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Effect of Hydrogen Peroxide Concentration on Zinc Ion Adsorption by Human Hair

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Abstract

Introduction: The global issue of heavy-metal pollution has extensive consequences for both the environment and human health. Traditional cleanup approaches usually fall short, encouraging the search for innovative methods that are both effective and sustainable. This study investigates the potential use of keratinous materials, specifically human hair, as a potential solution for heavy metal contamination.

Methods: Stock $Zn(NO_3)_2 \cdot 6H_2O$ solutions were prepared. Collected human hair was cleaned, rinsed, air-dried, and cut into small pieces. Hair was treated by soaking at pH 9.0 for 24 hours in H2O2 of concentrations ranging from 0% to 3%, then filtering and drying. The treated hair was placed in tubes with the zinc solution. Afterward, the hair was filtered, solutions were stored, and EDTA titration was performed after an indicator was introduced. The volume of EDTA solution was recorded, and this process was repeated for the remaining solutions following a control titration. The adsorption capacity was calculated using metal ion concentrations, allowing for a quantitative comparison of biosorption efficiency across treatments.

Results: Notably, as the hydrogen peroxide concentration increased, a gradual decrease in the required EDTA volume was recorded, thus suggesting that lower residual zinc concentrations are present.

Discussion: The control untreated hair had a biosorption capacity of 1.05 mg/g, whereas the 3% treated hair had a biosorption capacity of 12.95 mg/g. This indicates a 91.89% percent increase in biosorption capacity from the control to the 3% treated hair. Thus, the greater the hydrogen peroxide concentration is, the more zinc ions would be absorbed by the hair; so, its biosorption capacity would increase.

Conclusion: The idea of using hair as a biosorbent is gaining traction; however, it is still mostly unexplored. This study demonstrates that increasing hydrogen peroxide concentration enhances the zinc ion adsorption capacity of human hair, likely due to oxidative changes increasing the availability of binding sites, therefore supporting the potential application of treated keratinous waste for low-cost high-yield metal remediation systems. Future research may explore different biological substrates (e.g., wool, feathers, etc.) or optimize treatment conditions (e.g. pH, metal substrate, etc.) to further increase adsorption efficiency.

Keywords: adsorption capacity; binding site; biosorption; EDTA titration; functional group; hydrogen peroxide; keratin; keratinous biosorbent; oxidative pretreatment

Introduction

Biosorption is the removal of undesired substances, such as heavy metal ions, from aqueous solutions by using biological material such as keratin found in hair, where mechanisms such as adsorption and ion exchange are most utilized. Keratin is a fibrous and porous protein that makes up a significant portion of hair, wool, feathers, nails, etc. It contains many functional groups like amides and sulfhydryl, which can bind to metal ions [1, 2].

When substances containing keratin, such as hair, are treated by being soaked in hydrogen peroxide in alkaline conditions, many structural and chemical changes occur, thus allowing metal ions to be adsorbed. According to Arun Ghosh and Collie Stewart, since hydrogen peroxide is an oxidizing agent, it can break down disulfide bonds present in the protein structure, causing the cuticle scales present on the outer protective layer of the hair to open up, exposing the inner layers of the hair [3, 4]. This opening of cuticles also increases the surface area of the hair fibers, providing additional sites for metal ions to interact and be adsorbed. Keratin's cysteine amino acids contain thiol (-SH) groups, which are oxidized to form disulfide (-S-S-) bonds. This oxidation process alters the keratin structure by increasing its porosity and creating more binding sites for metal ions like zinc. This allows zinc ions (Zn^{2+}) to also form coordinate bonds with the oxygen atoms in the oxidized groups of keratins. This treatment also introduces and exposes functional groups on the hair's surface, like the hydroxyl (-OH) and carboxyl (-COOH) groups, which create binding sites for metal ions through interactions

such as ion exchange and coordination. Among these groups are sulfur-containing groups, particularly cysteine residues, that play a significant role in the formation of previously mentioned coordinate bonds, therefore facilitating their adsorption onto the hair. The chemical treatments, such as the alkaline solution, enhance the exposure of the functional groups and increase the affinity of the hair for the metal ions. The pH also affects the charge distribution on the surface of the hair which has a net negative charge. This change in the charge distribution facilitates the electrostatic interaction between the hair and positively charged metal ions [5].

EDTA is a hexadentate ligand with two amino groups and four carboxyl groups known as Lewis bases. It can form covalent bonds by donating six pairs of electrons, which can act as a hexadentate ligand. Even with small metal ions present, it induces a noticeable color change, indicating weak complex formations. EDTA titration, a form of complexometric titration, is a precise analytical technique for determining the concentration of metal ions in a solution. In an alkaline environment (pH ≈ 10), metal ions (e.g., Zn2+) within the analyte solution react with an indicator (HIn2-) to form a colored metal-indicator complex (ZnIn-) while releasing protons (H+). This reaction leads to a noticeable color change from wine-red to blue, indicating the endpoint.

$$Zn^{+2} + HIn^{2-} \overset{pH\ 10}{\longleftrightarrow} ZnIn^{-} + H^{+}$$
$$\underset{colorless}{analyte} + \underset{blue}{free\ indicator} \rightleftharpoons \underset{wine\ red}{metal\ ion\ indicater\ complex} + proton$$

The endpoint of the titration is detected using indicators such as Eriochrome Black T (EBT). These indicators change color when they form complexes with specific metal ions. This color shift serves as a clear indicator of the endpoint, which is the point at which all metal ions have formed complexes with the EDTA.

$$ZnIn^{-} + HY^{3-} \overset{pH\ 10}{\longleftrightarrow} ZnY^{2-} + HIn^{2-}$$
$$\underset{wine\ red}{metal\ ion\ indicater\ complex} + \underset{colorless}{titrant\ (EDTA)} \rightleftharpoons \underset{colorless}{metal\ ion\ EDTA\ complex} + \underset{blue}{free\ indicator}$$

[6]

The idea of using hair as a biosorbent is gaining traction; however, it is still mostly unexplored [1]. This experiment aims to deepen our understanding of how such keratinous substances interact with heavy metal ions, especially after hydrogen peroxide treatment. Specifically, we are investigating how varying the concentration of hydrogen peroxide (0%, 0.5%, 1%, 1.5%, 2%, 2.5%, and 3%) affects the adsorption of zinc ions from a 700 mg/L $Zn(NO_3)_2{\cdot}6H_2O$ (zinc nitrate hexahydrate; salt) solution when applied in the treatment of human hair with the goal of removing polluting zinc ions from contaminated water sources, as measured by EDTA complexometric titration.

Methods

1. Preparation of Zinc Nitrate Solution

Stock solutions of $Zn(NO_3)_2{\cdot}6H_2O$ (zinc nitrate hexahydrate) were prepared by dissolving 700 mg of the salt in 1L of distilled water, serving as the zinc ion source for all subsequent biosorption experiments.

2. Preparation of Human Hair

Human hair was collected, thoroughly washed with hair detergent to remove oils and residues, and rinsed with deionized water. After allowing the hair to dry at room temperature, it was cut into pieces with lengths ranging from 1mm to 20mm using scissors.

3. Hydrogen Peroxide Treatment of Hair

For each experimental condition, 5.0g of untreated hair was soaked in a hydrogen peroxide solution of varying concentrations (0.0%, 0.5%, 1.0%, 1.5%, 2.0%, 2.5%, 3.0%), each adjusted to a pH of 9.0 using an ammonia buffer for 24 hours. The hair was then filtered, rinsed with distilled water, and allowed to dry.

The control sample remains untreated, without any treatment solution (does not represent 0% H2O2). The control simply consists of the hair in its natural state, whereas the 0% H2O2 sample is soaked in water at pH 9.

4. Biosorption of Zinc Ions

From the treated hair, 0.3g was placed in test tubes where 25 mL of the $Zn(NO_3)_2{\cdot}6H_2O$ solution was added and left to interact under ambient conditions. The hair was then removed via filtration, and the resulting solutions were collected and stored separately for analysis.

5. Complexometric Titration with EDTA

To determine the residual zinc concentration, 20 mL of the zinc solution was measured using a graduated cylinder, diluted with 80 mL of distilled water to achieve an analyte of 100 mL, and transferred to an Erlenmeyer flask. The 0.01M EDTA solution was prepared and standardized against a Zn^{2+} standard before use, then loaded into a burette. Before adding the indicator, add 10

mL of ammonia buffer (NH_3/NH_4Cl of pH 10) to the analyte and verify pH ≈10 in the flask; individual pH values were not recorded. The indicator was prepared by mixing 0.2g of Eriochrome Black T with 20g of NaCl to form a stable blend complex with the zinc ions. Approximately 0.5g of the prepared indicator was added to the zinc solution until it turned a wine-red colour. While stirring the solution continuously with a magnetic stirrer, EDTA solution was slowly added from the burette into the flask, causing the wine-red colour of the solution to start to fade as the zinc ions reacted with the EDTA. The dropwise addition of the EDTA solution was stopped when the wine-red colour disappeared and the solution turned pure blue. The volume of EDTA solution used to reach the endpoint was recorded. This process was first conducted for the control sample and then repeated for each treated sample.

Note: No metal-masking agents (e.g., for Ca^{2+}/Mg^{2+}) were used; thus, complexometric measurements at pH ≈ 10 reflect total EDTA-complexable metals present in the aliquot. Because the prepared solutions contained reagent-grade $Zn(NO_3)_2{\cdot}6H_2O$ in distilled water as the only added salt, the analyte is expected to be zinc-dominated, but we cannot exclude contributions from other cations introduced by materials or leached from hair.

(see Appendix for details)

Table 1. Initial and Final Volumes of EDTA Esed to Titrate Solution for All Hydrogen Peroxide Concentrations.

Concentration of H_2O_2 (%)	Initial EDTA Volume (±0.05mL)	Final EDTA Volume (±0.05mL)
control	0.00	4.60
0.0	4.60	9.00
0.5	9.00	13.40
1.0	13.40	17.70
1.5	17.70	21.80
2.0	21.80	25.70
2.5	25.70	29.40
3.0	29.40	32.80

Results

EDTA titration was performed to quantify the residual concentration of zinc ions after solutions were exposed to hair treated with varying hydrogen peroxide concentrations. For each condition, the initial and final EDTA readings were recorded to calculate the volume of titrant used. Notably, as the hydrogen peroxide concentration increased, a gradual decrease in the required EDTA volume was recorded. As the hydrogen peroxide concentration increased from 0.0% to 3.0%, the volume of EDTA required to titrate the solution decreased progressively. The control sample, which contained untreated hair, required only 4.60 mL of EDTA, indicating the baseline concentration of zinc ions present in the solution.

Also, visual differences were noted during the experiment: hair samples treated with higher H_2O_2 concentrations appeared lighter in colour and produced more surface bubbling during treatment.

1. Volume of EDTA Added:

To find the volume of EDTA added to titrate the solution, the final EDTA volume measurement is subtracted from the initial one.

$$\Delta V\ mL = V_{final} - V_{initial}$$

The percentage uncertainties of these values were found by dividing the uncertainty of the measurement (found by adding the uncertainties of both the final and initial volumes, i.e. 0.05+0.05 = ±0.1 mL) by the measurement's value and multiplying the result by 100.

Table 2. Volumes of EDTA Used to Titrate Solution for All Hydrogen Peroxide Concentrations.

Concentration of H_2O_2 (%)	Volume of EDTA Added (±0.1 mL)	Percentage Uncertainty (%)
control	4.6	2.17
0.0	4.4	2.27
0.5	4.4	2.27
1.0	4.3	2.33
1.5	4.1	2.44
2.0	3.9	2.56
2.5	3.7	2.70
3.0	3.4	2.94

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2. Zinc Solution Concentration:

Note: the following calculations were applied to the titration volume for the control trial; however, the same calculations are followed for all hydrogen peroxide concentrations, with only the titration volume varying between each

Moles of Zinc:

The reaction involved in zinc titrations by EDTA is represented by this equation:

$Zn^{2+} + EDTA^{4-} \rightarrow ZnEDTA^{2-}$, so the ratio of nZn^{2+} to $nEDTA^{4-}$ is 1:1.

The ratio of nZn^{2+} to $Zn(NO_3)_2{\cdot}6H_2O$ is 1:1, since:

$Zn^{2+} + 2NO_3^- + 6H_2O \rightarrow Zn(NO_3)_2{\cdot}6H_2O$.

Thus, the ratio of $Zn(NO_3)_2{\cdot}6H_2O$ to nEDTA is 1:1

Therefore, the number of moles of $Zn(NO_3)_2{\cdot}6H_2O$ can be calculated by stoichiometry using the following method for the control titration;

$$n_{Zn(NO3)_2\cdot6H_2O} = n_{EDTA} = C_{EDTA}\ \text{mol}/dm^3 \times V_{EDTA}\ dm^3$$
$$n_{Zn(NO3)_2\cdot6H_2O} = 0.01 \text{ x } 0.0046 = 0.000046\ \text{mol}$$

The percentage uncertainty of this value would be equal to the sum of the percentage uncertainties of both values, thus equal to 2.17% (uncertainty of EDTA concentration is assumed to be zero).

Mass of Zinc:

The mass of $Zn(NO_3)_2{\cdot}6H_2O$ can be found by multiplying the number of moles of zinc nitrate hexahydrate by its molar mass, so;

$$m_{Zn(NO3)_2\cdot6H_20} = n_{Zn(NO3)_2\cdot6H_20} \times M_{Zn(NO3)_2\cdot6H_20}$$
$$m_{Zn(NO3)_2\cdot6H_20} = 0.000046 \times 297.49 = 0.01368g$$

Similarly, the percentage uncertainty of the above is 2.17%, thus the mass would be 0.01368 ±0.00029g.

Concentration of Solution:

The concentration of the zinc solution was found by finding the concentration in mol/dm^3 and converting that value to the concentration in mg/L.

$$C_{Zn(NO3)_2\cdot6H_20}(M) = n_{Zn(NO3)_2\cdot6H_20} \times V\ litres$$
$$C_{Zn(NO3)_2\cdot6H_20}(M) = 0.000046 \times 0.02 = 0.0023\ M$$

Using the same method, the percentage uncertainty of the concentration would be equal to 2.17% + 0.02/20*100 = 12.17%.

$$C_{Zn(NO3)_2\cdot6H_20}(mg/L) = C_{Zn(NO3)_2\cdot6H_20}(M) \times M_{Zn(NO3)_2\cdot6H_20} \times 1000$$
$$C_{Zn(NO3)_2\cdot6H_20}(mg/L) = 0.0023 \times 297.49 \times 1000 = 684.277\ mg/L$$

The percentage uncertainty of this value is equal to that of the concentration, i.e. 12.17%. Therefore, the concentration of the solution would be 684 ±83 mg/L (as zinc nitrate hexahydrate ($Zn(NO_3)_2{\cdot}6H_2O$) salt). The same method for all the above was applied to all the various solutions.

3. Percent Biosorption:

The percent biosorption for each was found by applying the percent change formula for biosorption, where Cf is the final concentration and C_i is the initial one of the solution.

$$\%\ biosorption = \frac{C_i - C_f}{C_i} \times 100$$
$$\%\ biosorption = \frac{700 - 684.227}{700} \times 100 = 2.25\%$$

The same method was applied to all the different concentrations. The percentage uncertainty of this value is equivalent to that of the concentration as the uncertainty of the concentration for the initial solution is assumed to be zero.

4. Biosorption Capacity:

Difference in Concentration:

The difference in concentration was found by subtracting the initial concentration of 700 mg/L as Zn(NO3)2•6H2O (salt) from the final one.

$$\Delta C = C\_i - C\_f$$

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Biosorption Capacity:

The biosorption capacity could be found as follows, where V is the total volume of the solution (0.02 L), and m is the mass of biosorbent used (0.3g)

$$Q_e = \frac{\Delta C \times V}{m}$$

$$Q_e = \frac{15.773 \times 0.02}{0.3} = 1.0515\ mg/g$$

The percentage uncertainty of this value can be calculated by adding the percentage uncertainty for the difference in concentration, volume and mass, e.g. 22.51% for the control.

The same formula was applied to the various concentrations of zinc solutions.

Table 3. Processed Data for All Hydrogen Peroxide Concentrations

H_2O_2 Concentration (%)	Zinc Nitrate Hexahydrate Solution Concentration (mg/L)		Difference in Zinc Solution Concentration (mg/L)		Percent Biosorption (%)		Biosorption Capacity (mg/g)	
control	684.22700	±83.29720	15.77300	±1.92019	2.25000	±0.27391	1.05150	±0.23666
0.0	654.47800	±80.32230	45.52200	±5.58679	6.50314	±0.79811	3.03480	±0.68605
0.5	654.47800	±80.32230	45.52200	±5.58679	6.50314	±0.79811	3.03480	±0.68605
1.0	639.60350	±78.83485	60.396500	±7.44422	8.62807	±1.06346	4.02643	±0.91235
1.5	609.85450	±75.85995	90.145500	±11.21322	12.87793	±1.60189	6.00970	±1.36855
2.0	580.10550	±72.88505	119.894500	±15.06367	17.12779	±2.15195	7.99297	±1.83019
2.5	550.35650	±69.91015	149.643500	±19.00877	21.37764	±2.71554	9.97623	±2.29813
3.0	505.7330	±65.44780	194.26700	±25.14044	27.75243	±3.59149	12.95113	±3.01431

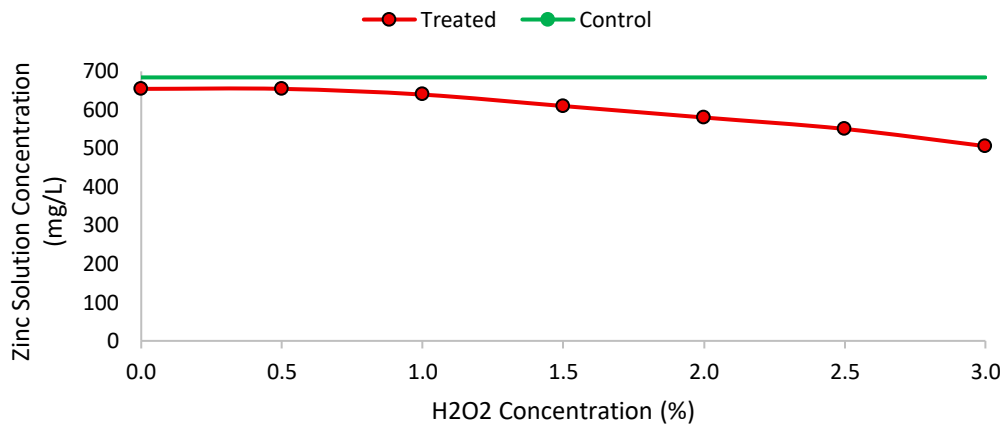

Figure 1. Residual Concentration (mg/L) Reported as $Zn(NO_3)_2 \cdot 6H_2O$ Across Hydrogen Peroxide Concentrations (%). (*Figure Created in Microsoft Excel)*

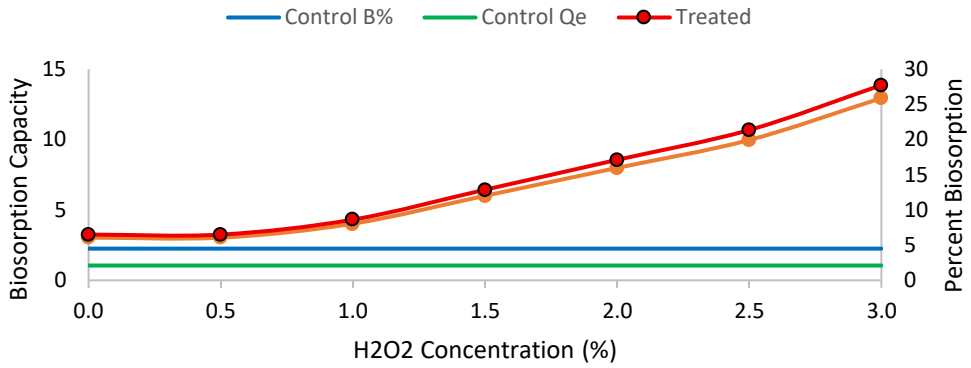

Figure 2. Biosorption Capacity (Q_e; $mg \cdot g^{-1}$) and Percent Biosorption (%) Across Hydrogen Peroxide Concentrations (%). (*Figure Created in Microsoft Excel)*

5. Percentage Uncertainty:

The percentage uncertainty for the control untreated hair was found by adding all the percentage uncertainties of all values pre-titration and that of the titration. The percentage uncertainty of the pre-titration values is 11.15% (equal across all concentrations). This was calculated by adding all the percentage uncertainties, which were found by dividing the uncertainty of the measurement by the value of the measurement and then multiplying the result by 100. The titration percent uncertainty was found by dividing the measurement, i.e. volume of solution used, by the uncertainty of the burette.

Table 4. Percentage uncertainty for all hydrogen peroxide concentrations

Hydrogen Peroxide Concentration (%)	Post-Titration Uncertainty Calculations	Percentage Uncertainty (%)
control	11.15 + 0.05 ÷ 4.6 × 100	12.24
0.0	11.15 + 0.05 ÷ 4.4 × 100	12.29
0.5	11.15 + 0.05 ÷ 4.4 × 100	12.29
1.0	11.15 + 0.05 ÷ 4.3 × 100	12.31
1.5	11.15 + 0.05 ÷ 4.1 × 100	12.37
2.0	11.15 + 0.05 ÷ 3.9 × 100	12.43
2.5	11.15 + 0.05 ÷ 3.7 × 100	12.50
3.0	11.15 + 0.05 ÷ 3.4 × 100	12.62

Discussion

In this investigation, the control untreated hair absorbed 2.25% of the zinc ions, so the biosorption capacity was 1.05 mg/g, whereas the hair that was treated with the 3% H_2O_2 solution absorbed 27.75% of the zinc ions, i.e. had a biosorption capacity of 12.95 mg/g. This indicates a 12.3-fold increase in biosorption capacity from the control to the 3% treated hair. In addition, the trend in Figure 1 shows a monotonic decrease in residual zinc with increasing H_2O_2, supporting the hypothesis that the greater the hydrogen peroxide concentration is, the more zinc ions would be absorbed by the hair, thus its biosorption capacity would increase.

By comparing the control hair and the hair treated with 0% hydrogen peroxide, we can also observe a clear increase in Qe from 1.05 mg/g to 3.03 mg/g. This is not contradictory: alkaline soaking alone can increase capacity by deprotonating surface groups, therefore promoting a negative surface charge, resulting in electrostatic attraction for Zn^{2+}. Oxidative pretreatment plausibly builds on this baseline by increasing porosity and introducing additional donor sites, which can enhance electrostatic interactions and complexation with Zn^{2+}, thereby explaining the stepwise rise in Qe from the control to the alkaline soak and then the oxidized fibers. Because residual zinc was quantified by EDTA titration, potential pH-driven zinc hydroxide precipitation could also reduce measured Zn^{2+} (discussed in limitations below).

Finally, the observed treatment effects substantially exceed the propagated measurement uncertainty, supporting that these differences reflect true adsorption changes rather than noise.

Limitations:

Titrations were carried out in NH_3/NH_4Cl (pH 10) ammonia buffer, with verified pH ≈10 in each flask, but individual pH values were not recorded. Masking agents were not used to suppress Ca^{2+}/Mg^{2+} during complexometric titration, and we could not perform selective validation (e.g., AAS/ICP-OES) on representative

samples. Consequently, the EDTA endpoint should be interpreted as total EDTA-complexable metal remaining (expected to be zinc-dominated in this $Zn(NO_3)_2 \cdot 6H_2O$ system rather than strictly zinc-specific), and alkaline precipitation may have contributed to apparent zinc losses. Recommended controls (i.e., no-hair solution, reagent-only solution, pH-matched blank) were not run, as well as contact time and temperature were not being fixed or measured. Because blanks were not available, adsorption calculations could not be blank-corrected; reported values, therefore, reflect apparent removal and cannot solely be attributed to adsorption. FTIR/XPS or other related surface-analysis data were not collected; mechanistic inferences are therefore tentative and should be confirmed in future work.

Conclusions

This investigation offers encouraging insights into the potential use of low-cost keratinous biowaste, such as human hair, as an environmentally sustainable biosorbent for water remediation techniques. A possible extension could be to investigate the effect of pH on the biosorption capacity of human hair and other keratinous substances, since it has been observed that the Qe of hair increases as pH increases [7]. Therefore, this extension could possibly identify the optimal pH at which hair has a maximum biosorption capacity. Additional exploration could employ surface characterization techniques (e.g. SEM, FTIR, and XPS) to confirm the mechanistic understanding of binding interactions involved. Future work should include appropriate blanks to distinguish adsorption from precipitation and selective zinc-specific validation (AAS/ICP-OES) to confirm quantification. Evaluating performance in realistic water matrices with competing ions (e.g. Ca^{2+}/Mg^{2+}) and assessing regeneration and simple column performance will support practical translation. Overall, this work contributes to the advancement of biosorption techniques and highlights new pathways for converting biological and eco-friendly waste into valuable environmental tools. Taken together, these findings motivate optimization and scale-up of simple pretreatments to enhance the remediation potential of keratinous biowaste.

List of Abbreviations

Cf: final concentration
Ci: initial concentration
–COOH: carboxyl group
EBT: eriochrome black t
EDTA: ethylenediaminetetraacetic acid
H_2O_2: hydrogen peroxide
HIn^{2-}: deprotonated indicator form
HY^{3-}: EDTA anion
NaCl: sodium chloride
NO_3^-: nitrate ion
–OH: hydroxyl group
Q_e: equilibrium adsorption capacity
SEM: scanning electron microscope
–SH: thiol group
–S–S–: disulfide bond
$Zn(NO_3)_2 \cdot 6H_2O$: zinc nitrate hexahydrate
Zn^{2+}: zinc ion
$ZnIn^-$: zinc–indicator complex
ZnY^{2-}: zinc–EDTA complex

Conflicts of Interest

The author declares that they have no conflicts of interest related to the conduct, interpretation, or presentation of this research.

Ethics Approval and/or Participant Consent

This study did not require approval from a research ethics board, as it did not involve human or animal subjects beyond the author. All human hair used in the experiment was collected from the author themself. No additional participants were involved, and no external biological samples were sourced. As such, participant consent was not applicable.

Authors' Contributions

BEK: made substantial contributions to the design of the study, the collection of data as well as interpretation and analysis of the data, revised the manuscript critically, and gave final approval of the version to be published.\

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