

A Computational Study of Removal of Heavy Metal Pollutants $\text{Cr}^{+3, +6}$, Ni^{+2} , Cu^{+2} , Zn^{+2} , As^{+3} , Hg^{+2} & Pb^{+2} from Soil Using Tri-Catecholate Protochelin Siderophore: A DFT Study

Hamza Khan ^{1,*} and Hania Khalid ²

¹ Department of Chemistry, Division of Science and Technology, University of Education, Lahore 54770, Pakistan

² School of Chemistry and Chemical Engineering, North-western Polytechnical University, Changan Campus, Xi'an 7170072, China; email1@email.com

* Correspondence: hamzakhanh625@gmail.com or bsf1900819@ue.edu.pk

Abstract

This research focuses on environmental challenges and has considerable importance as Ni, Cu, Zn, Cr, As, Hg, and Pb heavy metals have potential hazards for plants as well as for microorganisms that are useful for plants. The binding affinity of a Protochelin siderophore with transition metals is computationally analyzed utilizing density functional theory (DFT). The hybrid DFT functional PBE0, def2-SVP, and def2-TZSVP basis sets were utilized to optimize the complex geometry and determine the interaction energy of the various Protochelin-Metal complexes. Among the metal contaminants studied (Cr^{+6} , Cr^{+3} , Ni^{+2} , Cu^{+2} , Zn^{+2} , As^{+3} , Hg^{+2} , and Pb^{+2}), Cr^{+6} has a strong affinity for Protochelin, and the order of stability of the Protochelin-Metal complexes is ($\text{Protochelin-Cr}^{+6} > \text{Protochelin-As}^{+3} > \text{Protochelin-Cr}^{+3} > \text{Protochelin-Fe}^{+3} > \text{Protochelin-Ni}^{+2} > \text{Protochelin-Cu}^{+2} > \text{Protochelin-Pb}^{+2} > \text{Protochelin-Zn}^{+2} > \text{Protochelin-Hg}^{+2}$). Results are also supported by NCI, FMOs, DOS spectra, and RMSD analysis. Computational research suggests that bioremediation may be a great method for eliminating metal contaminants from groundwater and soil due to its eco-friendly use and environmental preservation.

Keywords: heavy metals; protochelin-metals complexes; DFT; phytoremediation; complex optimization; gibbs free energy; adsorption

Academic Editor(s): Name

Published: date

Citation: Khan, H.; Khalid, H. A Computational Study of Removal of Heavy Metal Pollutants $\text{Cr}^{+3, +6}$, Ni^{+2} , Cu^{+2} , Zn^{+2} , As^{+3} , Hg^{+2} & Pb^{+2} from Soil Using Tri-Catecholate Protochelin Siderophore: A DFT Study. *Chem. Proc.* **2025**, volume number, x.
<https://doi.org/10.3390/xxxxx>

Copyright: © 2025 by the authors. Submitted for possible open access publication under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

Rapid and continuous increase in industrialization and urbanization increases the release of heavy metals into soil, water, and air [1] Heavy metal poisoning in plants can lead to unhealthy situations and environmental issues, as they cannot be biologically or chemically destroyed and can remain in the environment for years and show detrimental result on the ecosystem [2]. Lancet-Commission on Pollution and Health, shows 9 million premature deaths worldwide in 2015, making pollution a leading environmental risk factor for diseases and early death. One in six deaths worldwide, or around 9 million deaths annually, are now attributed to pollution these current environmental risk factors have increased deaths by seven percent after 2015 and by more than sixty-six percent after 2000 [3]. After the Earth's origin, nearly every metal is present in the environment. Other mechanisms caused by human activities have accelerated the circulation of metals. Industrial contamination is the biggest factor responsible for the dispersal of metals over the

majority of the Earth's land surface [4]. Around 26% of the vegetables grown in Pakistan, Vehari district can be affected by the accumulation of heavy metals (Cd, Cr, Cu, Fe, Ni, Mn, Pb, and Zn) in the soil [5]. In past years several washing agents (inorganic and organic chelating agents and surfactants) have been used for heavy metal removal from soil. Ethylene diamine tetra acetic acid (EDTA) is highly effective in removing Cu, Zn, Pb, and Cd, but EDTA usage is restricted due to their detrimental effects on the well-being of living things. EDTA has a low biodegradability and may induce soil leaching is another significant disadvantage [6]. For eliminating pollutants from soil, electro-kinetic extraction has been explored by electrical adsorption. Toxic substances, anionic contaminants, and polar organics are targeted by electro-kinetic extraction in soils, sediment, sludge, and digging. Soils polluted with single, or several heavy metals have been tested. Insulating and conductive materials in soil, such as gravels, mineral deposits, and metallic objects, decrease the efficiency of electro-kinetic extraction. The total cost of electro-kinetic soil remediation varies greatly depending on pollution and field circumstances [7]. Plants may also be utilized to clean up the environment and this concept is viable, economical, and eco-friendly technology and is known as phytoremediation [7]. Phytoremediation offers cheaper installation and maintenance costs than other cleaning techniques. The cost of phytoremediation can be as little as 5% of that of traditional cleaning techniques. Moreover, planting plants on polluted soils reduces metal leaching and erosion [7,8]. Siderophores are organic substances that are produced by plants and microbes in reduced iron conditions. Siderophores main function is to absorb iron from various aquatic and terrestrial environments so that plants and microbial cells may use it. Siderophore has a variety of structural features, distinct properties, and the capacity to bind a number of metals in addition to iron. Siderophores serve as bio sensing, chelation agents, phytoremediation agents, biological control & promote plant development [9]. Iron must competes for siderophore binding sites with free-protons, divalent-cations, and other-metal ions (Zn^{2+} , Pb^{2+} , Ni^{2+} , Cd^{2+} , Cu^{2+} , and Cu^{2+}) [10]. Siderophores have been proposed as a safer replacement for harmful pesticides [11]. Catecholate-Siderophores are may produce by pathogenic bacteria and marine organisms [12]. Bioavailability of bacterial siderophore in plant rhizosphere is increased by their affinity to bind metals apart from iron. An improvement in growth of plants and metal adsorption would further boost the overall effectiveness of bioremediation processes [13]. Protochelin is produced by the free-living, gram negative, soil bacteria *Azotobacter vinelandii*, which is a safe catecholate siderophore & plays an important role in environmental nitrogen fixation. Iron adsorption into iron-deficient sections, demonstrating that *A. vinelandii* may use protochelin as a siderophore [14,15]. Protochelin is involve in the transport of *Molybdenum* and Vanadium along with Iron in *A. vinelandii*, for use as a co-factor for nitrogenase enzyme.

This research focuses on the environmental challenges and theoretical interaction of transition metal soil pollutants (Cr, Ni, Cu, Zn, As, Hg, and Pb) with protochelin siderophore through Density Functional Theory (DFT). Figure S1 representing the 2D, 3D-linear and 3D-Cage like structure and its orientation of the optimized tri catecholate protochelin siderophore. This research has considerable importance as Ni, Cu, Zn, Cr, As, Hg, and Pb metals have potential hazards for plants as well as for the microorganisms that are useful for plants. The theoretical study of soil samples for the separation of these contaminants reduces the cost and time of the real-time experimental techniques. In addition, these types of DFT calculations also provide a guess for the possible outcome of lab-based experiments.

2. Computational Methodology

DFT is a tool to determine the electronic structures of any compound or chemical system. In this study, a graphical user interface called "Gauss View 6.0" is utilized to

model and visualize graphical data as well as to design molecular shapes. 3D molecular structure visualization is also done using the CYLview computer program. Molecular structures are drawn by ChemBioDraw Ultra 14.0. All the molecular calculations are carried out on Gaussian 16 (Rev. B.01) [16] software. Hybrid DFT is functional Perdew–Burke–Ernzerhof (PBE0) has been used [17] with Ahlrichs def2-SVP and def2-TZVP basis sets [18] in conjunction with the SMD solvation model [19] and Grimme’s empirical D3 correction with Becke–Johnston damping (D3BJ), was used to obtain true low-energy structures for all optimization and frequency calculations. Siderophore, metal, and metal siderophore complexes were optimized using the double zeta basis set def2-SVP. After performing optimization calculation, the interaction energies of all metal complexes are computed using the following formula:

$$E_{\text{int}} = E_{\text{Complex}} - (E_{\text{PC}} + E_{\text{M}}) \quad (1)$$

Then Gibbs free energy calculations are performed on triple zeta basis sets (def2-TZVP) to support the results of interaction energy calculation which have been done on double zeta basis sets (def2-SVP). Frequency calculations have been performed using the def2-TZVP basis set on the structures following their optimization to verify them as minimum energy structures and Gibbs free energy is calculated for different Metal-Protochelin complexes using the following formula.

$$G^0 = E_{\text{Complex}} - E_{\text{PC}} + E_{\text{M}} \quad (2)$$

The non-covalent interaction (NCI) analysis was done using the Multiwfn software package version 3.80, and the Molecular graphic of the Isosurfaces was prepared using the visualization Molecular Dynamics (VMD) tool. DOS spectra were obtained from GaussSum software to determine the density of states spectra. AIMD simulations are conducted using ORCA 5.0.3 as the software, with the same theoretical level as optimization. The stability of the metal-protocelin complexes was determined through molecular dynamics simulations conducted to last 2000 fs at different temperatures.

3. Results and Discussion

3.1. Determination of Stable Multiplicity State of Metals

After choosing the best methodology, it’s essential to determine the binding energies of various heavy metals with the siderophore, i.e., Protochelin, to determine which metal has a stronger affinity for protochelin. First of all, the single point energies of heavy metals were calculated at a double zeta basis set [20]. Theoretically, all possible multiplicities were identified, and then those potential spin states were analytically investigated to identify stable multiplicity states which are given in Table 1. Single point energies of metals i.e., Fe^{+3} , Cu^{+2} , Cr^{+3} , Cr^{+6} , Ni^{+2} , As^{+3} , Hg^{+2} , Pb^{+2} and Zn^{+2} were determined to find out the stable multiplicity state, and those stable multiplicities were used for finding the interaction of metals with protochelin. According to the data mentioned below in table, sextet, doublet, triplet, quartet, singlet, singlet, singlet, singlet, and singlet are the stable multiplicities of Fe^{+3} , Cu^{+2} , Ni^{+2} , Cr^{+3} , Cr^{+6} , As^{+3} , Hg^{+2} , Pb^{+2} , and Zn^{+2} respectively [21].

Table 1. Interaction energy, Gibb’s Free Energy, and bond distances of the optimized geometries of metal-protocelin complexes calculated at def2-SVP & def2-TZVP basis set with PBE0 functional.

Metal-Protochelin Complexes	Multiplicity	Overall Charge	Bond Length Protochelin-M (Å)	Interaction Energy (kcal/mol)	Gibb’s Free Energy (kcal/mol)
Fe-Protochelin	Sextet	−3	1.90	−216.37	−331.91
Cr-Protochelin	Quartet	−3	1.95	−288.93	−388.06
Cr-Protochelin	Singlet	0	1.85	−2129.87	−1968.32
Cu-Protochelin	Doublet	−4	1.99	−133.61	−256.06

Zn-Protochelin	Singlet	-4	2.01	-115.07	-238.01
Ni-Protochelin	Triplet	-4	1.90	-194.09	-319.04
As-Protochelin	Singlet	-3	1.87	-387.62	-514.78
Hg-Protochelin	Singlet	-4	2.28	-109.87	-253.14
Pb-Protochelin	Singlet	-4	2.48	-125.74	-319.04

3.2. Stable Geometries, Interaction Energies & Gibb's Free Energy Calculation

Natural entities such as protochelin which belongs to siderophores have strong binding capacity [20] for shear metal ions and therefore are ideal for the removal of heavy metals [22,23]. The optimized protochelin and the protochelin-metal complex structures were determined before the interaction energy could be calculated. To achieve this, protochelin was first optimized, then the protochelin-metal compound was optimized, and metals in stable multiplicity states were used for the protochelin-metal complex optimization; to support this investigation, we employed Density Functional Theory (DFT), and to determine the different metal-protochelin interaction energies. Electronic structure predictions from PBE0 functional and def2-SVP double-zeta basis set are quite accurate where calculations were made. The stability of the other protochelin-metal complexes was compared with the protochelin-Fe complex because the siderophore is specialized to scavenge iron with greater affinity [23,24]. The stability of metal-protochelin complexes, with interaction energies following the order: Cr-Protochelin (-2129.87) > As-Protochelin (-387.62) > Fe-Protochelin (-216.37) > Ni-Protochelin (-194.09) > Cu-Protochelin (-133.61) > Pb-Protochelin (-125.74) > Zn-Protochelin (-115.07) > Hg-Protochelin (-109.87) (Table).

These results have confirmed the high selectivity of protochelin for toxic heavy metals such as chromium, arsenic, and lead in conformity with earlier reports on the high chelating efficiency of siderophores because of their hydroxamate and catecholate groups [21]. The prediction of high binding energy for Cr corroborates with the strong chelation observed for other siderophores such as deferoxamine and enterobactin which makes it capable of sequestering pollutants [25–28]. The varying affinities also imply that the coordination of protochelin with metals depends on the ionic size and electronic characteristics of the metal, which provides strong support for its use in environmental remedial applications for decontaminating waters containing potentially toxic metals [29]. The optimized geometries of the metal-protochelin complexes are presented in Figure S2. These geometries also depict the structural angles and coordination spheres that are responsible for different binding forces.

The computed bond lengths for metal-protochelin complexes comprise Pb-Protochelin (2.35 Å) > Hg-Protochelin (2.28 Å) > Zn-Protochelin (2.06 Å) > Cu-Protochelin (1.99 Å) > Cr (III)-Protochelin (1.95 Å) > Fe-Protochelin (1.91 Å) > Ni-Protochelin (1.90 Å) > As-Protochelin (1.87 Å), in decreasing order: Cr (VI)-Protochelin (1.85 Å). Shorter lengths are indicative of stronger interactions, ensuring greater stability and thus, potency while chelating toxic metals. Significantly, this is advantageous for environmental purposes, wherein improved bond strength with Cr and As allows for efficient sequestration.

In contrast, longer bond lengths, such as in Pb and Hg, denote weaker interactivity but still have relevance in applications that require reversible binding [30]. These trends match very well with the electronic and ionic size factors in promoting such metal-siderophore coordination.

The stability of the complex and the values for all metal complexes have been determined using the standard Gibbs free energy G^0 . On the other hand, Gaussian makes things easier by providing a shortcut. Without determining the enthalpies and entropies of the system, we calculate the ΔG of the reactions [31,32]. It is a key factor in figuring out if a chemical transition is thermodynamically possible. The findings of the Gibbs free energy calculations for multiple metal complexes support the interaction energy that we already

calculated. If the value of the sign is negative, the complex is stable, and this data follows the stability order based on interaction energy.

3.3. Electrostatic Potential (ESP) Analysis

ESP is particularly critical in analyzing how protochelin interacts with heavy metal ions. Thus, ESP allows the presentation of the charge distribution and determines more suitable areas for electrophilic or nucleophilic contacts. The ESP mappings of protochelin and its complexes with iron heavy metals present in Figure S3, provide important information concerning these interactions. The results of such maps are the red areas which represent the regions of the negative potential, or the areas that are rich in electrons and suitable for electrophilic bonding; and the blue areas, corresponding to the positive potential—the electron-poor areas susceptible to nucleophilic interactions.

In the case of protochelin-heavy metal complexes, red zones are mainly concentrated over electronegative atoms, and these are the binding sites revealed for metal ions, while blue zones commonly found near electron-deficient regions can be seen as possible sites for nucleophilic attack. When complexed, a significant change in the distribution of the charge density can be observed using ESP maps. These shifts indicate considerable alterations in the electronic environment due mainly to charge-transfer and inductive effects. This charge redistribution is evident from the higher dipole moment values recorded in the complexes. Interfacial charge transfer between the metal ion and protochelin either from the metal to the ligand or from the ligand to the metal was identified to be another significant strategy for boosting the dipole moment. Secondly, steric factors meaning inductive effects due to the metal ion also enhance polarization in the ligand leading to an increase in low-energy forms. The increase in ESP and dipole moment magnitude affirms the strength and type of interaction in the various complexes summarized in Table 2 based on the dipole moment values ranging from 4.45 to 21.21. Therefore, not only does the ESP analysis reveal the distribution of charge and the places of the strongest interactions but also the nature of the electronic effects that dictate the complexation. This approach answers the questions regarding the mechanisms through which the heavy metals react with protochelin and paves the way for the application of protochelin in chelating and sensing applications.

Table 2. Energies of HOMO, LUMO, band gap (eV), & dipole moment for all metal-protochelin complexes calculated at def2-SVP double-zeta basis set and def2-TZVP triple zeta basis set with PBE0 functional.

Complexes	Charge	E _{HOMO} (eV)	E _{LUMO} (eV)	Energy gap (eV)	μ (D)
Protochelin	−6	5.195473	12.39452	7.199048	4.454484
Fe-Protochelin	−3	−4.839	−0.59593	4.243074	13.607181
Cr-Protochelin	−3	−4.55464	−1.11621	3.438433	14.676050
Cr-Protochelin	0	−5.99712	−4.27192	1.725203	8.430779
Cu-Protochelin	−4	−4.33342	−0.09034	4.243074	20.067675
Zn-Protochelin	−4	−4.24199	0.02449	4.266475	17.615736
Ni-Protochelin	−4	−4.24743	−0.47293	3.774493	21.218394
As-Protochelin	−3	−3.94837	−0.61062	3.33775	17.198189
Hg-Protochelin	−4	−4.25913	−0.08408	4.175045	12.495942
Pb-Protochelin	−4	−4.22865	0.073199	4.30185	11.835909

3.4. Frontier Molecular Orbitals (FMO) Analysis

The electronic properties of protochelin and its complexes with various heavy metals were evaluated using Frontier Molecular Orbital (FMO) analysis. HOMO (Highest Occupied Molecular Orbital) acts as an electron donor, while LUMO (Lowest Unoccupied Molecular Orbital) accepts electrons. The HOMO energy, LUMO energy, and energy gap for bare protochelin were calculated as 5.195 eV, 12.395 eV, and 7.199 eV, respectively. Upon complexation with heavy metals, a significant reduction in the energy gap was observed, indicating enhanced interactions and alterations in electronic properties. The energy gaps for metal-protochelin complexes were determined as follows: Fe-Protochelin (4.243 eV), Cr (III)-Protochelin (3.438 eV), Cr (VI)-Protochelin (1.725 eV), Cu-Protochelin (4.243 eV), Zn-Protochelin (4.266 eV), Ni-Protochelin (3.774 eV), As-Protochelin (3.337 eV), Hg-Protochelin (4.175 eV), and Pb-protochelin (4.301) (Table 2). Among these, Cr (VI)-Protochelin exhibited the lowest energy gap (1.725 eV), signifying the strongest interaction and the highest potential for enhanced conductivity. These findings align with the values obtained from interaction and Gibbs free energy analyses, further supporting the observed trends and confirming the reliability of the electronic property evaluation. The observed reduction in the HOMO-LUMO gap upon metal complexation aligns with the principle that lower energy gaps correspond to increased and stronger metal-ligand interactions. The orbital density analysis further supports these findings. It is important to examine orbital densities to determine how metals interact with the protochelin surface. The orbital density of the bare protochelin and metals-protochelin complexes unit is shown in Figure S4. While the bare protochelin surface shows uniform HOMO-LUMO density distribution, complexes such as Cu-Protochelin, Ni-Protochelin, Zn-Protochelin, and Hg-Protochelin display minimal changes in density, indicating weaker perturbations. However, Fe-Protochelin, Cr (III & VI)-Protochelin, and Pb-protochelin exhibit significant redistribution of electron density, with the HOMO localized on the metal and the LUMO on the protochelin surface. This redistribution facilitates efficient electron transfer from the metal to protochelin, resulting in a reduced band gap and stronger bonding. These results underscore the unique electronic behavior of protochelin upon interaction with heavy metals and highlight its potential for applications requiring enhanced interaction for the removal of heavy metal pollutants.

3.5. Density of States (DOS) Analysis

Changes in the electronic transition of protocadherin-metals complexes provide a reliable method for determining the adsorption mechanism of heavy metals. The transfer of electrons from the valence band to the conduction band is central to this process, with the Fermi level lying between them. This electron transfer significantly influences the binding affinity of the system, which is closely related to the density of states (DOS). The macroscopic feature of “binding affinity” and the microscopic attribute “density of states (DOS)” exhibit a strong interdependence [33]. To investigate the formation of energy levels, DOS analysis was performed, and the results are depicted in Figure S5. The analysis reveals that while the HOMO energy remains relatively consistent across all complexes (approximately 4–5 eV), there is a significant reduction in LUMO energy levels upon complexation with heavy metals. The LUMO energy values for the complexes are as follows: bare protochelin (12.395 eV), Fe-Protochelin (−0.596 eV), Cr-Protochelin (−1.116 eV), Cu-Protochelin (−4.272 eV), Zn-Protochelin (−0.090 eV), Ni-Protochelin (0.024 eV), As-Protochelin (−0.473 eV), Hg-Protochelin (−0.611 eV), and Pb-Protochelin (0.073 eV).

This reduction in LUMO energy levels leads to a decrease in the HOMO-LUMO gap, thereby enhancing the interaction of the complexes. The DOS spectra clearly demonstrate the formation of virtualized energy levels during the complexation process, with the pronounced decrease in the HOMO-LUMO gap correlating to improved interaction. These

findings highlight the role of heavy metal interactions in modulating the electronic properties of protochelin, further validating the interaction with various heavy metals.

3.6. Non-Covalent Interaction (NCI) Analysis

Non-covalent Interaction (NCI) analysis was employed to provide further insight into the nature and strength of intermolecular forces between protochelin and the targeted metal pollutants (Cr, Ni, Cu, Zn, As, Hg, Pb). NCI visualizations are typically presented as 2D and 3D isosurfaces within the Reduced Density Gradient (RDG) spectrum, characterized by three distinct color regions signifying different interaction types. Blue isosurfaces represent attractive forces, such as hydrogen bonds, which play a crucial role in the complexation process. These stabilizing interactions are often characterized by low electron density and low RDG values, indicating minimal steric repulsion between the interacting moieties. In contrast, red isosurfaces depict repulsive steric clashes, typically arising from close proximity between bulky atoms or functional groups and are associated with high RDG values and positive isosurfaces intensities. Finally, green isosurfaces represent weaker van der Waals interactions, including London dispersion forces, which contribute to overall binding affinity but are generally less prominent than hydrogen bonding or steric repulsion. This color-coded scheme allows for intuitive visualization of the dominant intermolecular forces at play within the protochelin-metal complexes. Furthermore, the intensity of the isosurfaces correlates directly with the interaction strength, providing valuable insights into the relative contribution of different forces to the overall stability of the complexes [34].

Within the protochelin molecule itself, faint green isosurfaces emerge between the amide and benzene moieties, reflecting the presence of weak London dispersion forces. These non-directional, attractive interactions contribute to the overall stability of the molecule but are relatively minor compared to other forces at play. Additionally, subtle red patches appear both within the benzene ring and between the metal ions and oxygen atoms of the metal-binding rings. These low-intensity red regions hint at the presence of weak steric repulsion, suggesting potential steric clashes occurring due to the close proximity of bulky atoms or functional groups. Despite the weaker intensity, blue isosurfaces signifying hydrogen bonding interactions are observed between the amide groups and oxygen atoms on the metal-binding rings. These stabilizing hydrogen bonds, characterized by their low RDG values and minimal steric hindrance, play a crucial role in the attachment of the metal ions within the protochelin complex.

The 2D NCI plot provides further quantitative insights into the interaction types by visualizing the relationship between the electron density Laplacian ($\text{sign}\lambda_2(\rho)$) on the x-axis and the Reduced Density Gradient (RDG) on the y-axis. As with the isosurfaces, the color scheme remains consistent, with blue regions representing strong attractive interactions (e.g., hydrogen bonding and strong electrostatic contacts), green regions denoting weaker van der Waals forces, and red regions signifying repulsive interactions. Notably, the x-axis ($\text{sign}\lambda_2(\rho)$) values reflect the interaction strength, whereas increasingly negative values indicate stronger forces. Values falling on negative side of plot between 0.00 and -0.01 au for protochelin complex with As-iii, Cr-iii & Cr-vi extending to -0.02, -0.03, -0.04 au for Cu-ii, Fe-iii, Pb-iii and in some cases even up to -0.05 au for Ni-ii, Hg-ii specifically suggest the presence of strong electrostatic interactions, such as those arising from hydrogen bonds or cation- π interactions. Red and green spikes observed in the 2D NCI graphs potentially point towards significant electrostatic interactions between the metal ions and the protochelin molecule, a finding further corroborated by the presence of blue isosurfaces in the 3D RDG analysis.

The 2D NCI plots for all studied complexes reveal a consistent presence of non-covalent interactions, evidenced by prominent green and red spikes. The green spikes

correspond to weaker van der Waals forces, as visualized by the faint green patches in the 3D RDG isosurfaces, particularly between the amide and benzene moieties within the protochelin molecule. These interactions fall within the range of $(\text{sign}\lambda_2(\rho))$ values between 0.02 and 0.01 au, a finding further corroborated by the 2D NCI plots. However, the presence of mixed green and blue spikes in the 2D NCI plots in the region between 0 to -0.05 suggests the existence of stronger interactions involving both van der Waals and hydrogen bonding contributions. This observation aligns well with the results of the interaction energy analysis, highlighting the complementary nature of the two approaches in elucidating the dominant forces governing metal-protochelin complexation.

3.7. RMSD (Stability) Analysis of Protochelin and Metal-Protochelin Complexes via AIMD Calculations

The stability of protochelin and its metal-protochelin complexes was discussed using AIMD simulations, of which the details are described in the computational methodology section. RMSD analysis (as indicated in **Error! Reference source not found.**S7) showed minimal structural changes within a 1000 fs simulation at 25 °C. Additional simulation performed at 25 °C, 50 °C, 75 °C and 100 °C proved that both protochelin and its metal complexes do not exhibit any physical changes at any of the mentioned temperatures thereby indicating the stability of both protochelin and its metal complexes. This increase in RMSD suggests greater structural flexibility upon metal adsorption on protochelin.

4. Conclusions

In this work, we computationally investigated the effectiveness of heavy metal removal utilizing the siderophore, i.e., protochelin by calculating the siderophore's interaction energy & Gibb's free energy with various heavy metal pollutants. In this work, DFT was used to calculate the interaction energies & Gibb's free energy of Protochelin with several metals, including (Cr^{+6} , Cr^{+3} , Ni^{+2} , Cu^{+2} , Zn^{+2} , As^{+2} , Hg^{+2} , and Pb^{+2}) using hybrid DFT functional PBE0 along with basis set def2-SVP and def2-TZSVP. Cr shows a high chelating affinity for Protochelin compared to other metals in order of (Protochelin- Cr^{+6} > Protochelin- As^{+3} > Protochelin- Cr^{+3} > Protochelin- Fe^{+3} > Protochelin- Ni^{+2} > Protochelin- Cu^{+2} > Protochelin- Pb^{+2} > Protochelin- Zn^{+2} > Protochelin- Hg^{+2}). The research results indicate that a siderophore would be an excellent choice as a metal-chelating chemical. These results are further supported by various computational analyses such as FMOs analysis & DOS spectra (indicating there is a decrease in band gap in metal-protochelin complexes). Non-covalent Interaction (NCI) analysis was further employed to provide further insight into the nature and strength of intermolecular forces between protochelin and the targeted metal pollutants (Cr, Ni, Cu, Zn, As, Hg, Pb). The 2D NCI plots & the 3D RDG analysis for all studied complexes reveal a consistent presence of non-covalent interactions. Red and green spikes observed in the 2D NCI graphs potentially point towards significant electrostatic interactions between the metal ions and the protochelin molecule, a finding further corroborated by the presence of blue isosurfaces in the 3D RDG analysis. Additionally, RMSD analysis showed minimal structural changes within a 2000 fs simulation at 25 °C, 50 °C, 75 °C and 100 °C proving that both protochelin and its metal complexes do not exhibit any physical changes at any of the mentioned temperatures thereby indicating the stability of both protochelin and its metal complexes. It is now viable to create plants and bacteria that may be utilized efficiently to sequester metal pollutants from the soil and water using various approaches, such as genetic engineering.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/doi/s1>, Figure S1: title; Table S1: title; Video S1: title.

Author Contributions: H.K. (Hamza Khan): Benchwork, initial draft preparation, conceptualization, Visualization, Methodology, Investigation, Supervision, Writing, Reviewing, Editing, and Validation. H.K. (Hania Khalid): Benchwork, Reviewing, and Editing. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Institutional Review Board Statement:

Informed Consent Statement:

Data Availability Statement:

Conflicts of Interest: The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

1. Proshad, R.; Kormoker, T.; Mursheed, N.; Islam, M.M.; Bhuyan, M.I.; Islam, M.S.; Mithu, T.N. Heavy metal toxicity in agricultural soil due to rapid industrialization in Bangladesh: A review. *Int. J. Adv. Geosci.* **2018**, *6*, 83–88.
2. Nyiramigisha, P.; Komariah; Sajidan. Harmful Impacts of Heavy Metal Contamination in the Soil and Crops Grown Around Dumpsites. *Rev. Agric. Sci.* **2021**, *9*, 271–282. https://doi.org/10.7831/ras.9.0_271.
3. Fuller, R.; Landrigan, P.J.; Balakrishnan, K.; Bathan, G.; Bose-O'Reilly, S.; Brauer, M.; Caravanos, J.; Chiles, T.; Cohen, A.; Corra, L. Pollution and health: A progress update. *Lancet Planet. Health* **2022**, *6*, e535–e547.
4. Debnath, A.; Singh, P.K.; Sharma, Y.C. Metallic contamination of global river sediments and latest developments for their remediation. *J. Environ. Manag.* **2021**, *298*, 113378.
5. ur Rehman, K.; Bukhari, S.M.; Andleeb, S.; Mahmood, A.; Erinle, K.O.; Naeem, M.M.; Imran, Q. Ecological risk assessment of heavy metals in vegetables irrigated with groundwater and wastewater: The particular case of Sahiwal district in Pakistan. *Agric. Water Manag.* **2019**, *226*, 105816. <https://doi.org/10.1016/j.agwat.2019.105816>.
6. Ortega, L.M.; Lebrun, R.; Blais, J.-F.; Hausler, R.; Droguet, P. Effectiveness of soil washing, nanofiltration and electrochemical treatment for the recovery of metal ions coming from a contaminated soil. *Water Res.* **2008**, *42*, 1943–1952. <https://doi.org/10.1016/j.watres.2007.11.025>.
7. Zheng, X.; Li, Q.; Wang, Z.; Chen, M. Remediation of heavy metals contaminated soil by enhanced electrokinetic technology: A review. *Arab. J. Chem.* **2024**, *17*, 105773.
8. Riaz, U.; Athar, T.; Mustafa, U.; Iqbal, R. Economic feasibility of phytoremediation. In *Phytoremediation*; Elsevier: Amsterdam, The Netherlands, 2022; pp. 481–502.
9. Mukherjee, P.; Dutta, J.; Roy, M.; Thakur, T.K.; Mitra, A. Plant growth-promoting rhizobacterial secondary metabolites in augmenting heavy metal (loid) phytoremediation: An integrated green in situ ecorestorative technology. *Environ. Sci. Pollut. Res.* **2024**, *31*, 55851–55894.
10. Albrecht-Gary, A.; Crumbliss, A.J.M.D. New York. *Met. Ions Biol. Syst.* **1998**, *35*, 39–327.
11. Swarnalatha, G.V.; Goudar, V.; Reddy, E.C.R.G.S.; Al Tawaha, A.R.M.; Sayyed, R. Siderophores and their applications in sustainable management of plant diseases. In *Secondary Metabolites and Volatiles of PGPR in Plant-Growth Promotion*; Springer: Cham, Switzerland, 2022; pp. 289–302.
12. Harrington, J.M.; Bargar, J.R.; Jarzecki, A.A.; Roberts, J.G.; Sombers, L.A.; Duckworth, O.W.J.B. Trace metal complexation by the triscatecholate siderophore protochelin: Structure and stability. *Biometals* **2012**, *25*, 393–412.
13. Rajkumar, M.; Ae, N.; Prasad, M.N.V.; Freitas, H. Potential of siderophore-producing bacteria for improving heavy metal phytoextraction. *Trends Biotechnol.* **2010**, *28*, 142–149. <https://doi.org/10.1016/j.tibtech.2009.12.002>.
14. Cornish, A.S.; Page, W.J.J.B. Production of the triacetate siderophore protochelin by *Azotobacter vinelandii*. *BioMetals* **1995**, *8*, 332–338.
15. Timofeeva, A.M.; Galyamova, M.R.; Sedykh, S.E. Bacterial siderophores: Classification, biosynthesis, perspectives of use in agriculture. *Plants* **2022**, *11*, 3065.
16. Mohanapriya, E.; Elangovan, S.; Kanagathara, N.; Marchewka, M.; Janczak, J.; Revathi, P. Density functional theory calculations, structural and spectroscopic characterization, and solvent-dependent HOMO-LUMO studies of 2-nitro-4-methylanilinium benzenesulfonate. *J. Mol. Struct.* **2024**, *1317*, 139147.

17. Janesko, B.G. Projected Hybrid Density Functionals: Method and Application to Core Electron Ionization. *J. Chem. Theory Comput.* **2023**, *19*, 837–847.
18. Weigend, F.; Ahlrichs, R. Balanced basis sets of split valence, triple zeta valence and quadruple zeta valence quality for H to Rn: Design and assessment of accuracy. *Phys. Chem. Chem. Phys.* **2005**, *7*, 3297–3305. <https://doi.org/10.1039/B508541A>.
19. Marenich, A.V.; Cramer, C.J.; Truhlar, D.G. Universal Solvation Model Based on Solute Electron Density and on a Continuum Model of the Solvent Defined by the Bulk Dielectric Constant and Atomic Surface Tensions. *J. Phys. Chem. B* **2009**, *113*, 6378–6396. <https://doi.org/10.1021/jp810292n>.
20. Ren, J.; Zheng, L.; Su, Y.; Meng, P.; Zhou, Q.; Zeng, H.; Zhang, T.; Yu, H. Competitive adsorption of Cd (II), Pb (II) and Cu (II) ions from acid mine drainage with zero-valent iron/phosphoric titanium dioxide: XPS qualitative analyses and DFT quantitative calculations. *Chem. Eng. J.* **2022**, *445*, 136778.
21. Harvey, J.N. DFT Computation of Relative Spin-State Energetics of Transition Metal Compounds. In *Principles and Applications of Density Functional Theory in Inorganic Chemistry I*, Kaltsoyannis, N., McGrady, J.E., Eds.; Springer: Berlin/Heidelberg, Germany, 2004; pp. 151–184.
22. Xu, Y.-C.; Li, N.; Yan, X.; Zou, H.-X. DFT-based analysis of siderophore-metal ion interaction for efficient heavy metal remediation. *Environ. Sci. Pollut. Res.* **2023**, *30*, 91780–91793. <https://doi.org/10.1007/s11356-023-28854-6>.
23. Harrington, J.M.; Bargar, J.R.; Jarzecki, A.A.; Roberts, J.G.; Sombers, L.A.; Duckworth, O.W. Trace metal complexation by the triscatecholate siderophore protochelin: Structure and stability. *BioMetals* **2012**, *25*, 393–412. <https://doi.org/10.1007/s10534-011-9513-7>.
24. Gomes, A.F.R.; Almeida, M.C.; Sousa, E.; Resende, D.I.S.P. Siderophores and metallophores: Metal complexation weapons to fight environmental pollution. *Sci. Total Environ.* **2024**, *932*, 173044. <https://doi.org/10.1016/j.scitotenv.2024.173044>.
25. Xiao, W.; Zhang, Y.; Li, T.; Chen, B.; Wang, H.; He, Z.; Yang, X.J. Reduction kinetics of hexavalent chromium in soils and its correlation with soil properties. *J. Environ. Qual.* **2012**, *41*, 1452–1458.
26. Li, Q.; Liu, Y.; Yu, X.; Li, L.; Zhang, X.; Lu, Z.; Lin, J.; Yang, X.; Huang, Y. Removal of Cr(III)/Cr(VI) from wastewater using defective porous boron nitride: A DFT study. *Inorg. Chem. Front.* **2018**, *5*, 1933–1940. <https://doi.org/10.1039/C8QI00416A>.
27. Moreno, M.; Zacarias, A.; Porzel, A.; Velasquez, L.; Gonzalez, G.; Alegría-Arcos, M.; Gonzalez-Nilo, F.; Gross, E.K.U. IR and NMR spectroscopic correlation of enterobactin by DFT. *Spectrochim. Acta Part A Mol. Biomol. Spectrosc.* **2018**, *198*, 264–277. <https://doi.org/10.1016/j.saa.2018.02.060>.
28. Moreno, M.; Zacarias, A.; Velasquez, L.; Gonzalez, G.; Alegría-Arcos, M.; Gonzalez-Nilo, F.; Gross, E.K.U. Experimental and theoretical structural/spectroscopical correlation of enterobactin and catecholamide. *Data Brief* **2018**, *20*, 2054–2064. <https://doi.org/10.1016/j.dib.2018.08.114>.
29. Kraepiel, A.; Bellenger, J.; Wichard, T.; Morel, F.M.J.B. Multiple roles of siderophores in free-living nitrogen-fixing bacteria. *BioMetals* **2009**, *22*, 573–581.
30. Kraka, E.; Setiawan, D.; Cremer, D.J.J.o.C.C. Re-evaluation of the bond length–bond strength rule: The stronger bond is not always the shorter bond. *J. Comput. Chem.* **2016**, *37*, 130–142.
31. Eyube, E.; Onate, C.; Omugbe, E.; Nwabueze, C.J.C.P. Theoretical prediction of Gibbs free energy and specific heat capacity of gaseous molecules. *Chem. Phys. Sci.* **2022**, *560*, 111572.
32. Bhunia, K.; Chandra, M.; Sharma, S.K.; Pradhan, D.; Kim, S.-J. A critical review on transition metal phosphide based catalyst for electrochemical hydrogen evolution reaction: Gibbs free energy, composition, stability, and true identity of active site. *Coord. Chem. Rev.* **2023**, *478*, 214956.
33. Guo, M.; Ji, M.; Cui, W. Theoretical investigation of HER/OER/ORR catalytic activity of single atom-decorated graphyne by DFT and comparative DOS analyses. *Appl. Surf. Sci.* **2022**, *592*, 153237. <https://doi.org/10.1016/j.apsusc.2022.153237>.
34. Adamo, C.; Barone, V. Toward reliable density functional methods without adjustable parameters: The PBE0 model. *J. Chem. Phys.* **1999**, *110*, 6158–6170.

Disclaimer/Publisher’s Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.