsequent development of genetically enhanced plant lines for effective environmental remediation.

Keywords: phytoremediation, *Populus deltoides*, in- silico analysis, molecular docking, metal-binding proteins.

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List of Symbols and Abbreviations

1. **As:** Arsenic
2. **Cd:** Cadmium
3. **Hg:** Mercury
4. **Pb:** Lead
5. **Zn:** Zinc
6. **HM:** Heavy Metals
7. **BCF:** Bioconcentration Factor
8. **TF:** Translocation Factor
9. **ROS:** Reactive Oxygen Species
10. **ABC:** ATP-binding Cassette
11. **NRAMP:** Natural Resistance-Associated Macrophage Protein
12. **HMA:** Heavy Metal ATPase
13. **ZIP:** Zinc-Iron Permease
14. **MT:** Metallothioneins
15. **PCS:** Phytochelatin Synthase
16. **MD:** Molecular Dynamics
17. **ΔG:** Free Binding Energy
18. **PDB:** Protein Data Bank
19. **BLAST:** Basic Local Alignment Search Tool
20. **RMSD:** Root Mean Square Deviation

CHAPTER 1

INTRODUCTION

1.1 Recognizing Heavy Metal Pollution as a Worldwide Issue

A significant environmental issue, heavy metal contamination is caused by mining operations, urban activity, agricultural runoff, and industrial discharges. Due to their persistence in ecosystems and inability to biodegrade, metals including cadmium (Cd), lead (Pb), arsenic (As), zinc (Zn) and mercury (Hg) contaminate soil and water over time and may negatively impact a range of physiological and biochemical functions in crop plants, reduce agricultural output, and pollute the environment [1]. While the biological half-life of cadmium (Cd) is around 10–30 years [2], lead (Pb) can stay in soil for more than 150–5,000 years and at high concentrations for up to 150 years after sludge application [3]. Like other contaminants, heavy metals deteriorate, making their removal from the environment very difficult. As heavy metals, elements having densities more than 5 g/cm3 are regarded as universal contaminants [4]. Approximately 10 million individuals globally have been impacted by HM-contaminated soil, according to previously published research [5]. Anthropogenic industrialization, urbanization, and agricultural practices have all substantially raised the amount of heavy metals released into the environment.

- **Industrial Activities:** The primary causes of heavy metal pollution of soils and water systems are the mining, metal smelting, and manufacturing sectors [6]. The problem is made worse by improper industrial waste disposal.
- **Agricultural practices:** Sewage sludge, chemical fertilizers, and pesticides all bring metals like Cd, As, and Pb into the soil.
  These metals contaminate drinking water sources when they leach into the groundwater over time.
- **Urbanization:** Heavy metal pollution of water bodies is mostly caused by urban runoff that contains metallic contaminants from automobiles, construction materials, and municipal trash.
- **Human health:**The health of humans, plants and livestock is greatly threatened by them as they can pass through the food chain through crops and build up in the body through biomagnification [7] [8].

Long term exposure to such pollutants inducing ecological imbalance, bioaccumulate in living things, and endanger human health by having neurotoxic, myocardial infractions, harm to organ systems, elevated blood pressure, skin lesions, and consequences for the reproductive, urinary, and cancerous implications. Therefore, remedial actions must be taken to minimize the polluted area and stop heavy metals from entering terrestrial, atmospheric, and aquatic habitats [9].

1.2 Role of Plants in Phytoremediation

The traditional techniques of eliminating environmental contaminants are linked to a number of problems, including incomplete removal, high energy requirements, the production of large amounts of toxic sludge, restricted application, and high costs. The financial cost of physically cleaning dirt is detailed in the Salt et al. (1995) research. Compared to phytoremediation, physical remediation is four to six times more expensive to clean, which only costs between 60,000 to 1,000,000 US $. An ecologically friendly, economically feasible, and quickly developing substitute for conventional remediation techniques has drawn attention throughout the past ten years. This method is called phytoremediation uses plants to either capture and eliminate elemental contaminants or reduce their bioavailability in soil [11]. The most desirable plant species for phytoremediation should be strong, producing lots of biomass, be resistant of adverse impacts of metallic substances and contaminants, are easy to cultivate, have a high absorption capacity, and are not preferred by herbivores. Depending on the type of plant and its surroundings, several methods are used by plants to remove both organic and inorganic contaminants from soil and water. Pollutant removal depends on how plants interact with microorganisms and the environment (soil, water, and air). The success of remediation depends on various factors such as the type of plant, the characteristics of the soil, and the specific toxin involved. Key influences include soil pH, electrical conductivity, organic content, microbial activity, and enhancements to the soil's properties. Additionally, plant biomass and metabolic processes play a significant role in determining the overall effectiveness of the remediation.

The Bioconcentration Factor (BCF), which is the ratio of pollutant concentration in plant parts to that in the media, and the Translocation Factor (TF), which is the ratio of elemental accumulation in the plant's shoot to that in the root, are typically used to assess the phytoremediation potential of plants [12].

1.3 Molecular understanding of plants Tolerance to Heavy Metals

The high concentrations of heavy metals (HMs) in soil and water demonstrate the impact of human activity on the environment and the significant environmental danger that goes along with it. Heavy metals (HMs) may move across great distances in both gaseous and particle form, which makes them accumulated rapidly in soil, water and biological systems. All numbers of elements up to No. 53 having a relative atomic mass smaller than 180 have been classified as HMs, because of their density greater than 5 g/cm3[13]. Plants have developed various detoxification strategies to circumvent the toxic effects of heavy metal, including metal immobilization, sequestration, transportation, and uptake that are effective strategies in reaction to stress from heavy metals. Toxic metal transport and buildup in plant cells are regulated by proteins, enzymes, and signaling pathways. Plants can maintain cellular homeostasis through the potential and active involvement of vital molecular actors such as stress responsive transcription factors, heavy metal ATPases, metal transporters, phytochelatins and metallothioneins which intensify the defence responses to equilibrium the system [1]. Using in silico approaches, scientists have identified conserved motifs, key genes and metal-binding proteins involved in the tolerance to metals. In order to design rational approaches to improving phytoremediation capacity, this study also applies new technologies, such as molecular docking and structural modelling, to estimate metal binding affinities with plant proteins [14]. An increasingly popular and economical solution for cleaning up heavy metal-contaminated locations is phytoremediation, or the use of plants. Their rapid development, large biomass, and capacity to absorb and withstand with excess heavy metals, species such as *Populus deltoides* are of great interest. With the continuous progress in omics techniques (transcriptomics, metabolomics, genomics) the exploration of key regulatory mechanism and genes in phytoremediation has been facilitated.

1.4 In Silico Analysis as a Predictive Tool

In silico analysis is becoming an increasingly important tool for understanding the interaction between heavy metals and plant proteins. Phytochelatins and metallothioneins, some of the proteins found in *Populus deltoides*, for example, are proteins that are necessary for eliminating several harmful elements, including zinc, lead, and cadmium. The strong association of these proteins with heavy metals, as revealed by docking studies, shows that these proteins are involved in phytoremediation. Additionally, metal transport related genes, including the ZIP, NRAMP and HMA families were also identified through a transcriptome study. These genes are more potential tools for genetic manipulation for the purpose of enhancing the plant's ability for metal removal [15] [16].

1.5 Applications in *Populus deltoides* for Phytoremediation

The study of *Populus deltoides* growing along diverse habitats has been significantly accelerated using in silico techniques. Despite disparate soil pH, metal content and climatic conditions these sets of data have shown that the plant is an extremely versatile plant, thriving on soils that are riddled with heavy metal contaminants. These methodologies which produce accurate predictions assist in narrowing down the promising sites for the utilisation of P. deltoides in polluted area, thus saving time and costs of conducting extensive field trials. Computational studies have advanced our understanding of *P. deltoides* and its heavy metal tolerance. Evolutionary analysis has revealed conserved regions in heavy metal-binding proteins while protein modelling has established how these proteins undergo structural changes in order to bind different metals. These findings bring closer the day when metal-tolerant *P. deltoides* have been manipulated so that they can remove metals from whatever soil is contaminated. [17] [18]

1.6 Research Objectives and Scope

By improving the effectiveness and sustainability of these applications, however, one can achieve real benefits which are impossible to achieve by direct environmental control. With this research, which reveals molecular processes for metal tolerance and detoxification, it is possible to accomplish the environmental treatment function of these phytoremediation techniques.

Using advanced computer methods, the study sets out to determine if and how Populus deltoides can be a natural effective plant for cleaning up heavy metal pollution. Based on in silico research approaches (such as protein modeling and molecular docking), this paper examines how plants generate genes as well as their interactions with the hazardous elements of arsenic, lead, cadmium. Thus, these molecular mechanisms that allow a plant to survive trauma from certain toxic metals--be it arsenic, for example. They may eventually be used for such things as growing 'biotechnological bombs'. [19].

In order to determine how the environment impacts the activity of *Populus deltoides*, for instance changes in pH or the concentration of metals in the soil, this study uses computer simulations to create similar environments. Through this method, performance of the plant can be estimated to some degree in actual polluted conditions without having to rely solely on backbreaking field trials. An extensive understanding of how the plant copes with heavy metal stress is given by utilizing a mix between molecular biology and bioinformatics as a result of these findings, the goal of our research has shifted to develop improved *Populus deltoides* lines with stronger performance in phytoremediation and that last longer [20].

1.7 Socioeconomic and Ecological Perspectives on Integration

To achieve the success in field conditions, phytoremediation should be in accordance with the overall social-economic and eco-systematic context. This involves interaction with communities, consideration of cost-effectiveness and concern for minimising environmental consequences. Joint efforts will facilitate more acceptance and effectiveness of phytoremediation technology [21].

Adoption of these novel strategies will enable researchers and practitioners with a total scope of *Populus deltoides* to tackle heavy metals pollution efficiently and in an environmental-friendly manner.

CHAPTER 2

REVIEW OF LITERATURE

2.1 An overview of the process of phytoremediation

Plants are used in the phytoremediation process to purify toxic media, including soil and water. Toxins from contaminated soil and water can be retained, sequestered, or detoxified using a technique called phytoremediation. It has benefits for the environment and the economy. Among the methods used in phytoremediation to break down, remove, or immobilize the contaminants are degradation (rhizo-degradation, phytodegradation), accumulation (phytoextraction, rhizofiltration), dissipation (phytovolatilization), and immobilization (hydraulic control and phytostabilization). Depending on the contaminants, plants employ one or more of these strategies to reduce their concentrations in soil and water. For example, plants absorb and collect heavy metals (HMs) in their tissues and decompose organic contaminants to reduce the toxicity of soil and water resources.

Populus deltoides or Eastern Cottonwood is one of those species that have been widely researched for this use. It is a good phytoremediation candidate since it grows rapidly, yields high biomass, and is also resistant to poor conditions. Therefore, it is an ideal candidate for extensive cleanup. *Populus deltoides* not only remediates the soil of contaminants but also stabilizes wet soils and even improves the degradation of toxic compounds by microorganisms in its roots [22]. Based on the processes of phytoremediation involved in the removal of hazardous pollutants from soil and water, the general mechanisms of phytoremediation depicted in Fig. 1 are thoroughly explained.

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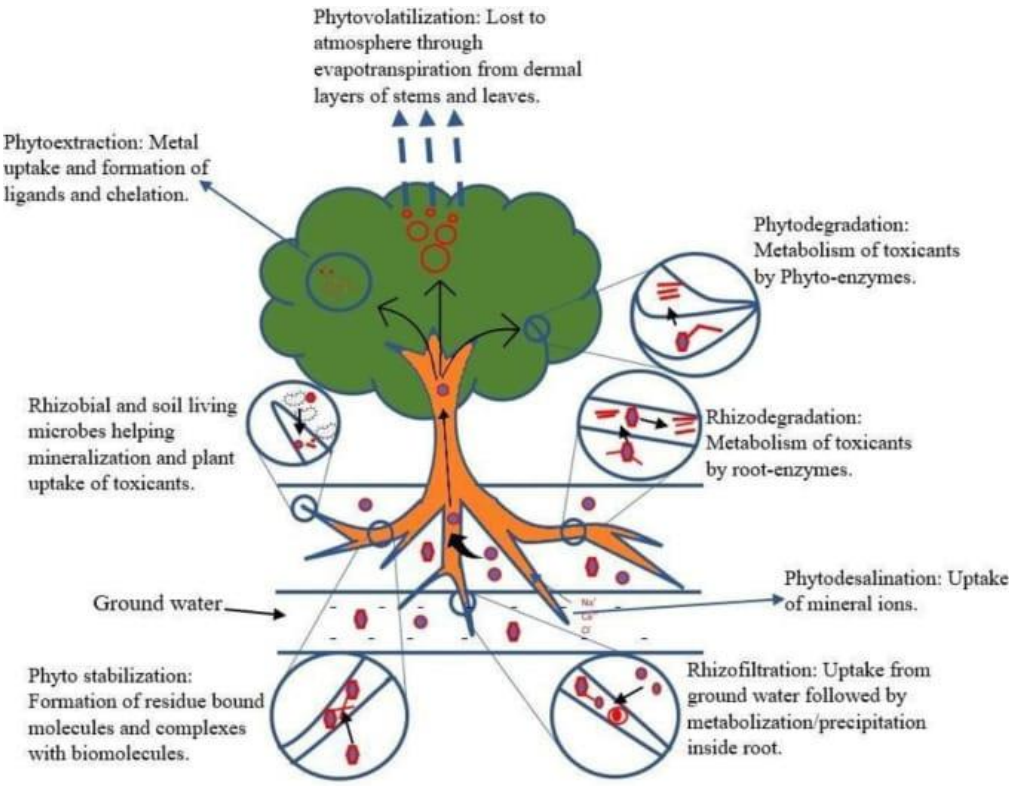

Figure 1. The mechanisms of phytoremediation and the roles they perform

2.2 Mechanism of Phytoremediation in *Populus deltoides*

- Phytoextraction

  Heavy metals like arsenic and cadmium can be taken up by the tree through its roots and stored in its leaves and shoots. Nature accomplishes this in the same manner it detoxifies toxins. The tree employs some chemicals to immobilize the metals and reduce their toxicity in order to survive within polluted environments.

- Phytostabilization

Populus deltoides helps to retain the pollutants. Heavy metals that chelate in the soil and impede their migration to groundwater or wind transport away are excreted by the tree through the roots. Where soil erosion is an issue, this is of significant benefit [23].

- phytovolatilization

Populus deltoides will transmute some poisons, such as mercury, into a less toxic gas that can be released into the environment. This, while less common, illustrates the way in which the tree can transmute many different kinds of pollution.

- Rhizofiltration

Roots of *Populus deltoides* also possess the ability to absorb heavy metals from water. It is thus ideal for purifying industrial effluent and polluted rivers, among other sources of polluted water. It has a large root system that acts as a natural filter, which separates impurities from the water [24].

- Rhizodegradation

Populus deltoides association with soil microbes is arguably one of its most intriguing features. These microbes are fed by nutrients released by the tree's roots, and they subsequently decompose organic pollutants such as hydrocarbons and pesticides. The relationship is mutually beneficial and expedites the cleanup process.

2.3 The advantages of employing *Populus deltoides* in phytoremediation

Populus deltoides is a potential phytoremediation due to its particular characteristics. It can quickly cover and cleanse large areas of land due to its fast rate of growth, and can decontaminate a large number of pollutants due to its high biomass. It is highly resistant due to its tolerance to a broad variety of pollutants. To further improve its ability, researchers are also considering genetic modification which would increase the synthesis of metal-binding proteins [25].

We can combat environmental degradation in a cost-effective and sustainable manner by utilizing *Populus deltoides* in remediation agendas. It is a living example of the fact that if utilized by humans in the right manner, nature can restore itself.

2.4 Heavy Metal Pollution and Its Environmental Impact

2.4.1 Sources of heavy metal contamination

- **Agricultural practices:** Toxic substances like arsenic and chromium enter the soil as a result of overuse of chemical fertilizers and pesticides. elucidate how the metals then make their way into the food chain.
- **Industrial Activities:** Industrial Operations as Sources of Heavy Metal Pollution: Lead, Cadmium, and Mercury are examples of heavy metals present in the environment from unprocessed industrial wastes that have been released into the environment, e.g., those of the mine and factories [26].
- **Urban runoff:** which transports impurities like zinc and nickel from the roofs, roads, and industrial sites, also contaminates the surrounding water bodies.
- **Natural Sources:** Volcanic activity and weathering of metal rocks are some of the natural sources of heavy metals in the environment.

2.4.2 Impact of Heavy Metals on Human Health and the Environment

- **Soil erosion**

Metallic substances are toxic to the soil health by destabilizing the microbial communities and lowering fertility, hence lowering soil productivity.

- **Contamination of Water**

The leached metals enter bodies of water and accumulate in aquatic life, which is toxic to biodiversity and to the human food chain.

- **Plant Toxicity:**

Plants Cadmium and lead toxicity inhibits plants' nutrient uptake and photosynthesis, leading to stunted growth and yield.

- **Health risk**

Human health issues include neurological diseases, renal injury, and the susceptibility to cancer due to long-term exposure to heavy elements.

2.5 Role of *Populus deltoides* in Phytoremediation

- ***Populus deltoides* traits for phytoremediation**

Spontaneous multiplication and maximum biomass yields

P. deltoides grows at 3 to 5 meters per annum and is an ideal species for mass removal.

There is intense biomass production, and this is beneficial in phytoextraction and potential utilization in bioenergy production. [27]

- **Deep Root System and High Transpiration Rate**

The plant species can access pollutants in deeper levels of the soil and groundwater due to its large root system.

Its high rate of transpiration (up to 100 liters/day in mature trees) enables the uptake and movement of water-soluble contaminants [28].

- **Acceptance of Contaminated Environments**

P. deltoides is tolerant to several toxic metals such as manganese (Mn), cadmium (Cd), lead (Pb), and zinc (Zn) and maintains growth and physiological processes under metal stress.

- **Improved Phytoremediation through Symbiotic Relationships**

Two benefits of symbiotic association with ectomycorrhizal fungus, for example, Paxillus involutus, include aluminium (Al) toxicity resistance and improved nutrient uptake. [27]

2.6 Adaptability and growth in contaminated environments

In highly polluted environments with heavy metals like nickel (Ni), chromium (Cr), and mercury (Hg), Populus deltoides can thrive. *Populus deltoides'* deep root system and efficient nutrient acquisition mechanisms allow it to obtain water and nutrients in contaminated environments.

Despite the hyper-environmental conditions of contamination, the innate capacity of the species to detoxify contaminants through the process of chelation and sequestration ensures that it can thrive. Its physiological flexibility and genetic variation also make it a favourite when it comes to remediation processes responsible for the removal of a variety of forms of pollution.

In addition, to improve its growth and efficiency in pollutant removal, *P. deltoides* has been reported to be promising when supplemented with organic soil amendments like compost and biochar [29].

2.7 Heavy Metal Accumulation in Plants

- Heavy Metal Accumulation in Plants: Mechanisms of Uptake, Transport, and Sequestration. Divalent metal ions such as cadmium, lead, and zinc are accumulated by plant roots mainly by specific transport proteins such as ZIP and NRAMP family members [30]. by avoiding toxiciy, these transport proteins help to maintain metal homeostasis.
- Phytochelatins and metallothioneins are some of the molecules that chelate heavy metals intracellularly, binding with them and mitigating their toxic effects within plant cells. Heavy metals transport to aerial plant parts occurs through xylem and phloem after uptake. This transport involves heavy metal ATPases (HMAs) and other transport proteins that control metal distribution in plant tissues [31].
- By suppressing metal-induced oxidative damage by sequestration into vacuoles or binding with cell wall constituents—often with the help of organic acids and sulfur-containing ligands—plants further detoxify the metals [32].
- The suitability of Populus deltoides for phytoremediation for the detoxification of soils contaminated with heavy metals, as evidenced by its rapid growth and metal-accumulation capacity.

2.8 Molecular Basis of Heavy Metal Tolerance

- **Gene Expression and Regulation:**

genes that encode proteins called metal transporters HMA (Heavy Metal ATPase) [1] to pump toxic metals in or out of cells provide plants with a vital first line of defense.

Stress-responsive transcription factors. Such as MYB (a subset related to c-MYB and v-Myb forms which are involved in cell cycle control), WRKY, and bZIP (bZIP proteins have leucine zipper motifs followed by basic regions, for example these proteins moderate the expression levels of genes that confer metal resistance on plant cells.

- **Signal Transduction Pathways:**
  Hormones such as Ethylene, Salicylic Acid, and Jasmonic Acid have roles in modulating plant responses to heavy metal stress [32].

Signalling through reactive oxygen species, ROS, is part of the cell's response to stress and defense [1].

Cellular Antioxidant Systems: ROS generated as a result of heavy metal stress are detoxified by the enzymatic antioxidants catalase (CAT) and superoxide dismutase (SOD) (Yadav, 2010). Non-enzymatic antioxidants, like ascorbate and glutathione, are additional cellular protectors.

- **Genes involved in metal tolerance**

Metallothionein:

MTs, also known as the class of metallothionein identified by Margoshes and Vallee, are small molecules with an abundance of cysteines that shield cells from harm by binding heavy metals to their thiol groups.

ABC Transporters on Cassettes (ATP-Binding):

ATP-binding cassette (ABC) transporters, allow cells to remove heavy metal complexes and store metals in vacuoles.

ATM

ATM (Ataxia Telangiectasia Mutated) protein plays a role in phytoremediation by regulating plant responses to environmental stress, including DNA damage caused by pollutants. Its activation helps enhance plant resilience and efficiency in detoxifying and accumulating harmful substances.

The ZIP and NRAMP transporters:

In the case of the NRAMP and ZIP transporters, for example, the movement of metals across cell membranes depends on this pathway.

The pathway of glutathione (GSH):

Glutathione (GSH) is responsible for this. In addition to assisting the synthesis of phytochelatin, a small peptide called GSH protects against oxidative stress from within the cell.

2.9 In Silico Studies in Phytoremediation Research

2.9.1 Importance of computational modelling in phytoremediation

- **Systems Biology Approach to Heavy Metal Uptake**

The eco-physiological response of plants to native heavy metal levels was model by systems biology modelling within the systems biology approach of heavy metal uptake. This work measured the capacity of copper binding per cell in saturated state and the copper affinity of CuSA using the language of growth: the authors of the work described the copper accumulation of cells using an equation of the logistic type to the Verhulst model. This approach led to considerable knowledge of the processes behind plant heavy metal homeostasis.

- **Applying Machine Learning to Predict Phytoextraction Efficiency**

Predicting the variables affecting the phytoextraction efficiency of hyperaccumulators was performed in a recent study by means of machine learning techniques. Based on 173 data points, we found that, the family of plants and heavy metal were significant parameters affecting the metal accumulation in shoots, in which metal ionic radius was the most critical one. This method offers a knowledge-based tool to aid in selecting efficient phytoremediators.

- **Model-Assisted Phyto-Remediation Strategies**

After conducting an extensive analysis, several modeling techniques were applied for the support of phytoremediation, e.g., Decision Support Systems (DSS), Response Surface Methodology (RSM), and regression models. ” These models help us make truly efficient plans and save time and money through the phytoremediation process by showing how the plants, soil and climate interact with one another.

- **Mathematical Modelling of Heavy Metal Transfer**

A mathematical model for the transport of heavy metals from soil to plants was developed. The model included several of the factors influencing the uptake of metals and provided a basis for predicting the plant metal accumulation, which was used for assessing the applicability and optimization of phytoremediation.

2.9.2 Tools and techniques for in silico analysis

Protein Sequence Analyses and Preparation

- **BLAST (Basic Local Alignment Search Tool)**: Its purpose was to look for homologous sequences between Populus deltoides proteins and between proteins in general.

Multiple sequence alignment using Clustal Omega or MUSCLE.

Swiss-Prot or UniProt: Protein sequence databases to get reliable sequence and functional information.

- **Protein Structure Modeling**

AlphaFold: These are used in the prediction of the 3D protein structures if the experimental structures do not exist.

Swiss-Model or Modeller: Homology modeling to generate protein structures using known templates.

PDB (Protein Data Bank): A resource for obtaining atomic 3D structures of proteins solved experimentally.

- **Ligand Preparation**

PubChem: The databases for retrieving information on the ligands, such as molecular structures and SMILES strings.

- **Molecular Docking**

AutoDock for Molecular Docking:

AutoDock is a suite of automated docking computer programs that have been designed to forecast the affinity of tiny molecules, such substrates or possible treatments, for a receptor whose three-dimensional shape is known as AutoDock

PyRx is a computer-aided drug design virtual screening program that may be used to dock compounds with known crystal structures. the software converts these outputs of pK values (-log of activity) to estimate the activity of molecules, and larger pK values are supposed to have higher predicted activity. PyRx offers a docking wizard for virtual screening, a chemical spreadsheet to add/modify chemical information, and a chemical 3D viewer.

Molecular Operating Environment (MOE): To provide a docking, visualization and interaction analysis.

- **Visualization and Analysis**

PyMOL or Chimera: Programs for visualization of protein ligand interactions, binding surfaces, and molecular structures.

LigPlot+: For generating 2D interaction diagrams in order to identify hydrogen bonds and hydrophobic contacts.

Discovery Studio Visualizer: For docking results inspection and binding site interactions exploration.

- **Validation Techniques**

RMSD (ROOT MEAN SQUARE DEVIATION) Analysis: Assures the validity of the Docking pose.

Molecular Dynamics (MD) To examine the stability of protein-ligand interactions over time for confirming the docking results.

2.10 Binding Affinity of Heavy Metals in *Populus deltoides*

Molecular docking and dynamics studies

- Molecular docking is applied to predict the heavy metal ions and plant proteins like ABC, ATM, HMA and ATM interactions.
- Insights into the dynamic stability of metal protein complexes under physiological conditions can be profitably obtained in Molecular Dynamics (MD) simulations.
- By the means of MD,[34] for other plant species had stable complexes with root cell membrane proteins, supporting the notion of detoxification by sequestration.
- Predicting interaction sites and binding affinities
- The three-dimensional structure of the heavy-metal-binding proteins of P. deltoides can be predicated by the method of homology modelling.

- Free binding energy (ΔG) is estimated by various software such as AutoDock and SwissDock to quantify the heavy metal interactions strength.
- In contrast to lead, which chelates strongly with carboxylic and hydroxyl groups, cadmium favours a sulphur-rich milieu (thiol groups) the literature reports.

2.11 Strategies for Enhancing Phytoremediation Efficiency

Techniques for Improving Phytoremediation Efficiency a. Genetic Engineering Approaches

- Overexpression of Metal-Binding Genes:

The cadmium uptake of the transgenic *P. deltoides* plants overexpressing metallothioneins was 1.5–2 times higher than that of wild type plants [35].

Metal chelation and reduced oxidative damage were further enhanced by overexpressing phytochelatin synthase (PCS) in transgenic lines.

- Biostimulants and soil amendments

Biostimulants :

Metals like lead and cadmium were more biologically available, in part due to fulvic and humic acids, which also promoted uptake through roots and shoots.

As a byproduct, organic biostimulants indirectly improved the efficiency of phytoremediation through enhancing the activities of soil microorganisms and root growth.

Chelation Agents**:**

The ubiquitous chelator EDTA solvates heavy metals in the soil. Although effective, it may be considered hazardous to the environment when irresponsible treated and causing leachability.

Natural chelators: safer alternatives also enhancing the bioavailability such as citric acid.

Soil Modifications:

Biochar reduces leaching and enhances plant availability by adsorbing heavy metals, enhancing soil aeration and reducing loss of moisture.

As studied in [36] observed 75% higher cadmium uptake using biochar.

2.12 **Research Gaps**

Limitations in current studies

limited computational techniques Only a few computational and in silico models are used in the majority of experimental studies on heavy metal accumulation in *Populus deltoides*. Dynamic simulations and molecular docking are two computational methods that are still underutilized for comprehending molecular-level binding processes. Since different studies use different methodologies to detect heavy metal buildup, it is difficult to compare results and draw reliable conclusions. Often, factors like Populus deltoides genetic diversity, environmental impacts, and soil composition are not taken into account in a methodical manner [37].

Genomic and proteomic gaps: Although omics technology has advanced, minimal is known about the specific genes and proteins that Populus deltoides uses to bind and transport heavy metals. Designing or selecting genotypes with enhanced phytoremediation abilities becomes more challenging as a result.

investigation into the impacts on the ecosystem and environment: Few studies fully evaluate how using *Populus deltoides* for phytoremediation affects the ecology and environment. The potential for bonded metals to leak back into the environment or have other unanticipated consequences is still unknown.

CHAPTER 3

MATERIALS AND METHODOLOGY

Literature search Using the Google Scholar and PubMed databases, a thorough literature analysis was conducted to identify the high affinity metal grabbing genes among hyperaccumulator plant species that have been implicated in the detoxification of heavy metals from soil. Heavy metal, gene, protein, hyperaccumulator, plant, phytoremediation, metal absorption, metal transport, sequestration, and soil detoxification were among the free words and topic keywords used in the search-in literature study.

3.1 Sequences and Retrieval of Genomic Annotation Data

Protein sequences from the literature-derived genes and the genome annotation data file were downloaded from the UniProt and the National Center for Biotechnology Information (NCBI). Furthermore, the AUGUSTUS [38] program was employed, with the Oryza sativa, Nicotiana attenuata, Nicotina tabacum, Medicago sativa, Medicago truncatula, Hordeum vulgare, Glycine max, Camella sinensis, Arabidopsis thaliana and Arabidopsis arenosa, species as the source of the training gene, to predict the genes based on the genome FASTA file. This resulted in the anticipated genes and their genomic locations are included in a General Gesture Format (GFF3) file. The program AUGUSTUS is another well-known gene predictor. A comprehensive protein sequence is UniProt.

and annotation repository, while NCBI is a collective biological data and information repository.

3.2 The BLAST Sequence Analysis of *populus deltoides*

Homologous genes were identified by BLAST sequence analysis based on the sequence similarity of the proteins. The protein database was created by using BLAST+ software for the putative *Populus deltoides* proteins. The query used during the BLAST database search was the proteins found in the literature responsible for metal uptake in different plant species. From that point, "blastp" was run using alternative parameters including e-value cutoff of 1e-30, and maximum alignments of 1.

E-value, which is the expected number of alignments with a score of S in a single database search, is an indicator of alignment quality. The searching is more rigid, and there will be more potential hits, if you increase the value from the default of 10.

Using protein-based homology searches an $E < 1e-5$ was considered the threshold, lower values indicated the clearer homology [39] E values of 1e-25 or less were also used. Also, the restricted number of alignments basically ensures that only one optimal hit for each query is displayed, e.g. the highest scoring hit is determined for each protein. Thereafter, the final results were rearranged in table like fashion and hits IDs were stored in a separate text file. It was also used to retrieve the top hits based on a protein database search of by the NCBI BLAST+ "blastdbcmd" command line tool [40]. In the present investigation homologs of populus deltoides species metal up-take associated proteins have been identified. Functional Annotation and Homology Modeling. In the next step, we predicted the three-dimensional structures of the detected protein sequences. This was accomplished by applications of AlphaFold, a 3D model application commonly recognized by its quality in predicting the structure of proteins, ab initio. AlphaFold runs on an NVIDIA A100 graphics card with 80 GB of RAM and can process protein sequences as long as 4096 amino acids. SWISS-MODEL was used to predict sequences particular to amino acids longer than column 1500, while the popular homology modelling programme is designed with a template for sole-homologous proteins and is online as free software. For functional annotation of new proteins, web-based BLAST searches against other plant species were carried out. If no equivalent sequences were found. Protein families and domains were identified by sequence search in the InterPro database. A large collection of protein families, superfamilies and domains can be found in InterPro.

3.3 Virtual Screening and Ligand Retrieval

Ligands such as $CdCl_2$, $ZnCl_2$, MnS, and CdS. were retrieved from the PubChem compound library. The canonical SMILES Using the Python RDkit module, which is widely used for molecular representation, substructure searches, and property prediction, ligands were obtained and transformed into pdb.file format. The candidate proteins were virtually screened for heavy metals using the Pyrx program. and this gives a mean unsigned error of MNINA: reveals accuracy rate that could be obtained. CNNs are used as a scoring function for pyrx tool, which combines prediction biases from different CNNs and uses an ensemble of layers to view entire protein structures simultaneously.

CHAPTER 4

RESULTS AND DISCUSSION

RESULTS

To determine whether genes are involved in metal intake in various animals, a thorough examination of the literature was conducted.

9 different species were found to have 93 genes involved in metal absorption. Furthermore, the literature study identified numerous species that were implicated in the absorption of different primarily composed metals, such as $CdCl_2$, $ZnCl_2$, MnS, and CdS. Each species genes and metal uptake are shown in Table 1.

Table 1: Analysis of Gene Associated with Metal Absorption in Species.

Genus/Species	**Genes**	**Metals**
Arabidopsis thaliana	AtIRT1, AtPIC1, PAA1, AtZIP2, YSL4, AtABCC1, AtHMA4, AtECA3, PAM71, AtPCR2, HMA1, AtZIP1, NRAMP1, AtYSL3, AtMTP8, AtABCC2, AtVIT1, RAN1, IRT1, HMA3, FRO3, AtCAX3, COPT1, AtMTP1, TpNRAMP5, AtNRAMP6, AtABCC3, AtZIP1, HMA8, ZTP29, MTP11, AtIRT2, YSL6, MOT1, AtHMA2, HMA4, EAC4, CMT1, PAA2, AtYSL1, AtATM3, HMA2, PAM71	Co, Mn, Cd, Cu, Zn, Hg, Fe, As, Mo, Ca, Pb
Camella sinensis	BHLH2, BHLH3	Zn, Fe, Cd, Mn

Glycine max	GmZIP1, GmHMA8, GmIRT1, GmHMA3	Cu, cu
Hordeum vulgare	HvZIP3, HvIRT1, HvHMA1, HvZIP5, HvZIP5	Zn, Fe+3, Mn+2, Cu, Fe+2, Zn+
Medicago truncatula	MtVTL4, MtVTL8, TaVTL2, TaVTL3, GST	Fe, Co,
Medicago sativa	ERFs, POD, GST, WRKY, IRT, SOD, CDFs	pb
Nicotiana tabacum	NtMTP2, NtPIC1, AhNRAMP1, MTP1	Co, Ni, Mn, Zn, Fe
Nicotiana attenuata	IGER2	Cd
Oryza sativa	OsZIP1, OsZIP8, OsMTP1, OsHMA2, OsNRAMP1, MIT, OsHMA3, YSL, OsIRT1, OsZIP4, ARG1, OsZIP3, MAPK, OsHMA5, OsLCT1, OsABCC1, OsNRAMP5	Mn, Fe+2, Zn, Cd, Fe+3, Zn+2, Cu, Co, Cr, Mn+2, Ni, As, Fe

4.1.1 Sequence Alignment, Sequence Retrieval, and Genomic Annotation Data for populus deltoides Using BLAST

The UniProt database was used to acquire the gene protein sequences identified by the literature study. Since certain protein sequences were not accessible, 32 sequences of the 93 genes in different species were used from the UniProt database. Protein sequences that were collected from the literature were compared to the BLAST database in order to find populus deltoides. BLAST's database search produced 21 results. Table 2 lists the gene names and IDs of aligned sequences.

Table 2: The BLAST database was used to match the genes of Populus deltoides with those of other species.

Genus/ organisms	**Gene**	**Identifiers (populus deltoides)**
Arabidopsis thaliana	EAC4	A0A2KIZPT8
Arabidopsis thaliana	AtATM3	B9HUH9
Arabidopsis thaliana	ZIP11	BPGFK8
Arabidopsis thaliana	MTP11	A0A4U5PYQ6
Arabidopsis thaliana	HMA	B9I1M3
Arabidopsis thaliana	AtABC1	A0A4U5QZ9
Camella sinensis	BHLH2	A0A3N7FPN1
Camella sinensis	BHLH3	A0A3N7EXB7
Glycine max	GmHMA8	A0A2K12YJ4
Glycine max	GmHMA3	A0A2K2AKB7
Glycine max	GmIRT1	A0A4U5QWX1
Hordeum vulgare	HvHMA1	A0A4U5P506
Hordeum vulgare	HvIRT1	A0A2K1YTI7
Medicago truncatula	GST	A0A193KWY8
Medicago sativa	WRKY	U5GAH7

Nicotiana tabacum	NtMTP1a	A0A4U5PYQ6
Nicotiana attenuata	IREG2	U5G956
Oryza sativa	OsMTP1	B9GRR6
Oryza sativa	OsZIP8	B9GFK8
Oryza sativa	OsZIP3	A0A2K2BY18
Oryza sativa	OsZIP1	A0A2KIZ254

4.1.2 3D Structure Forecasting for Similar Sequences

AlphaFold and SWISS-MODEL were used to predict the 3D protein structures of the 21 aligned sequences; AlphaFold predicted 9 structures and SWISS-MODEL predicted 12. The protein structures predicted by AlphaFold and SWISS-MODEL are listed in Table 3.

Table 3: Protein Sequence Prediction and Identity Analysis Using SWISS-MODEL And AlphaFold

S. No.	**Species**	**Genes**	**Gene of (*Populus deltoides*)**	**Sequence length(Amino acid)**	**Sequence identity**
1	*Oryza sativa*	OsZIP1	H0E87_017906	107	99%
2	*Oryza sativa*	OsZIP3	H0E87_002345	324	98%
3	*Oryza sativa*	OsZIP8(Zinc transporter 8 precursor)	H0E87_000717	347	93%
4	*Oryza sativa*	OsMTP1(Metal tolerance protein 1)	H0E87_005127	554	98%
5	*Nicotiana attenuata*	IREG2(Solute carrier family 40 member)	H0E87_009632	591	99%

6	*Nicotiana tabacum*	NTMTP1a	H0E87_005357	515	98%
7	*Medicago sativa*	WRKY (WRKY domain-containing protein)	H0E87_012693	358	99%
8	*Medicago truncatula*	GST (Glutathione S-transferase)	H0E87_005388	217	98%
9	*Hordeum vulgare*	HVIRT1(Fe (2+) transport protein 1-like)	H0E87_019170	414	99%
10	*Hordeum vulgare*	HvHMA1(Calcium-transporting ATPase)	H0E87_014503	754	99%
11	*Glycine max*	GmIRT1(Fe (2+) transport protein 1-like)	H0E87_019178	359	96%
12	*Glycine max*	GmHMA3(Calcium-transporting ATPase)	H0E87_011179	1052	100%
13	*Glycine max*	GmHMA8(P-type Zn (2+) transporter)	H0E87_013174	1140	98%
14	*Camella sinensis*	BHLH3-BHLH domain-containing protein	H0E87_010237	319	97%
15	*Camella sinensis*	BHLH2- BHLH domain-containing protein	H0E87_018449	211	98%
16	*Arabidopsis thaliana*	AtABC1(ABC transporter B family member 25)	H0E87_025932	654	94%

17	*Arabidopsis thaliana x Arabidopsis arenosa*	HMA3(HMA domain-containing protein)	H0E87_020568	142	99%
18	*Arabidopsis thaliana*	MTP11	H0E87_005357	515	98%
19	*Arabidopsis thaliana*	ZIP11(Zinc transporter 11)	H0E87_000717	347	93%
20	*Arabidopsis thaliana*	AtATM3(ABC transporter domain-containing protein)	H0E87_019845	1119	99%
21	*Arabidopsis thaliana*	ECA4(Calcium-transporting ATPase)	H0E87_014503	1016	99%

4.1.3 An explanation of the similar sequences' function

To identify Web-based techniques like BLAST and InterPro were used to determine the gene or protein domain/family names associated with each of the 21 putative gene structures of *Populus deltoides*. Eleven of these gene names were identified accurately by using BLAST searches, while ten protein family/domain names were discovered for the sequences that did not match those obtained by BLAST. Table 4 lists the domain identities and gene and protein families.

Table 4: The names of the protein families and genes that make up the models.

Genes Identifiers	**Identified Protein Family/Domains**
A0A2KIZPT8	Calcium-transporting ATPase
B9HUH9	Protein with transporter domain-containing
BPGFK8	Zinc transporter
A0A4U5PYQ6	cytoplasmic domain-associated cation efflux protein
B9I1M3	Protein containing an HMA domain
A0A4U5QZ9	member 25 of the ABC transporter B family
A0A3N7FPN1	Protein with BHLH domain
A0A3N7EXB7	Protein with BHLH
A0A2K12YJ4	P-type Zn (2+) transporter
A0A2K2AKB7	Calcium-transporting ATPase
A0A4U5QWX1	Fe (2+) transporter, protein 1-like
A0A4U5P506	Calcium-transporting ATPase
A0A2K1YTI7	Fe (2+) transporter, protein 1-like
A0A193KWY8	Glutathione S-transferase
U5GAH7	WRKY domain-containing protein
A0A4U5PYQ6	Cation efflux protein cytoplasmic domain-containing protein
U5G956	Solute carrier protein family 40 member
B9GRR6	Metal tolerance protein 1
B9GFK8	Zinc transporter 11
A0A2K2BY18	Uncharacterized protein
A0A2KIZ254	Zinc transporter (Fragment)

4.1.4 Virtual screening and ligand recovery

The PubChem database provided the ligands (such as metals) that were found in the literature research. The 4 ligands that were retrieved in SMILES format and transformed into pdb format were CdCl2, ZnCl2, MnS, and CdS. Table 5 lists the ligands along with their PubChem ID and SMILES.

Table 5: List of Ligands, SMILES, and Their PubChem Compound Identifier

Ligands	PubChem Compound Identifier	SMILES
ZnCl2	5727	[Cl-].[Cl-].[Zn+2]
CdCl2	24947	[Cl-].[Cl-].[Cd++]
MnS	87809	[Cd++].[S--]
CdS	14783	[Mn+2].[S-2]

4.1.5 Molecular docking

Docking studies are a powerful approach in molecular biology to explore protein-ligand interactions, providing insights into the structural and functional dynamics of proteins. This study focuses on proteins from various species that share sequence similarity with Populus deltoides proteins, particularly those involved in calcium and zinc transport. These proteins include the Calcium-transporting ATPase, P-type Zn (2+) transporter, and Zinc transporter 11, among others. The investigation aims to understand how these proteins interact with ligands such as $CdCl_2$ and $ZnCl_2$, which, because of their functions in energy transport and toxic metal tolerance, are physiologically significant.

To perform the study, protein homologs were selected based on sequence alignment to identify conserved domains and functional similarities. Docking simulations were conducted using advanced molecular docking tools to evaluate the binding affinities and interaction patterns of the selected ligands with the proteins. The docking results were analyzed to identify critical residues in the binding sites, their contributions to ligand specificity, and their implications for protein function. Findings revealed conserved interaction patterns across species, emphasizing the evolutionary conservation of metal transport mechanisms in these proteins.

The 21 predicted protein structures with four ligands were virtually screened using the molecular docking tool Pyrx. The results showed dockings with affinity for binding ranging from -1.9 kcal/mol to -2.8 kcal/mol. Some proteins had the top 7 best binding affinities, with AtATM3 with cd displaying binding affinities of -2.8 kcal/mol. Based on their distinct interactions with the ligands $CdCl_2$, $ZnCl_2$, the OsZIP8, HvHMA1, GmHMA8, OsZIP3, OsZIP8(1), and HvHMA1(1)

proteins were notably shortlisted; their corresponding affinity scores were -2.7, -2.4, and -2.5, -2.7, -2.6, and -2.4 respectively. Table 6 lists the interacting proteins, the ligands that go with them, and the affinity scores.

Table 6: Interaction binding affinities of various proteins with ligands $CdCl_2$, $ZnCl_2$, MnS, and CdS.

Ligand	**Proteins**	**Affinity**
CdCl2	ATPase involved in calcium transport	-2.6
	Protein with a transporter domain	-2.8
	cytoplasmic domain-related cation efflux protein	-2.1
	HMA domain-containing protein	-2
	Member 25 of the ABC transporter B family	-2.4
	Protein with a BHLH domain	-1.9
	Protein with a BHLH domain	-1.9
	P-type Zn (2+) transporter	-2.5
	Calcium -transporting ATPase	-2.1
	Fe (2+) transporter, protein 1-like	-2.2
	Calcium -transporting ATPase	-2.4
	Fe (2+) transporter protein 1-like	-2.2
	Glutathione S-transferase	-2.4
	WRKY domain-containing protein	-2.5
	Cation efflux protein cytoplasmic domain containing protein	-2.4
	Solute carrier family 40 member	-2.3

	Metal tolerance protein 1 Zinc transporter 11 Uncharacterized protein Zinc transporter (fragment)	-2.3 -2.7 -2.5 -2.3
ZnCl2	Calcium-transporting ATPase Protein with a domain transporter Cation efflux protein cytoplasmic domain-containing protein ABC transporter B family member 25 Protein with a BHLH domain Protein with a BHLH domain P-type Zn(2+) transporter Calcium -transporting ATPase Fe (2+) transporter, protein 1-like Calcium -transporting ATPase Fe (2+) transporter, protein 1-like Glutathione S-transferase WRKY domain-containing protein Cation efflux protein cytoplasmic domain containing protein Solute carrier family 40 member Zinc transporter 11 Uncharacterized protein Zinc transporter (fragment)	-2.6 -2.4 -2.4 -2.4 -1.9 -1.9 -2.3 -2.4 -2.4 -2.4 -2.3 -2.3 -2.4 -2.3 -2.3 -2.6 -2.7 -2.3
MnS	Calcium -transporting ATPase Zinc transporter (fragment) Zinc transporter 11	-1.7 -1.6 -1.6
CdS	Calcium -transporting ATPase Metal tolerance protein 1 Zinc transporter 11 Uncharacterized protein	-1.6 -1.9 -1.6 -1.6

4.1.6 Results of Molecular Docking Analysis

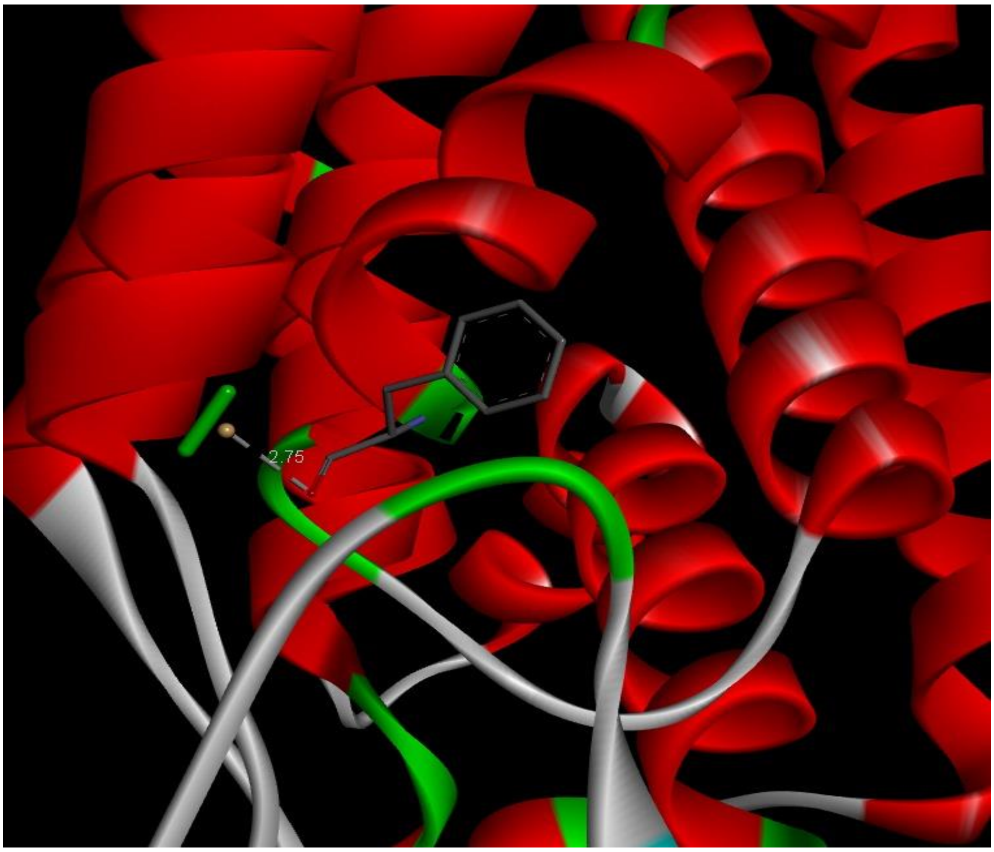

Figure 2: 3D structure of docked AtATM3 and $CdCl_2$

Table 7: Docking Results for AtATM3 with $CdCl_2$

Ligand	**Binding Affinity**	**RMSD/UB**	**RMSD/LB**
AtATM3_model1_24947	-2.8	0	0
AtATM3_model1_24947	-2.6	45.535	45.216
AtATM3_model1_24947	-2.5	12.603	12.488
AtATM3_model1_24947	-2.5	34.447	34.279
AtATM3_model1_24947	-2.5	1.625	0.817
AtATM3_model1_24947	-2.5	7.302	7.302
AtATM3_model1_24947	-2.3	41.52	41.304
AtATM3_model1_24947	-2.3	80.309	80.304
AtATM3_model1_24947	-2.2	41.094	40.735

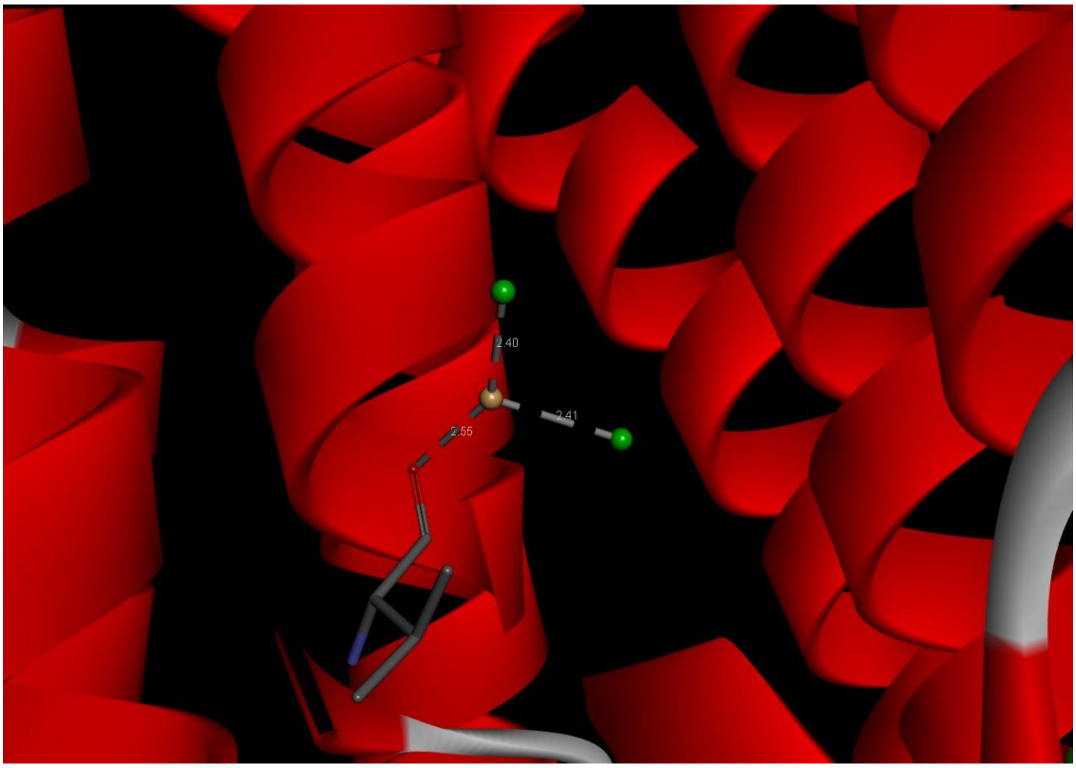

Figure 3: 3D structure of docked HvHMA1 and $CdCl_2$

Table 8: Docking Results for HvHMA1 with $CdCl_2$

Ligand	**Binding Affinity**	**RMSD/UB**	**RMSD/LB**
HVHMA1_model1_24947_uff_E=0.03	-2.6	0	0
HVHMA1_model1_24947_uff_E=0.03	-2.3	55.854	54.796
HVHMA1_model1_24947_uff_E=0.03	-2.3	36.651	36.38
HVHMA1_model1_24947_uff_E=0.03	-2.3	2.576	2.163
HVHMA1_model1_24947_uff_E=0.03	-2.3	62.325	61.931
HVHMA1_model1_24947_uff_E=0.03	-2.3	71.012	70.282
HVHMA1_model1_24947_uff_E=0.03	-2.3	55.937	55.211
HVHMA1_model1_24947_uff_E=0.03	-2.3	70.957	70.279
HVHMA1_model1_24947_uff_E=0.03	-2.2	36.683	36.448

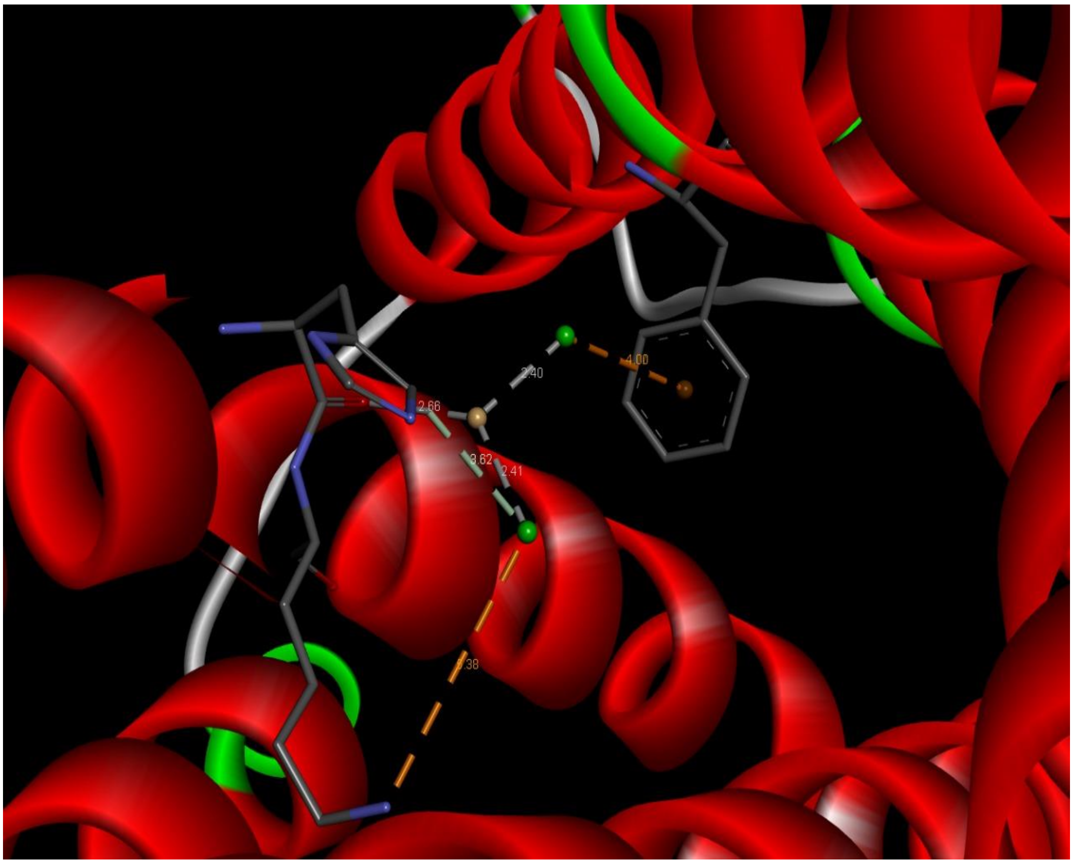

Figure 4: 3D structure of docked OsZIP8 and $CdCl_2$

Table 9: Docking Results for OsZIP8 with $CdCl_2$

Ligand	**Binding Affinity**	**RMSD/UB**	**RMSD/LB**
OsZIP8_model1_24947_uff_E=0.03	-2.7	0	0
OsZIP8_model1_24947_uff_E=0.03	-2.7	3.192	0.037
OsZIP8_model1_24947_uff_E=0.03	-2.4	10.987	10.129
OsZIP8_model1_24947_uff_E=0.03	-2.3	11.201	10.552
OsZIP8_model1_24947_uff_E=0.03	-2.2	14.575	14.074
OsZIP8_model1_24947_uff_E=0.03	-2.1	10.702	9.999
OsZIP8_model1_24947_uff_E=0.03	-2.1	15.665	15.078
OsZIP8_model1_24947_uff_E=0.03	-2	14.202	13.902
OsZIP8_model1_24947_uff_E=0.03	-2	16.992	16.525

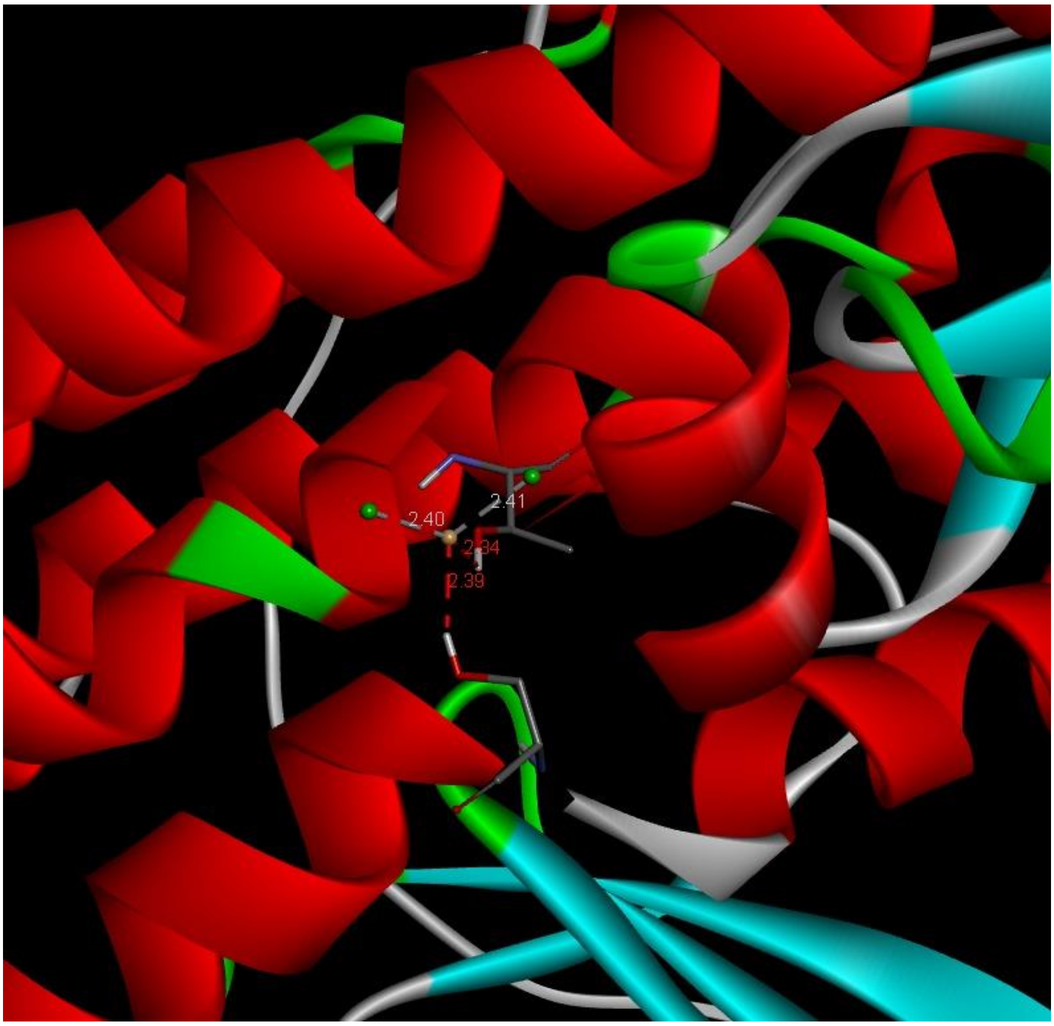

Figure 5: 3D structure of docked GmHMA8 and $CdCl_2$

Table 10: Docking Results for GmHMA8 with $CdCl_2$

Ligand	**Binding Affinity**	**RMSD/UB**	**RMSD/LB**
GMHMA8_model1_24947_uff_E=0.03	-2.5	0	0
GMHMA8_model1_24947_uff_E=0.03	-2.4	20.871	20.438
GMHMA8_model1_24947_uff_E=0.03	-2.3	21.183	20.587
GMHMA8_model1_24947_uff_E=0.03	-2.3	21.143	20.578
GMHMA8_model1_24947_uff_E=0.03	-2.2	19.865	19.827
GMHMA8_model1_24947_uff_E=0.03	-2.1	24.544	24.496
GMHMA8_model1_24947_uff_E=0.03	-2.1	44.625	43.755
GMHMA8_model1_24947_uff_E=0.03	-2.1	20.481	19.517
GMHMA8_model1_24947_uff_E=0.03	-2	20.315	19.515

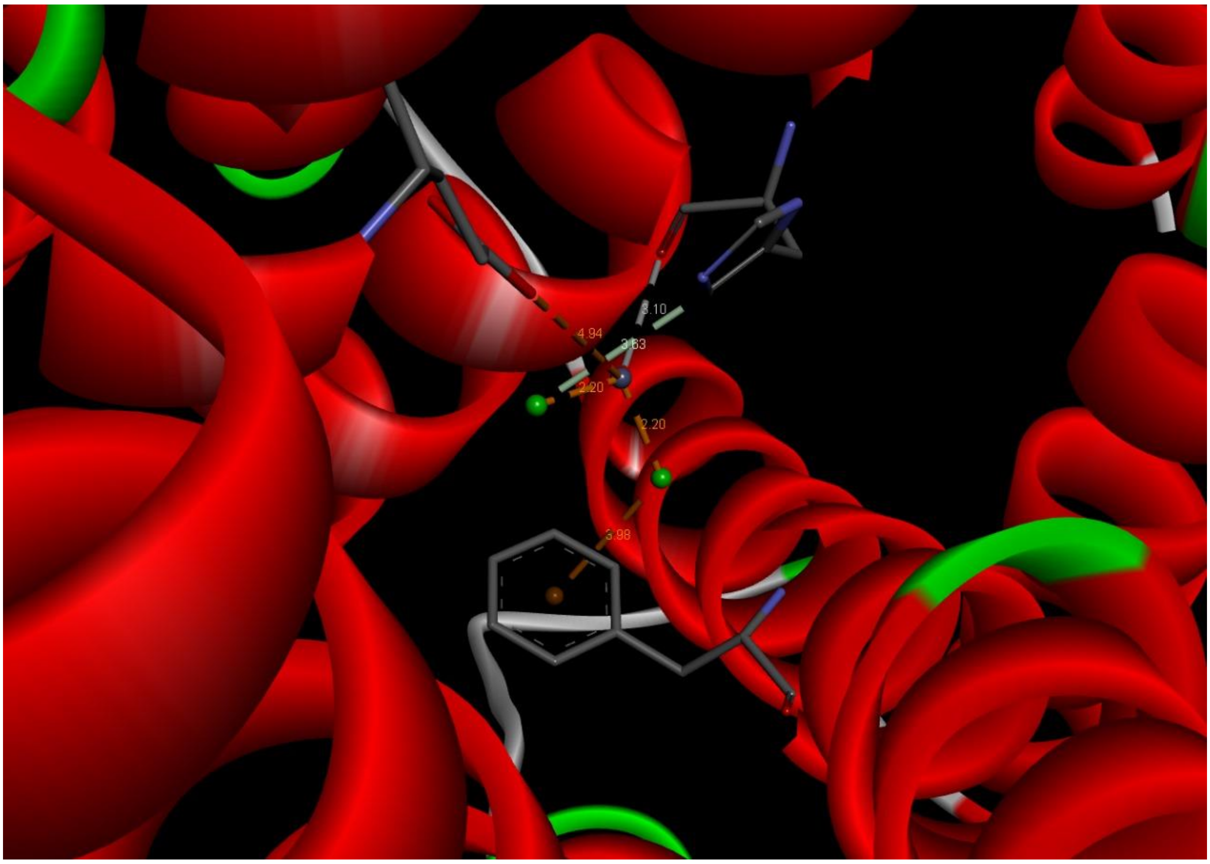

Figure 6: 3D structure of docked OsZIP8 and $ZnCl_2$

Table 11: Docking Results for OsZIP8 with $ZnCl_2$

Ligand	**Binding Affinity**	**RMSD/UB**	**RMSD/LB**
OsZIP8_model1_5727_uff_E=0.00	-2.6	0	0
OsZIP8_model1_5727_uff_E=0.00	-2.6	2.935	0.067
OsZIP8_model1_5727_uff_E=0.00	-2.4	11.294	10.375
OsZIP8_model1_5727_uff_E=0.00	-2.4	11.144	10.423
OsZIP8_model1_5727_uff_E=0.00	-2.2	17.479	17.213
OsZIP8_model1_5727_uff_E=0.00	-2.2	25.041	24.788
OsZIP8_model1_5727_uff_E=0.00	-2.2	14.559	14.273
OsZIP8_model1_5727_uff_E=0.00	-2.1	16.48	16.012
OsZIP8_model1_5727_uff_E=0.00	-2.1	17.969	17.906

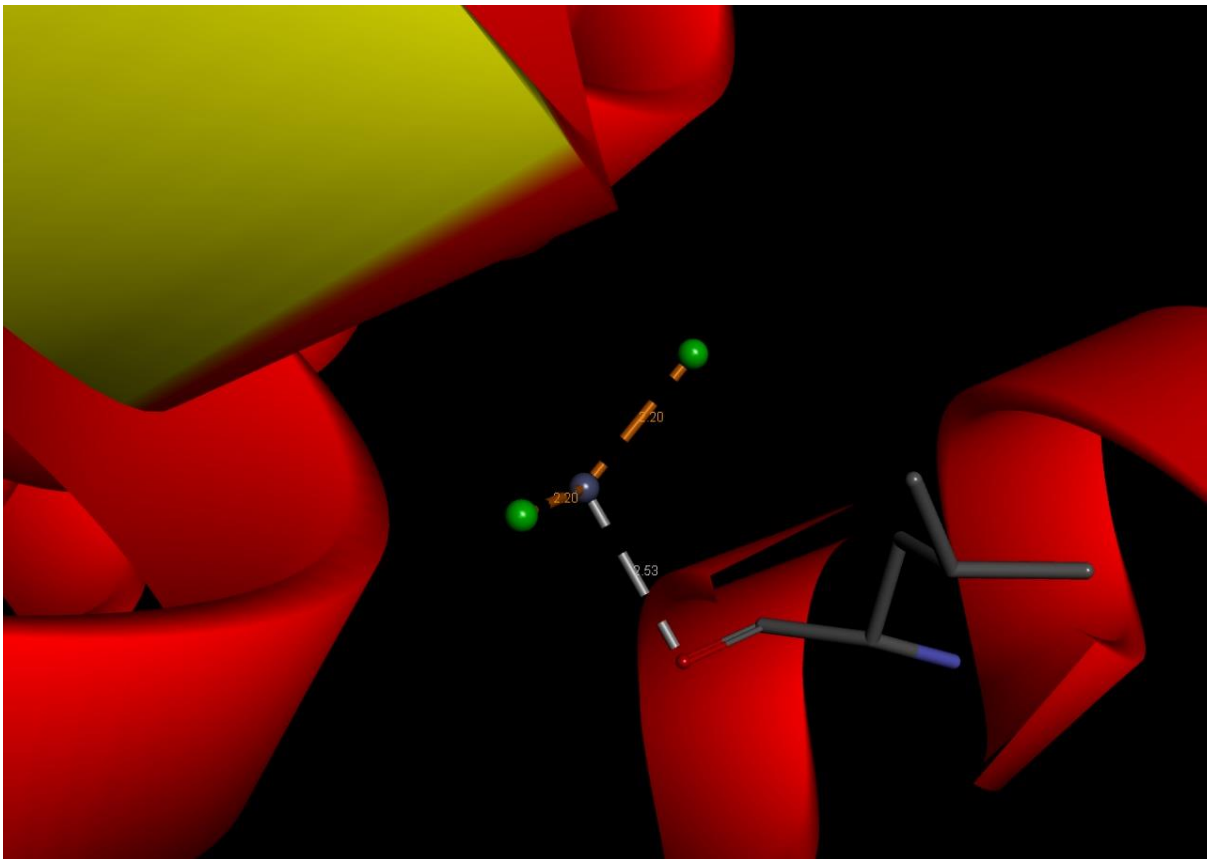

Figure 7: 3D structure of docked OsZIP3 and ZNCl$_2$

Table 12: Docking Results for OsZIP8 with ZnCl$_2$

Ligand	Binding Affinity	RSMD/UB	RMSD/LB
OsZIP3_model1_5727_uff_E=0.00	-2.7	0	0
OsZIP3_model1_5727_uff_E=0.00	-2.4	23.988	23.504
OsZIP3_model1_5727_uff_E=0.00	-2.4	12.214	11.236
OsZIP3_model1_5727_uff_E=0.00	-2.4	14.653	13.758
OsZIP3_model1_5727_uff_E=0.00	-2.3	14.935	13.826
OsZIP3_model1_5727_uff_E=0.00	-2.3	12.239	10.894
OsZIP3_model1_5727_uff_E=0.00	-2.2	20.476	20.125
OsZIP3_model1_5727_uff_E=0.00	-2.2	11.421	10.66
OsZIP3_model1_5727_uff_E=0.00	-2.2	20.433	20.154

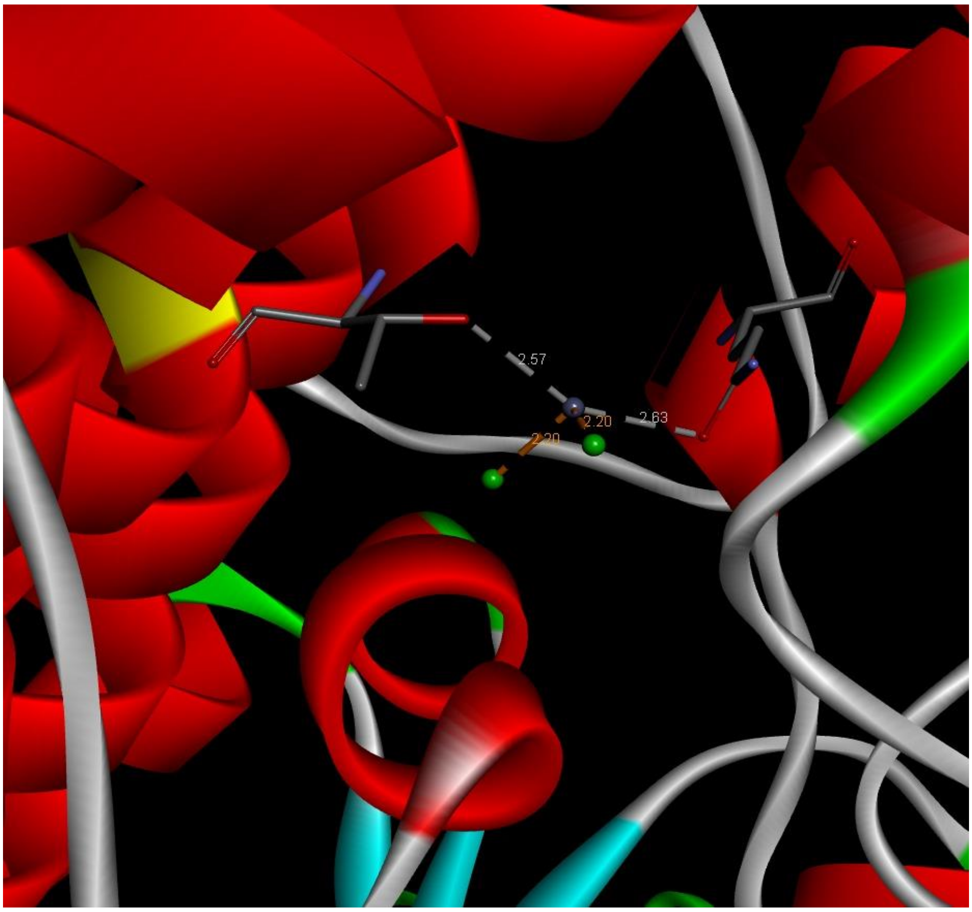

Figure 8: 3D structure of docked HvHMA1 and $ZNCl_2$

Table 13: Docking Results for OsZIP8 with $ZnCl_2$

Ligand	Binding Affinity	rmsd/ub	rmsd/lb
HVHMA1_model1_5727_uff_E=0.00	-2.4	0	0
HVHMA1_model1_5727_uff_E=0.00	-2.3	30.581	30.199
HVHMA1_model1_5727_uff_E=0.00	-2.3	50.227	49.751
HVHMA1_model1_5727_uff_E=0.00	-2.2	27.161	27.132
HVHMA1_model1_5727_uff_E=0.00	-2.2	49.344	49.074
HVHMA1_model1_5727_uff_E=0.00	-2.2	24.312	24.15
HVHMA1_model1_5727_uff_E=0.00	-2.2	33.576	33.218
HVHMA1_model1_5727_uff_E=0.00	-2.1	2.721	2.545
HVHMA1_model1_5727_uff_E=0.00	-2.1	33.84	33.358

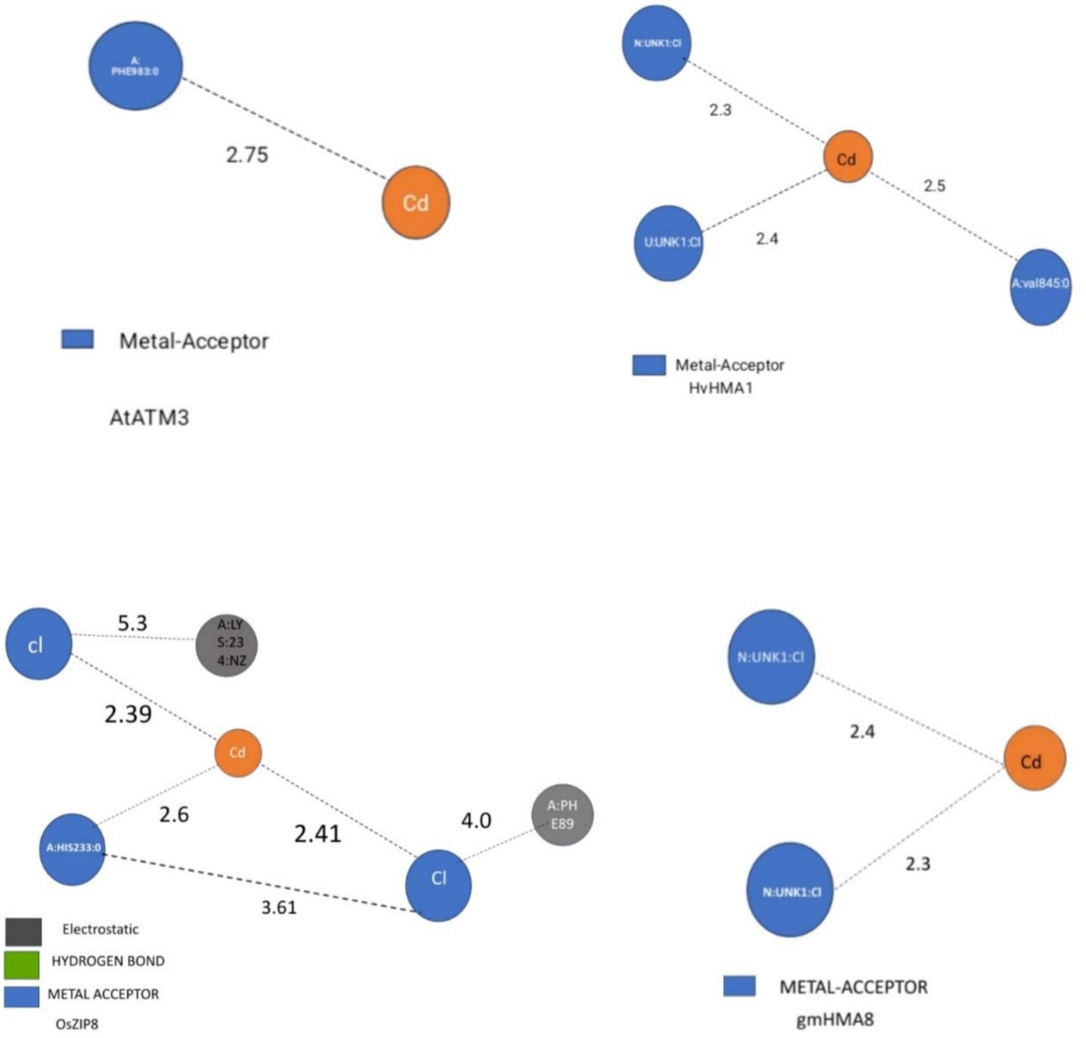

Figure 9: 2D Structural Interactions of Selected Proteins with $CdCl_2$

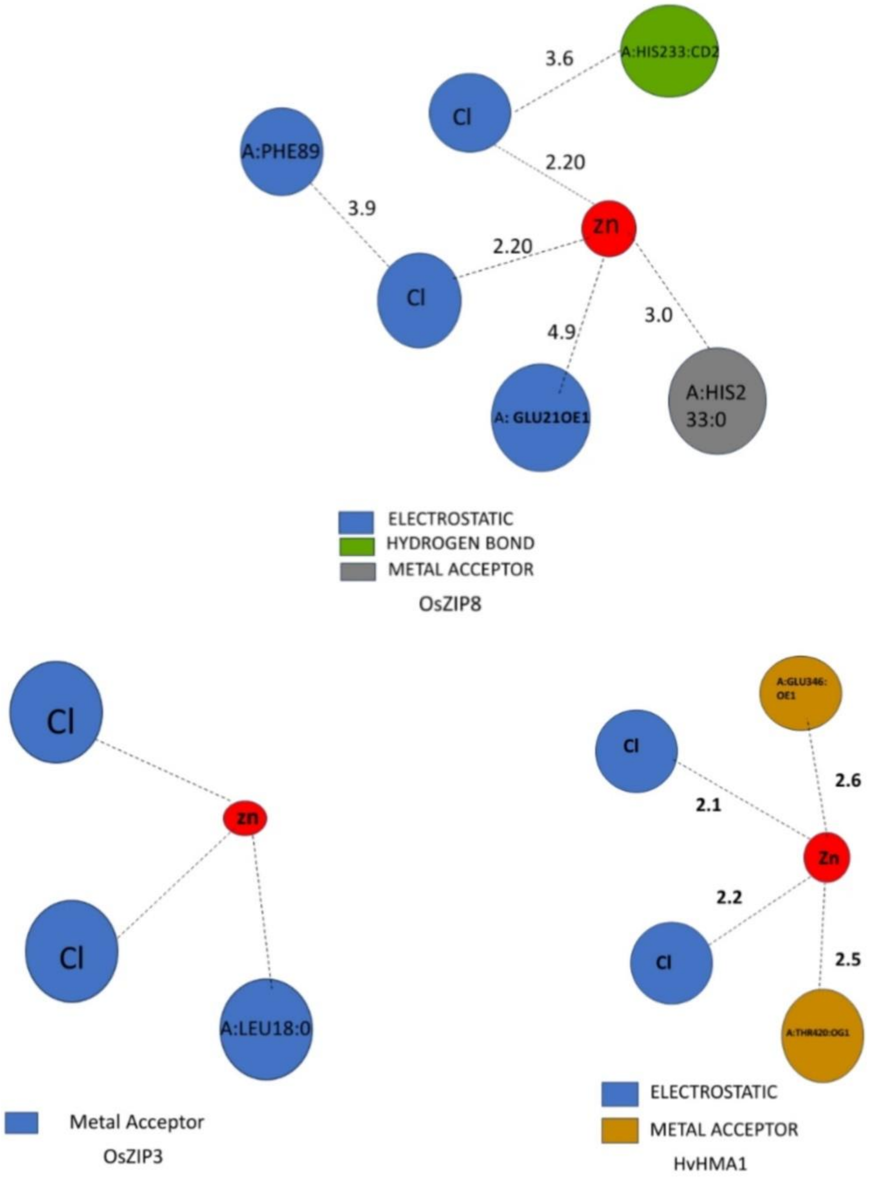

Figure 10: 2D Structural Interactions of Selected Proteins with $ZnCl_2$

4.2 Discussion

The findings of this study can provide a novel insight into hyperaccumulator plants, genes that absorb heavy metals are involved in both high density metal accumulation and detoxification. 93 genes from 9 plant species were found by a comprehensive literature search; these genes are mostly linked to the transport and sequestration of heavy metals like copper, manganese, zinc, and cadmium. These results also highlight the genetic complexity and the adaptation capacity for managing the heavy metal stress of hyperaccumulators as well as their value in bioremediation source. Consequently, a complete review of literature being carried out in order to identify metal capturing genes found in several plant species that involved in the translocation and transport in alloyed soil (*Arabidopsis thaliana, Arabidopsis thaliana x Arabidopsis arenosa, Camella sinensis, Glycine max, Medicago sativa, Medicago truncatula* and *Medicago sativa, Nicotiana tabacum, Hordeum vulgare, Nicotiana attenuata* and *Oryza sativa),* reduced heavy metals $CdCl_2$, $ZnCl_2$, MnS and CdS. populus deltoides, which is a metal-tolerant species having low Translocation Factor (TF) and significantly high Bioconcentration Factor (BCF), seems to be extremely promising for the phytostabilization of these contaminated ecosystems [41]. Homology search (BLAST) using amino acid sequences resulted in 21 genes in *Populus deltoides*, and subjected to functional annotation and 3D structural modelling. Predictive programs such as AlphaFold and SWISS-MODEL showcased their usefulness in predicting protein structures.

necessary for elucidation of protein-ligand interactions. Functional annotation of these genes was further strengthened with the identification of specific protein families and domains through tools such as InterPro. The molecular docking of the ligands was performed against the predicted proteins, and was able to gain an insight regarding the protein-ligand binding affinities and proposed metal - protein interactions. The studied proteins, AtATM3 showed highest binding affinity against Cd ($CdCl_2$) with binding energy of -2.8 kcal/mol, indicating its efficiency in cadmium detoxification. Several other proteins such as OsZIP8 and HvHMA1, were also found to interact with $ZnCl_2$ and $CdCl_2$ and thus may also be considered as possible models for further functional investigations.

The hypothesis of high affinity metal capturing genes being one of the key players in metal detoxification is further supported by molecular docking predictions showing strong binding affinity with heavy metals individually. In addition, ligands can be retrieved from databases such as PubChem and format-converted for the docking simulation, which also demonstrates integrative analysis of gene-environment interactions. This study provides further insights into the molecular basis of phytoremediation and serves as an impetus for the production of transgenic glands with improved detoxification potential in planta. Further efforts should concentrate on experimental confirmation of these interactions, and considerations of the ecological consequences of planting these plants in contaminated areas.

CHAPTER 5

CONCLUSION AND FUTURE PERSPECTIVE

The genetic makeup of hyperaccumulator plants, or those that can take in and detoxify toxic metals from the soil, is examined in detail in this study. Through the analysis of previous studies, as well as with the help from advanced bioinformatic tools and molecular docking analysis, the research identified important genes and proteins that regulate metal transport and storage. Their importance was validated by a combination of sequence analysis, 3D modeling of proteins, and interaction studies with heavy metal ligands. AtATM3, OsZIP8 and HvHMA1 were identified as exemplary proteins, all having high-affinity for metal-compounds and are thus directly claimed as potential targets for the method of cleaning up polluted soils with plants is known as phytoremediation. These results demonstrate the potential application of genomic and proteomic approaches in identifying the molecular mechanisms of heavy metal detoxification. Through this knowledge of how plants already accumulate and tolerate toxic metals naturally, we can start to develop better, more sustainable solutions for cleaning up polluted land. For the future, functional examination of these genes and proteins will be important. Exciting new opportunities abound for improving how plants detoxify by unique genetic interventions such as CRISPR-Cas9 technologies. Additionally, researchers need to study new genes, make use of nanotechnology in conjunction with the findings and follow the performance of these plants in response to climate changes. This combined approach may help towards developing sustainable, green technologies for heavy metal decontamination under diverse environments.

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