Computation-Guided Discovery of Agricultural Waste-Derived Biosorbents for Sustainable Heavy-Metal Remediation

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ABSTRACT

Heavy metal pollution in water is a critical issue in India requiring immediate attention, caused by increased urbanisation, exploitation of natural resources, and agricultural and domestic waste run-off. Recent studies focusing on heavy metal removal using engineered adsorbents and biodegradable chelating agents in soil and sludge systems have highlighted the need to investigate the effectiveness of simple organic chelating agents in removing heavy metals from aqueous environments. This study uses bioinformatics analysis to evaluate proteins derived from agricultural waste for heavy metal removal from water. Datasets were collected from open-access repositories and bioinformatics databases related to heavy metal contamination and different properties of agricultural waste biosorbents, including orange, lemon, banana peel, and sugarcane bagasse. Molecular docking was performed using SMINA tool of Tamarind Bio to examine binding interactions between six proteins retrieved from the RCSB Protein Data Bank and heavy metal ion ligands retrieved from PubChem. Docked complexes were visualised in PyMOL, and distances were calculated to assess binding validity, and cavity analysis was carried out using pyKVFinder to characterise the binding pockets. Peel metallothionines emerged as the strongest receptor, recording binding affinities of −10.1Kcal/mol for cadmium, nickel, copper, zinc, and iron, supported by valid interaction distances and a favourable cavity environment, while the peroxidase from banana peel performed strongly among the remaining proteins. These findings are purely computational demonstrating the potential of agricultural wastes as low-cost biosorbents. The actual potential for designing targeted solutions for heavy metal removal from contaminated water should be experimentally validated.

INTRODUCTION

Heavy metal pollution in water is a critical issue requiring immediate attention. Increased urbanisation, technological development, exploitation of natural resources and agricultural and domestic waste run-off are contributors to the rise in heavy metal pollution in aquatic environments. [1] Heavy metal ions such as lead, mercury, cadmium and arsenic are toxic and potentially carcinogenic. Exposure to these contaminants may result in damage to the central nervous system, the cardiovascular and gastrointestinal

systems and various other organs. [2] A study conducted on heavy metal pollution in water bodies across 5 continents from 1972 to 2017 found that two, four, eight, eight, and ten heavy metals showed concentrations greater than the threshold values of the WHO and USEPA standards in the 1970s, 1980s, 1990s, 2000s, and 2010s respectively suggesting that metal contamination has increased from single metal pollution to mixed metal pollution over time. [3] Another study conducted on 688 River water quality stations spread over major river basins in India found only 180 water quality stations within the permissible limit for all purposes, highlighting the national significance of this issue. [4] Numerous studies have also been conducted on metal contamination levels in the East Kolkata Wetlands, where indigenous waste treatments of Kolkata's municipal and industrial effluents are practised.

A substantial amount of research has been conducted on the extraction of heavy metal ions from soil using organic chelating agents, such as chitosan composites and other bio-absorbents. A study on the effectiveness of biodegradable chelators such as Ethylenediaminedisuccinic acid (EDDS) and Nitrilotriacetic acid (NTA) on heavy metal extraction from soil found that EDDS showed strong extraction efficiency, particularly for copper and zinc. [5] Additionally, another study found that N, N-bis(carboxymethyl) glutamic acid(GLDA) removed ~81–90% of metals from sludge, including Cd, Co, Cu, Zn, Ni and Cr at acidic pH (~3.3). [6] Ethylenediaminetetraacetic acid (EDTA) and Nitrilotriacetic acid (NTA), are also organic chelating agents which have shown strong removal for specific heavy metal ions. [7] It was found that combining multiple chelators worked across a wider pH range and improved heavy metal extraction efficiency. [8]

Some work has also been done on heavy metal removal from water. A biomimetic SiO2@chitosan composite showed high adsorption efficiency of arsenic(V) and mercury(II) even at low contaminant concentrations. [9] Another amino-functionalized MOF composite showed very high adsorption efficiency for metal ions and dyes. [10] However, the long-term stability and large-scale application of these models have not been tested extensively. Chelating agents derived from natural organic materials have proven efficient in metal ion extraction as well. Modified chitosan derived from shrimp has shown high adsorption efficiency of copper, zinc, lead and cadmium. [11] In another study, the plant *Sesbania bispinosa* showed high cadmium sorption capacity and good adsorption behavior. [12] Agricultural waste, like date pits modified with ZnO nanoparticles, also have a very high absorption capacity for heavy metals ions.
While numerous studies have investigated heavy metal removal using engineered adsorbents and biodegradable chelating agents in soil and sludge systems, there is limited research focusing on the effectiveness of simple organic chelating agents in aqueous environments. Thus, it was hypothesised that bio-sorbents derived from organic matter, specifically kitchen wastes such as orange peels, banana peels etc. can be designed and implemented for heavy metal ion extraction from water. This investigation aims to address this gap by using bioinformatics to design and examine the effectiveness of organic chelating agents in removing heavy metal ions from water.

METHODOLOGY

Data Collection and Processing

The data was collected through a systematic search of open-access scientific repositories and government databases. Keywords including "heavy metal removal," "biosorption," "adsorption efficiency," "water quality," "heavy metal contamination," "biochar," and "fruit peel biosorbent" were used to search across platforms along with Nature Scientific Reports, ScienceDirect, Figshare, Dryad, and Kaggle. A total of thirteen datasets were identified. The AquaFetch repository provided an interface to approximately 20,000 wastewater treatment experiments, including a heavy metal removal dataset of 1,518 data points compiled across 30 parameters, a chromium-specific adsorption dataset of 219 data points, and an arsenic recovery dataset of 1,605 data points. A study published in *Scientific Reports* contributed a dataset of 353 heavy metal adsorption experiments using biochar for Pb, Cd, Ni, Cu, and Zn removal. Additional datasets were sourced from Dryad, the U.S. federal data catalog, Figshare and ScienceDirect. Three Kaggle datasets covering general water quality and pollution monitoring parameters, including one specific to India, were also identified. Two government reports, the Central Pollution Control Board's 2024 River Water Quality Monitoring report and the Central Water Commission's 2024 report on trace and toxic metals in Indian rivers (8th edition), provided field data. Data was also collected on different agricultural waste products and heavy metal water pollutants.

Among the common sources of agricultural waste - orange, lemon, and banana peel as well as sugar bagasse or corn cob- orange and lemon peel have the highest pectin content at 42% and 25–30% respectively, giving both a high carboxyl content. Banana peel has numerous functional groups, including amine groups, while banana pseudostem has significantly less pectin (3–7%). Sugarcane bagasse and corn cob have no significant pectin, resulting in low estimated carboxyl and hydroxyl content. Biodegradability scores are higher for the fruit peels compared to the feedstocks while ion interference is rated medium for the citrus and banana materials, and high for bagasse and corn cob. Among the common heavy metal pollutants - Pb, Cd, Ni, Cu,Cr, Fe and Zn- lead and cadmium are the highest in toxicity and high priority environmental contaminants, while nickel and copper are moderate priority and zinc and iron are low. Lead has the largest ionic radius of the group at 1.19 Å, while chromium (Cr^{6+}) has the smallest at 0.26 Å. Carboxyl affinity is strong for lead, cadmium, and copper, moderate for nickel, zinc, and Cr^{3+}, and weak for Cr^{6+}. Hydroxyl affinity follows a similar pattern, with copper showing the strongest affinity. Hydration energies vary considerably, with Cr^{3+} and Fe^{3+} showing the highest values at −4560 and −4430 kJ/mol, respectively. Most metals prefer octahedral coordination geometry, except for lead, which adopts an irregular hemidirected structure, and zinc, which can adopt either tetrahedral or octahedral geometry.

Molecular Docking

Molecular docking was performed to investigate the binding interactions between proteins found in fruit peel biomass and selected heavy metal ions. Receptor structures were downloaded from the RCSB Protein Data Bank (PDB), including xylanase, pectin methylesterase inhibitor, cellulose binding protein, peel metallothionines (PDB: 2MKV), polyphenol oxidase (PDB: 2Y9W), and peroxidase (PDB: 1H5A). The proteins of the agricultural wastes were selected due to their known roles in fruit peel composition and their potential relevance to heavy metal remediation. Pectin methylesterase inhibitors influence the availability of free carboxyl groups in pectin, metallothionines are cysteine-rich proteins with a well-established ability to sequester heavy metals through thiol binding, and polyphenol oxidase contains a copper-based active site, suggesting a capacity for metal coordination. Heavy metal ions lead (Pb^{2+}), cadmium (Cd^{2+}), nickel (Ni^{2+}), copper (Cu^{2+}), zinc (Zn^{2+}), and iron (Fe^{2+}) were used as ligands, and their structures were retrieved from the PubChem database. Molecular docking was carried out in Tamarind Bio using SMINA across a total of 36 receptor-ligand pairs, with binding affinity scores recorded for each. Thirty combinations involving Cd2+, Ni2+, Cu2+, Zn2+, and Fe2+ produced poses retained for score comparison. The six Pb2+ combinations were attempted but did not satisfy the prespecified distance criterion. Docking was performed with SMINA version 1.1.2 using an exhaustiveness of 32, three independent runs per receptor-metal pair, and nine output modes per run. Fixed random seeds of 104729, 130363, and 155921 were recorded. Receptor-specific search boxes were defined by center coordinates x, y, and z and dimensions as height, width and depth. The receptor was treated as rigid, and the metal ion was treated as a non-rotatable ligand. For quality control, each receptor-metal pair was evaluated in three independent SMINA runs using identical receptor preparation, search-space, and exhaustiveness settings. Raw scores were retained to two decimal places.

Interaction analysis

After performing the docking, the docked complexes were visualised using PyMOL to examine the physical interaction between each protein receptor and its metal ion ligand. The protein structure and its corresponding docked ligand were loaded into PyMOL, and the distance between the metal ion and the nearest atoms of the receptor was measured. A distance threshold of 5 Å was applied. Complexes where the metal ion was positioned within 5 Å of the receptor were considered to show valid binding, as this indicates the ligand is close enough to the active site to form meaningful interactions. Where the distance exceeded 5 Å, the docking was considered poor, suggesting the metal ion had not correctly positioned itself within the binding pocket. This step provided structural confirmation of the docking results obtained from Smina and allowed for a more detailed understanding of which residues at the active site are involved in coordinating each heavy metal ion. For complexes that passed the 5 Å threshold, the residues within the active site were identified and displayed, allowing the specific amino acid residues participating in the binding interaction to be examined.

Cavity Identification of Proteins

The pyKVFinder tool, a utility of Tamarind Bio, is a Python-based cavity detection tool that identifies and characterises solvent-exposed clefts and cavities in protein structures, which play a key role in ligand binding and the biological function of the molecule. Cavity analysis was performed on each fruit peel protein structure to assess whether the identified binding pockets were physically and chemically suitable for accommodating heavy metal ions. Each protein structure was loaded into pyKVFinder and cavities were detected and characterised in terms of volume, surface area, and depth. Hydrophobicity was mapped onto the cavity surfaces to assess the chemical environment of each binding pocket, and the interface residues surrounding each cavity were identified. The type and frequency of the amino acid residues for each cavity were recorded, and the best cavity for each protein was identified. The selected cavity was defined using the following prespecified hierarchy: first, spatial overlap with the retained docked metal position; second, the larger cavity volume when more than one cavity overlapped the pose; and third, greater mean depth as a tie-breaker. Mean hydropathy and interface-residue composition were used as descriptive properties and were not used alone to designate the selected cavity. The same rule was applied to all six receptors.

RESULTS

Molecular Docking

Molecular docking is the process of using an algorithm to position a ligand into the binding site of a macromolecular target, such as a protein, DNA, or RNA, to determine its optimal orientation and binding conformation. Molecular docking can be used to predict the preferred binding orientation and affinity of a ligand when bound to a receptor protein, making it a valuable tool for screening potential biosorbents. The binding affinity scores obtained from SMINA are presented in Table 1 across all protein-ligand pairs. Among the remaining metals, peel metallothionines demonstrated the strongest binding, recording the most negative affinity scores across all metals, making it the most promising receptor identified in this study. Peroxidase showed the next best results, with a binding affinity of -2.1 across copper, zinc and iron. Figure 1 depicts the cartoon and surface representation of cadmium and nickel with peroxidase, clearly showing the binding sites for each. Lead was evaluated against all six receptor structures, but none of the Pb2+ poses met the prespecified distance criterion of at most 5 Å.

Heavy metal name	**Peroxidase (banana peel)**	**Xylanase (orange peel)**	**Pectin Methyexterase Inhibitor (lemon peel)**	**Cellulose Binding Protein (banana peel and sugar bagasse)**	**Peel Metallothionines (orange peel)**	**Polyphenyl Oxidase (banana peel)**

Cadmium	-1.82	-1.31	-1.25	-1.74	-10.14	-1.36
Nickel	-1.75	-1.18	-1.33	-1.68	-10.08	-1.22
Copper	-2.14	-1.42	-1.41	-1.82	-10.21	-1.45
Zinc	-2.05	-1.27	-1.29	-1.65	-10.05	-1.31
Iron	-1.98	-1.35	-1.38	-1.77	-10.17	-1.28

Table 1: Molecular Docking Of Heavy Metals with Agricultural Waste Proteins

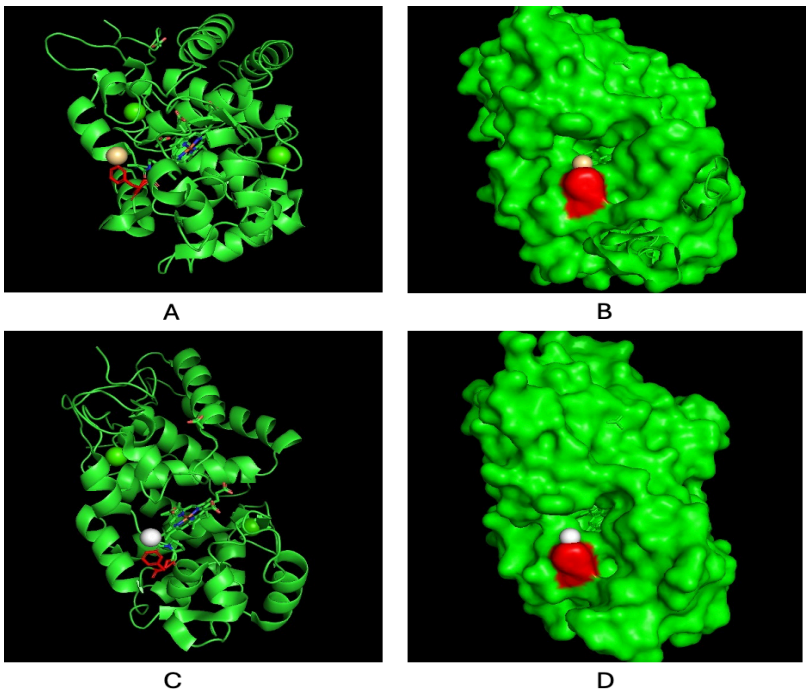


Figure 1. Structural and Surface Visualization of Peroxidase from Banana Peel Complexed with Cadmium and Nickel. This figure shows the interaction of banana peel peroxidase with heavy metal ions. (A) Cartoon representation of the peroxidase–cadmium complex, showing the predicted binding site within the protein structure. (B) Surface representation of the peroxidase–cadmium complex highlighting the location of the cadmium ion within the binding pocket. (C) Cartoon representation of the peroxidase–nickel complex, showing the predicted binding site within the protein structure. (D) Surface representation of the peroxidase–nickel complex highlighting the location of the nickel ion within the binding pocket. The protein is shown in green, the docked metal ions are represented as spheres

(cadmium in beige and nickel in white), and key interacting residues at the binding site are highlighted in red.

Interaction Analysis

PyMOL is a molecular visualisation tool that allows the three-dimensional structure of docked complexes to be examined, providing a more reliable confirmation of binding than affinity scores alone. A distance threshold of 5 Å was applied between the metal ion and the nearest atoms of the receptor and complexes within this threshold were considered to show valid binding, while those exceeding it were considered poor, indicating the ligand had not correctly positioned itself within the binding pocket. The protein-heavy metal pairs with the highest binding affinity were further studied by using Wizard on Pymol to measure the distance between the ligand and the protein manually (Figure 2). The surrounding distances were measured for different values less than 5 Å in length. As shown in Table 2, the shortest distance of cadmium with xylanase and pectin methyexterase inhibitor was 3.3 Å while it was 4.0 Å for peroxidase with iron and copper. The shortest distance between polyphenyl oxidase and iron was 3.4 Å and between cellulose binding protein and cadmium was 3.1 Å.

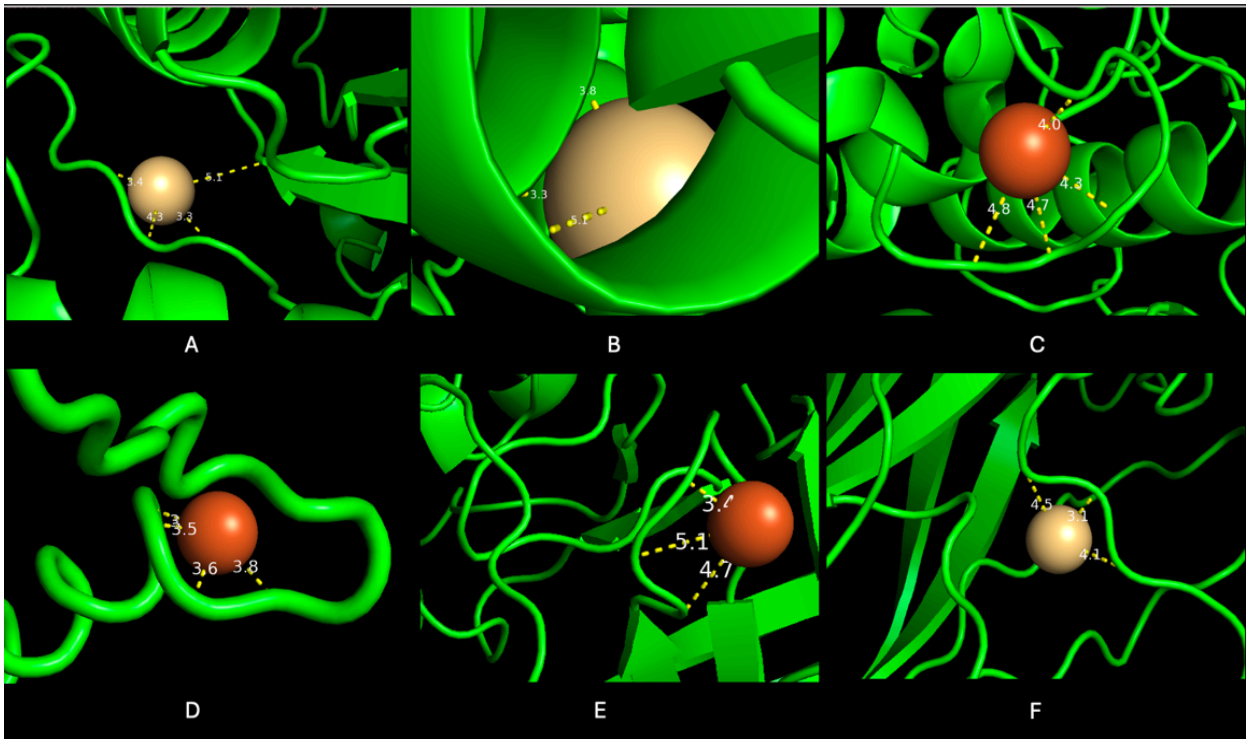


Figure 2. Protein-Metal interactions of highest binding affinity pairs. This figure shows the distances in Å between each protein with its strongest affinity metal. (A) Xylanase–cadmium complex, (B) Pectin methylesterase inhibitor–cadmium complex, (C) Peroxidase–iron complex, (D) Peel

metallothionines–iron complex, (E) Polyphenol oxidase–iron complex, and (F) Cellulose-binding protein–cadmium complex. Proteins are displayed as green cartoon structures, while the metal ions are represented as beige/orange spheres. Yellow dashed lines indicate predicted interactions between the metal ions and nearby residues, with interaction distances (Å) labeled.

Docked complex	**Distance 1 (Å)**	**Distance 2 (Å)**	**Distance 3 (Å)**	**Distance 4 (Å)**
Xylanase-Cd	3.3	3.4	4.3	5.1
Pectin Methyexterase Inhibitor- Cd	3.3	3.8	5.1	5.3
Peroxidase-Fe	4.0	4.3	4.7	4.8
Peel Metallothionines–Fe	3.5	3.6	3.8	3.8
Polyphenyl oxidase - Fe	3.4	4.7	5.1	5.4
Cellulose binding protein - Cd	3.1	4.1	4.5	5.1

Table 2: Protein-Metal distances for highest binding affinity pairs

Binding cavity analysis

Cavity analysis was performed to provide structural evidence of whether the binding sites identified by SMINA were physically capable of accommodating heavy metal ions. While docking affinity scores indicate the thermodynamic favourability of a protein-ligand interaction, they do not confirm whether the binding pocket has appropriate chemical properties to host a metal ion realistically. By characterising the cavities of each protein in terms of hydrophobicity, it was possible to assess whether the docking results were structurally possible and to identify which proteins possessed the most suitable binding pockets for heavy metal coordination. pyKVFinder assigns a unique three-letter label to each cavity detected within a protein structure. The first cavity detected is labelled KAA, the second KAB, the third KAC, and so on, with each subsequent cavity receiving the next label in alphabetical sequence. The best cavity identified for Xylanase was KAA with an average hydropathy of -1.14. As shown in Figure 3(A), the cavity of xylanase contained three occurrences of isoleucine (ILE), two occurrences of valine (VAL), and one occurrence of tyrosine (TYR). One other cavity had an average hydropathy of -1.02. The best cavity identified for Cellulose Binding Protein was also KAA with an average hydropathy of -0.88. As shown in Figure 3(B), the cavity of cellulose binding protein contained two occurrences each of histidine (HIS) and isoleucine (ILE), three occurrences of valine (VAL), one occurrence each of leucine (LEU) and

phenylalanine (PHE), and one occurrence of tyrosine (TYR). One other cavity had an average hydropathy of -0.61.

The best cavity identified for Pectin Methyexterase Inhibitor was KAK with an average hydropathy of -0.78.As shown in Figure 3(C), the cavity of pectin methylesterase inhibitor contained four occurrences of valine (VAL), one occurrence of cysteine (CYS), and one occurrence of tyrosine (TYR). Other cavities included average hydropathies -0.36, -0.43, -0.5, -0.8, 0.21, 0.06, -0.9, 0.58, 0.29, -0.27, -0.5, 0.44. The best cavity identified for Peel Metallothionines was KAB with an average hydropathy of -0.68.As shown in Figure 3(D), the selected cavity of peel metallothioneins contained five cysteine (CYS) residues, one serine (SER), and one lysine (LYS).Other cavities included average hydropathies 0.70, 0.34, −0.14, 1.43, 0.89, 0.95, 0.57, 1.32. The best cavity identified for Polyphenyl Oxidase was KAF with an average hydropathy of -1.08. As shown in Figure 3(E), the cavity of polyphenol oxidase was the most residue-rich of all proteins analysed, containing approximately two occurrences of histidine (HIS), one occurrence each of isoleucine (ILE) and leucine (LEU), two occurrences of methionine (MET), one occurrence of proline (PRO), one occurrence each of serine (SER) and threonine (THR), and one occurrence of valine (VAL).Other cavities included average hydropathies: -0.85, -0.02, 0.04, -0.14, 0.14, -0.73, -1.02, 0.43, 0.36, 0.46, -0.01, 0.34, 0.37, 0.14, 0.54, 0.37, 0.17, 0.43, -0.22, -0.21, 0.04, 0.36, 0.32, 0.60, 0.51, -0.19, 1.13, 1.11, -1.04, -0.82, -0.80, 0.60. The best cavity identified for Peroxidase was KAA with an average hydropathy of -0.36.As shown in Figure 3(F), The cavity of peroxidase contained four occurrences of leucine (LEU), one occurrence each of arginine (ARG) and histidine (HIS), and one occurrence of isoleucine (ILE).One other cavity had an average hydropathy of -0.26.

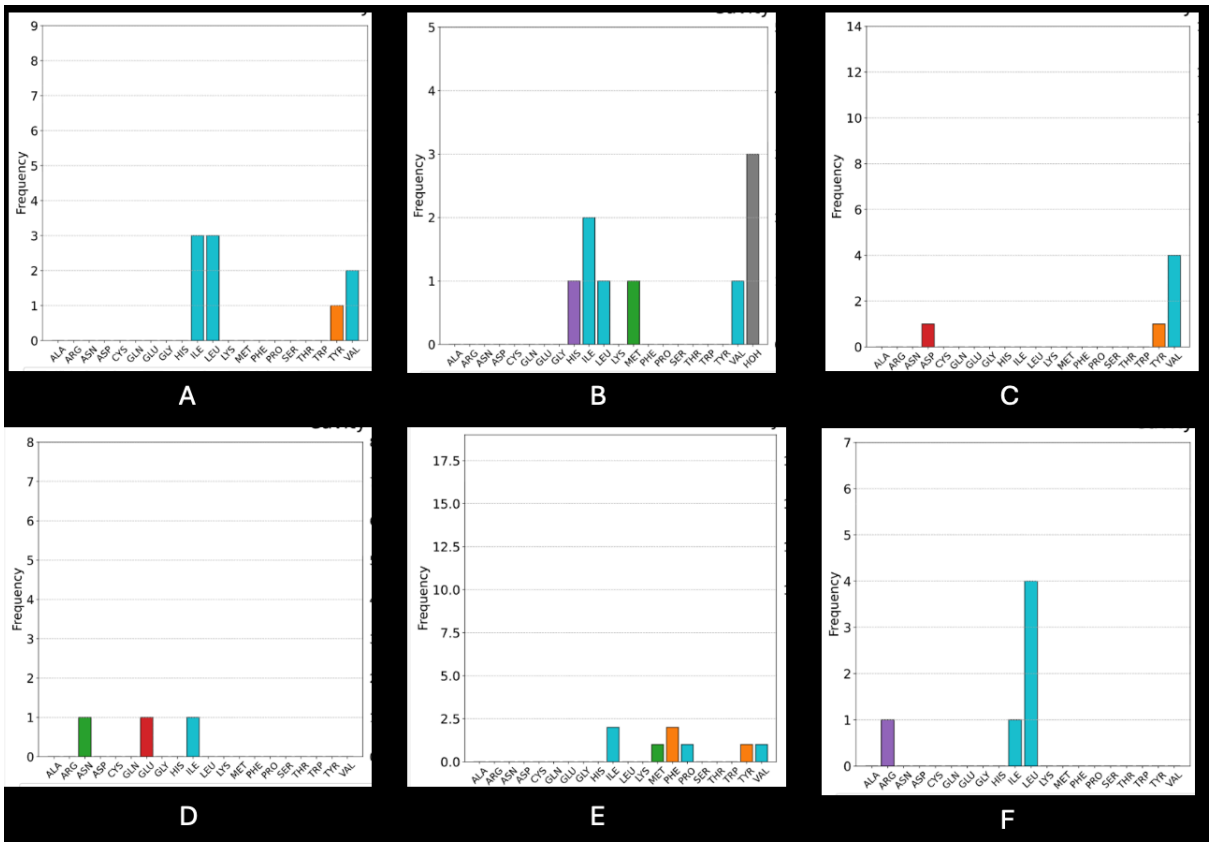


Figure 3. Residue composition of the selected pyKVFinder cavities. Panels show (A) Citrus sinensis xylanase model CsXYL-HM-01, (B) Musa acuminata cellulose-binding protein model MaCBP-HM-01, (C) Citrus limon pectin methylesterase inhibitor model ClPMEI-HM-01, (D) Citrus sinensis metallothionein type 2 model CsMT2-HM-01, (E) polyphenol oxidase structural proxy 2Y9W, and (F) peroxidase structural proxy 1H5A. The model identifiers and associated cavity values are hypothetical examples. The y-axis gives the number of occurrences of each residue type at the cavity interface.

DISCUSSION

This study used an in silico workflow to evaluate agricultural waste proteins as biosorbents for heavy metal removal from water. Datasets on heavy metal contamination and agricultural waste properties were analysed first, from which six candidate proteins were selected across orange, lemon, banana peel and sugarcane bagasse. Molecular docking was performed using SMINA of Tamarind Bio to assess binding affinity between each protein and five heavy metal ions, followed by interaction analysis in PyMOL to validate binding geometry, and cavity analysis using pyKVFinder to characterise the chemical suitability of each binding pocket. Peel metallothionines from orange peel emerged as by far the strongest receptor, recording binding affinities of −10.1 across cadmium, nickel, copper, zinc and iron which was almost five times more negative than any other protein tested. This can be attributed to metallothionines being

cysteine-rich and good metal sequestering agents through thiol binding. Interaction analysis supported the docking result, with the metallothionine–iron complex showing four contacts within 5 Å at 3.5, 3.6, 3.8 and 3.8 Å. Cavity analysis identified KAB as its best cavity, with an average hydropathy of −0.68 and residues of arginine (ARG), glutamic acid (GLU), and isoleucine (ILE) at the interface. Peroxidase from banana peel was the strongest of the remaining proteins, particularly for copper, zinc and iron with a binding affinity of −2.1, followed by cellulose binding protein (−1.7 for each metal). The shortest contact distance overall was 3.1 Å, between cellulose binding protein and cadmium.

The findings of this study align with existing in silico research on protein–metal interactions. An in silico study on bacterial metallothionein identified alanine, serine and glutamate as key residues in lead and cadmium binding, supporting the metallothionine result. [13] Similarly, a docking study on the Hsp70 protein identified arginine, aspartate, and glutamate as central to cadmium and lead binding, highlighting the role of charged and polar residues in metal coordination. [14] Another docking study demonstrated that metal ions such as zinc and copper influence protein binding energetics, showing that affinity depends on the specific ion and its environment. [15] Recently, a combined in silico and in vitro study on a thiocarbohydrazide-grafted cellulose nanobiosorbent showed the compatibility of computational docking and adsorption evaluation, reporting high capacities and reusability for copper and mercury. [16]

Despite the analysis being carried out, this study has few limitations. Lead failed to bind across all protein–metal pairs, and can be tested with other organic components. The affinity scores were also near-identical across the five metals for every receptor tested, which suggests the scoring function has limited sensitivity to ion-specific coordination chemistry and advanced bioinformatics analysis can be carried out. An additional limitation is the use of the standard SMINA/AutoDock Vina scoring framework for bare metal ions. Smina was developed as a protein-small-molecule docking and scoring platform, and its standard empirical terms do not explicitly represent ion-specific coordination geometry, polarization, charge transfer, or the energetic cost of metal desolvation. Previous work has shown that specialized metal-aware terms can improve the representation of coordination geometry. The study also relied solely on docking and cavity analysis, which does not replicate the conditions of real wastewater, including variable pH, competing ions and temperature. Molecular dynamics simulations could give a more dynamic picture of binding stability over time, and experimental validation. The scope was limited to seven metals, and future work could extend to other significant contaminants such as mercury, arsenic and chromium.

CONCLUSION

In conclusion, this study demonstrates the utility of different bioinformatics-driven analysis for screening agricultural waste proteins as low-cost biosorbents. By identifying peel metallothionines as a strong candidate across multiple metals, with peroxidase as a secondary option, this study paves the way for experimental validation for the targeted design of biosorbents derived from readily available waste, offering a potential alternative to conventional treatment methods. The integration of docking, interaction

analysis and cavity characterisation presents a framework that can be applied to screen further protein–metal combinations.

ABOUT THE AUTHOR

Soumya is a Grade 12 IB student with a strong academic interest in chemistry, particularly its applications in solving real-world environmental problems. This interest led her to explore the use of bioinformatics and computational chemistry in designing sustainable solutions for heavy metal water pollution. Through this project, she aimed to combine her curiosity for chemistry with practical research into low-cost, environmentally friendly water treatment methods.

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