The Journal of Phytopharmacology 2026; 15(2):126-134
Online at: www.phytopharmajournal.com

The Journal of Phytopharmacology
(Pharmacognosy and phytomedicine Research)

Research Article

ISSN 2320-480X
JPHYTO 2026; 15(2): 126-134
March- April
Received: 16-01-2026
Accepted: 12-04-2026
Published: 17-04-2026

doi: 10.31254/phyto.2026.15202

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Development and characterization of a fungal melanin hydrogel with potential in environmental bioremediation

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ABSTRACT

Background: Heavy metal contamination of aquatic systems, particularly by copper (Cu^{2+}) and nickel (Ni^{2+}), poses a serious environmental and public health challenge due to their toxicity, persistence, non-biodegradable nature, and tendency to bioaccumulate. Although conventional remediation techniques are available, they are often expensive, energy-intensive, and environmentally unsustainable, necessitating the development of eco-friendly and cost-effective alternatives. **Objective:** The present study aimed to evaluate the potential of melanin-based hydrogels as efficient bio-derived adsorbents for the removal of Cu^{2+} and Ni^{2+} ions from aqueous solutions. **Materials and Methods:** Melanin was extracted from *Thermothelomyces hinnuleus* SP1 and incorporated into an agarose hydrogel matrix to improve structural stability and adsorption functionality. The swelling behaviour of the hydrogel was assessed to determine its water retention capacity. Adsorption experiments were conducted using 1 ppm solutions of Cu^{2+} and Ni^{2+} over a 36-hour period. Agarose-only hydrogels were used as controls. Metal ion uptake was monitored at regular time intervals to evaluate adsorption efficiency and kinetics. **Results:** The melanin-based hydrogel exhibited a high swelling ratio of 77%, indicating excellent water absorption and suitability for ion adsorption. Significantly higher metal ion adsorption was observed in melanin-incorporated hydrogels compared to controls. The hydrogel achieved 31.24% adsorption of Cu^{2+} and 17.65% adsorption of Ni^{2+}, whereas agarose-only hydrogels showed only 5.66% and 7.59% uptake, respectively. Time-dependent studies revealed faster adsorption kinetics, with maximum uptake occurring at approximately 13 h for Cu^{2+} and 17 h for Ni^{2+}. The enhanced adsorption performance was attributed to the presence of functional groups in melanin, such as carboxyl, hydroxyl, and amine groups, which facilitate strong metal ion chelation. **Conclusion:** Melanin-based hydrogels demonstrate significant potential as sustainable, eco-friendly, and cost-effective adsorbents for heavy metal remediation. Their superior adsorption efficiency, favorable swelling characteristics, and bio-derived origin highlight their applicability in wastewater treatment and environmental cleanup. This study underscores the promise of fungal melanin hydrogels as an effective alternative to conventional heavy metal adsorbents.

Keywords: *Thermothelomyces Hinnuleus*, Melanin Hydrogel, Heavy Metal Adsorption, Copper and Nickel Remediation, Fungal Melanin, Bioremediation.

INTRODUCTION

Agricultural production is increasingly challenged by various abiotic stresses, including drought, soil salinity, and extreme temperature fluctuations. These stresses not only reduce crop productivity but also threaten global food security, particularly as urbanization, industrialization, and land degradation continue to intensify environmental pressures [1]. Efficient management of water resources has therefore become a priority in modern agriculture, with technologies aimed at improving water use efficiency gaining global attention. Among such innovations, hydrogels three-dimensional networks of hydrophilic polymers with high water absorption capacity have emerged as promising materials for agricultural applications. Hydrogels demonstrate excellent water-retention abilities, functioning as soil conditioners to enhance crop establishment and resilience under water-limited conditions [2]. Their network structure enables them to absorb and gradually release water, nutrients, and agrochemicals, thereby supporting sustainable irrigation practices and improving soil fertility [3]. In response to minor changes in their environment, hydrogels demonstrate a remarkable physical chemical change. These changes are reversible, however, once the trigger is removed after a reaction, Hydrogel may return to its original position [4]. With dual cross-linked network hydrogels demonstrating enhanced mechanical properties and nanocomposite-formed supramolecular networks exhibiting exceptional stretchability and tensile strength, the majority of chemically linked hydrogels have respectable mechanical strength and toughness, expanding the range of applications for hydrogels [5]. Hydrogel provides versatile solutions to deal with water scarcity and soil fall. Recent research explained hydrogel for optimal agricultural performance, increased soil water retention and plant growth [6]. Beyond water management, hydrogels can act as carriers for fertilizers, pesticides, and bio stimulants, aligning with the principles of smart and sustainable agriculture [7]. Importantly, they have also been studied for their role in environmental remediation, including the immobilization of heavy metals in contaminated soils.

Heavy metal contamination is a major environmental concern, primarily resulting from industrial activities such as mining, smelting, fossil fuel combustion, and excessive agrochemical use [8]. Once released, these metals accumulate in soils and water systems, disrupting microbial diversity, reducing soil fertility, and posing serious risks to plants and human health through food chain transfer [9]. At the cellular level, heavy metals interfere with nutrient uptake, photosynthesis, and respiration, often inducing oxidative stress and growth inhibition [10].

Among these, copper (Cu^{2+}) and nickel (Ni^{2+}) are of particular concern. Although essential micronutrients, their excess levels lead to metabolic toxicity, copper disrupting protein synthesis and photosynthetic efficiency, and nickel inhibiting enzymatic functions and nitrogen fixation in legumes [11,12]. Consequently, Cu^{2+} and Ni^{2+} contamination not only affects soil health and crop productivity but also contributes to long-term ecological and human health risks [13].

To mitigate these challenges, eco-friendly strategies such as bioremediation are gaining importance. One biopolymer of particular interest is melanin, a naturally occurring pigment widely distributed among microorganisms, animals, and plants. Melanin is produced in fungi through oxidative polymerization of precursors such as 1,8-dihydroxynaphthalene (DHN) or L-3,4-dihydroxyphenylalanine (L-DOPA) [14,15]. Structurally, melanins are heterogeneous, water-insoluble polymers with a broad light absorption spectrum and remarkable physicochemical stability [16]. They are classified into eumelanin, pheomelanin, and allomelanin, with fungi often synthesizing DHN-derived melanin [17]. With a high absorbance in the UV area, melanins absorb light at a wide range of wavelengths, from ultraviolet to red [18].

Melanin also exhibits excellent insulation efficiency due to its conjugated polymeric backbone and broadband absorption capacity. It effectively dissipates thermal and UV energy, which enhances the thermal stability and photoprotection of the hydrogel matrix [19]. This characteristic can potentially improve hydrogel resilience in varying temperature and light conditions during field applications.

The unique functional groups present in melanin, including hydroxyl, carboxyl, and amine moieties, enable metal-binding through hydrogen bonding, electrostatic interactions, and complexation [20]. This property makes melanin an excellent natural candidate for sequestering toxic metals such as Cu^{2+} and Ni^{2+}. Additionally, melanin displays antioxidant activity, radical scavenging ability, and photoprotective functions, further highlighting its ecological and biotechnological potential [21]. Despite these advantages, the large-scale application of melanin remains limited due to difficulties in extraction and purification from natural sources. However, fungal melanins offer a sustainable alternative, as they can be produced cost-effectively through microbial fermentation [22].

Recent studies have demonstrated that melanin-based composite materials, particularly when integrated with polymeric supports such as hydrogels, combine the water retention properties of the polymer matrix with the heavy metal adsorption capacity of melanin [23]. Such hybrid materials hold promises not only for remediating heavy metal-contaminated soils but also for improving soil Condition and supporting sustainable agricultural practices. In this context, the present study focuses on the synthesis and characterization of melanin-infused agarose hydrogels and their application in the adsorption of nickel and copper ions from aqueous solutions. By integrating the multifunctional properties of melanin with the structural advantages of hydrogels, this work aims to develop a cost-effective and environmentally friendly material for heavy metal remediation and agricultural sustainability.

MATERIAL AND METHODS

Materials

Agarose powder, melanin stock solution (biosynthesized in-house, not commercially purchased), glutaraldehyde, ammonium persulfate (APS), sodium hydroxide pellets, cupric sulfate pentahydrate ($CuSO_4 \cdot 5H_2O$), nickel chloride hexahydrate ($NiCl_2 \cdot 6H_2O$), ethanol, chloroform, ethyl acetate, hydrochloric acid (HCl), and potassium hydroxide (KOH) were obtained from standard laboratory suppliers. All chemicals used were of analytical grade, and distilled water was used for solution preparation.

Extraction and Purification of Fungal Melanin

Melanin was extracted from dried mycelia of *Thermothelomyces hinnuleus* SP1following an alkali–acid precipitation method with modifications [24]. Briefly, dried mycelia were soaked in KOH (1 N) overnight at room temperature, followed by autoclaving at 121 °C for 20 min to disrupt cell walls. The biomass was homogenized in double-distilled water using a mortar and pestle, and the slurry was centrifuged at 5000 rpm for 15 min. the remaining supernatant was collected followed by removal of the pellet obtained. The supernatant was acidified with concentrated HCl until the pH reached 2.5, resulting in melanin precipitation. The precipitate was collected by centrifugation at 8000 rpm for 15 min and washed three times with distilled water. To remove impurities, the crude melanin was further purified using an ethanol:chloroform:ethyl acetate mixture (2:3:2, v/v/v). The purified melanin was dried in a hot air oven at 60 °C until a fine black–brown powder was obtained and stored in airtight containers for further use.

Synthesis of Melanin-Integrated Hydrogel

A 2% (w/v) agarose solution was prepared by dissolving agarose powder in distilled water at 90-100 °C under continuous stirring until a transparent solution was obtained. The solution was cooled to 35-40 °C. Separately, a 0.01% (w/v) melanin solution was prepared by dissolving melanin in 0.5 N NaOH with mild heating. To the cooled agarose solution, 1% (w/v) glutaraldehyde was added as a cross-linking agent, followed by dropwise addition of freshly prepared 0.1% (w/v) APS as a polymerization initiator under constant stirring. The reaction mixture was poured into molds and allowed to solidify at room temperature. The resulting melanin–agarose hydrogel was washed repeatedly with distilled water to remove unreacted chemicals. The hydrogels were cut into uniform cubes (0.5cm x 0.5cm) and stored in hydrated form until further use. The ratio of hydrogel to solution used in adsorption studies was maintained at 0.1 g per 10 mL solution. All experiments were conducted at 28 ± 2 °C with continuous orbital shaking at 100 rpm.

Characterization of Hydrogel

Fourier Transform Infrared Spectroscopy (FTIR)

FTIR spectra of dried melanin and melanin–hydrogel samples were recorded in the 4000–400 cm^{-1} range. The characteristic functional groups (O–H, N–H, C=O, and aromatic bonds) were identified to confirm melanin incorporation and structural integrity of the hydrogel. Samples were outsourced to an external laboratory.

Scanning Electron Microscopy (SEM)

The surface morphology of the melanin–hydrogel was examined using SEM. Dried hydrogel samples were sputter-coated with gold and imaged at various magnifications to analyze morphology, surface roughness, and porosity. The samples were analyzed at an external laboratory.

Swelling Behavior

Swelling behavior was determined by immersing dried hydrogel cubes in distilled water at room temperature. After defined time intervals, swollen hydrogels were blotted and weighed. The swelling ratio (SR) was calculated as: The swelling ratio (SR) was calculated as:

$$SR = \frac{(w_s - w_d)}{w_d} \times 100\%$$

where W_s is the swollen weight and W_d is the dry weight of the hydrogel.

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Each experiment was performed in triplicate, and data were expressed as mean ± standard deviation (SD). Statistical significance was determined at $p < 0.05$.

Heavy Metal Adsorption Studies

Calibration Curve Preparation

Standard solutions of $NiCl_2 \cdot 6H_2O$ and $CuSO_4 \cdot 5H_2O$ were prepared in the range of 0.1–1 ppm. Absorbance values were measured spectrophotometrically to generate calibration curves for each metal ion.

Adsorption Experiments

Hydrogel cubes (with and without melanin) were immersed in 1 ppm solutions of Ni^{2+} and Cu^{2+} ions. All adsorption studies were carried out at 28 ± 2 °C, under continuous shaking at 100 rpm for uniform mixing**.** Experiments were conducted in triplicates at room temperature (28 ± 2 °C) with continuous shaking. Absorbance of the supernatant was measured at hourly intervals up to 36 h to monitor residual ion concentrations using the calibration curves. Metal ion uptake efficiency was compared between melanin–hydrogels (test) and plain agarose hydrogels (control).

All experiments were conducted in triplicates (n = 3), and mean values with standard deviations were calculated.

Statistical analysis

Statistical analysis was performed using one-way ANOVA to compare adsorption efficiencies between control and melanin–hydrogel groups, with $p < 0.05$ considered significant.

RESULTS

Extraction and Purification of Fungal Melanin

Melanin was successfully extracted from fungal mycelia using alkali–acid precipitation. The purified pigment appeared as a fine black–brown powder, confirming typical characteristics of fungal melanin [24].

Synthesis of Melanin Based Hydrogel

A melanin-agarose hydrogel was synthesized using agarose (2% w/v) as the main polymer and melanin (0.01% w/v) as a co-polymer. Crosslinking was facilitated by glutaraldehyde (1% w/v) and APS (0.1% w/v). The resulting material was a stable, dark-brown hydrogel (Figure not shown), confirming successful incorporation of melanin.

Characterization of Synthesized Material

FTIR Analysis

FTIR spectra of control agarose hydrogel (RW01), metal-incubated controls (RW02, RW03), melanin–agarose hydrogel (RW04), and metal-incubated melanin hydrogels (RW05, RW06) are (summarized in figure 1 and 2). Pure agarose hydrogel (RW01) showed typical bands: broad –OH/–NH stretching (~3335 cm^{-1}), C=O/C=C stretching (~1636 cm^{-1}), and strong C–O/C–OH stretching (~1100–1000 cm^{-1}). After incubation with Cu^{2+} (RW02) or Ni^{2+} (RW03), only slight band shifts occurred, confirming agarose has little interaction with metal ions. Incorporation of melanin (RW04) introduced new characteristic peaks: C–N stretching (~1400 cm^{-1}, indolic/amide structures). Aromatic C–H out-of-plane bending (~840 cm^{-1}). Upon exposure to Cu^{2+} (RW05) and Ni^{2+} (RW06), distinct new peaks appeared: ~630 cm^{-1} (Cu–O stretching). ~680 cm^{-1} (Ni–O stretching). These peaks were absent in agarose-only hydrogels, demonstrating active chelation of metal ions by melanin. Thus, melanin served not only as a filler but as a multifunctional scaffold, providing phenolic, aromatic, and indolic binding sites for Cu^{2+} and Ni^{2+} coordination.

SEM Analysis

The surface morphology of agarose-only (RW01) and melanin–agarose hydrogels (RW04) was examined by FE-SEM at magnifications ranging from 1,000x to 50,000x. RW01 (Agarose hydrogel): Rough, irregular structure with large cracks and loosely arranged pores. The porous architecture was coarse and poorly distributed, indicating weak structural integrity. RW04 (Melanin hydrogel): Compact, smoother surface with finely distributed micro-pores. Higher magnifications revealed a uniform porous network, providing increased surface area and enhanced stability. These observations confirm that melanin incorporation improved hydrogel uniformity, porosity, and structural robustness, which are advantageous for heavy metal adsorption (Shown in figure 3). Average pore size, estimated from SEM images, ranged from 15–40 µm, confirming micro-porous architecture suitable for efficient ion diffusion and adsorption.

While FTIR and SEM provided indirect confirmation of structural integrity and surface morphology, additional analyses such as XRD, BET surface area, and rheological testing could offer deeper insights into cross-linking density and metal-binding mechanisms. These characterizations are proposed for future optimization of hydrogel formulation.

Swelling Behaviour

The swelling behavior of the hydrogel was evaluated by immersing it in distilled water for 36 h. The hydrogel exhibited a significant increase in weight, from its initial dry weight of 4.6112 g to a final swollen weight of 8.1927 g. This corresponds to a swelling ratio of 77% and a swelling index of 0.77, indicating a moderate-water absorption capacity. Such a pronounced swelling ability highlights the hydrogel's strong potential for water retention and ion absorption, making it highly suitable for environmental applications such as wastewater treatment, soil moisture management, and pollutant remediation.

Although the swelling behavior was extensively analyzed, the rate of water release from the hydrogel was not quantified in this study. Preliminary qualitative observation indicated gradual desorption within 24-36 h, suggesting slow and sustained moisture release. Detailed kinetic analysis of water release under different humidity conditions is planned for future work and can be provided as supplementary data in subsequent studies.

Heavy metal adsorption studies

Calibration Curves

Standard calibration curves for Cu^{2+} and Ni^{2+} were prepared at 628 nm and 294 nm, respectively (Shown in figure 4 and 5)

Dosage Study

The dosage study was conducted using 1 ppm solutions of Cu^{2+} and Ni^{2+} incubated with agarose-only (control) and melanin-loaded hydrogels for 36 h, with hourly absorbance measurements used to calculate residual ion concentrations (shown in figure 6 and 7). For Cu^{2+}, the control hydrogel showed 5.66% adsorption, whereas the melanin hydrogel achieved 31.24% adsorption, representing a marked improvement of +25.58%. Similarly, for Ni^{2+}, the control exhibited 7.59% adsorption, while the melanin hydrogel reached 17.65%, showing an enhancement of +10.06%. Time-course analysis (figures 6 and 7) revealed a distinctly faster adsorption rate in melanin hydrogels compared to the control, with maximum Cu^{2+} uptake observed around 13 h and maximum Ni^{2+} uptake around 17 h. These findings demonstrate the superior ion-binding capacity of melanin hydrogels, particularly for copper, and their potential for effective heavy metal remediation.

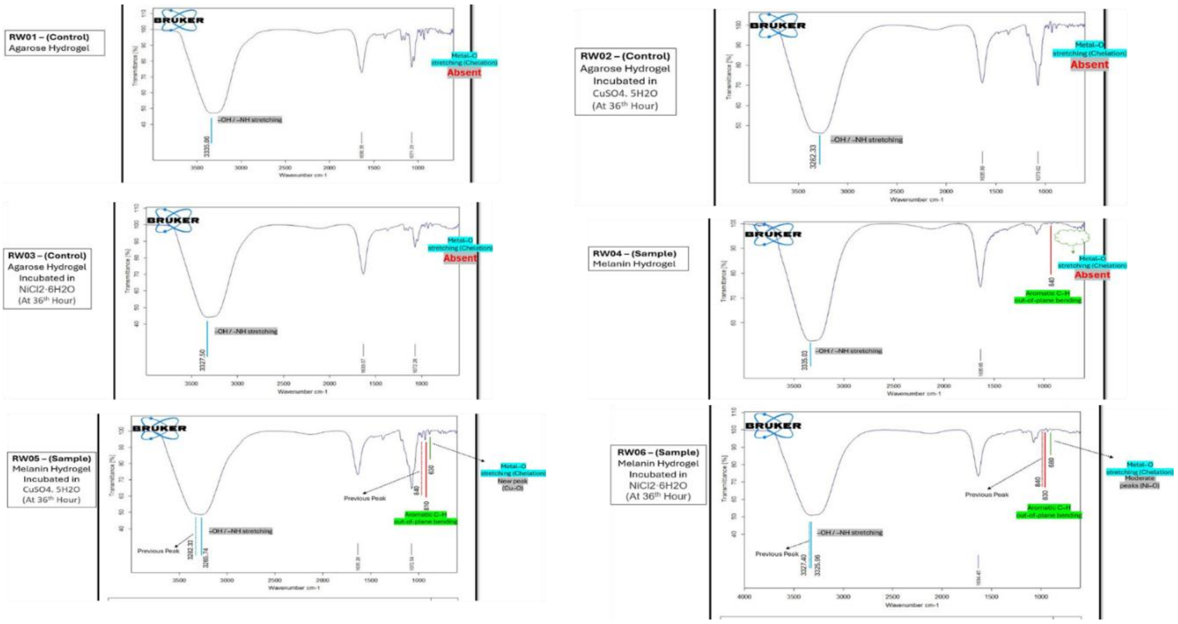

Figure 1: Showing FTIR spectra of agarose (control) and melanin hydrogels before and after incubation in Cu^{2+} ($CuSO_4 \cdot 5H_2O$) and Ni^{2+} ($NiCl_2 \cdot 6H_2O$) solutions for 36 h. Control hydrogels (RW01–RW03) showed absence of metal–ligand coordination peaks, while melanin hydrogels (RW04–RW06) exhibited characteristic shifts and new absorption bands corresponding to metal–ligand interactions, confirming successful Cu^{2+} and Ni^{2+} binding

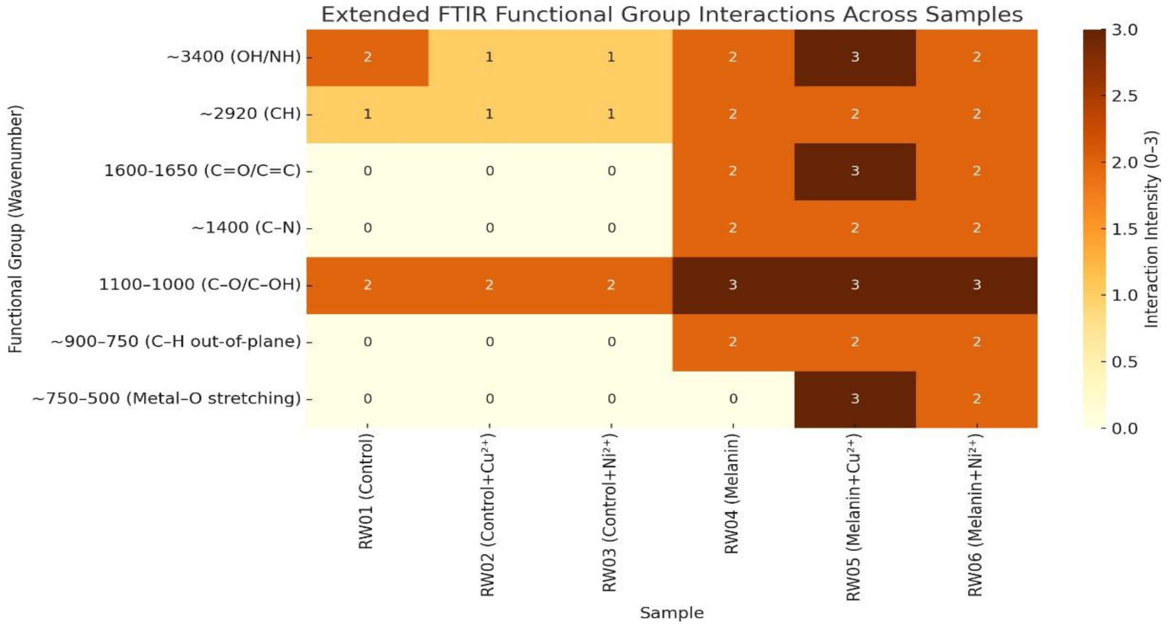

Figure 2: Extended FTIR functional group interactions across agarose (control) and melanin hydrogels before and after metal exposure. The heatmap represents interaction intensities (0–3 scale) of major functional groups: hydroxyl/amino (~3400 cm^{-1}), aliphatic C–H (~2920 cm^{-1}), carbonyl/unsaturated (~1600–1650 cm^{-1}), C–N (~1400 cm^{-1}), C–O/C–OH (~1100–1000 cm^{-1}), out-of-plane C–H (~900–750 cm^{-1}), and metal–O stretching (~750–500 cm^{-1}). While control samples (RW01–RW03) showed weak or no interaction, melanin hydrogels (RW04–RW06) exhibited strong shifts and new peaks, particularly in OH/NH, C–O, and metal–O regions, confirming coordination of Cu^{2+} and Ni^{2+} with melanin functional groups.

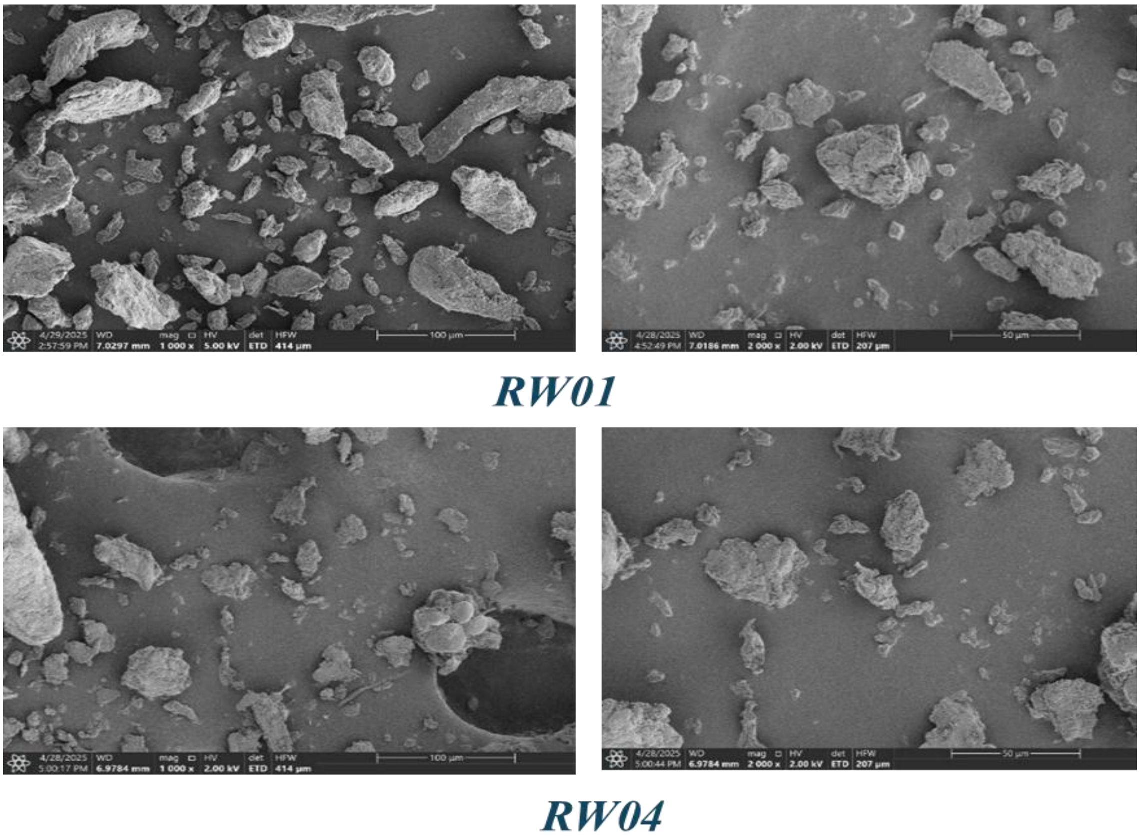

Figure 3: Scanning Electron Microscopy (SEM) images of control agarose hydrogel (RW01) and melanin-incorporated hydrogel (RW04) after metal exposure. RW01 exhibited irregular, loosely aggregated particles with rough surfaces, while RW04 showed more compact clusters with smoother morphology and partial surface coverage, indicating structural modification and enhanced interaction due to melanin incorporation. Images captured at 1,000× and 2,000× magnification

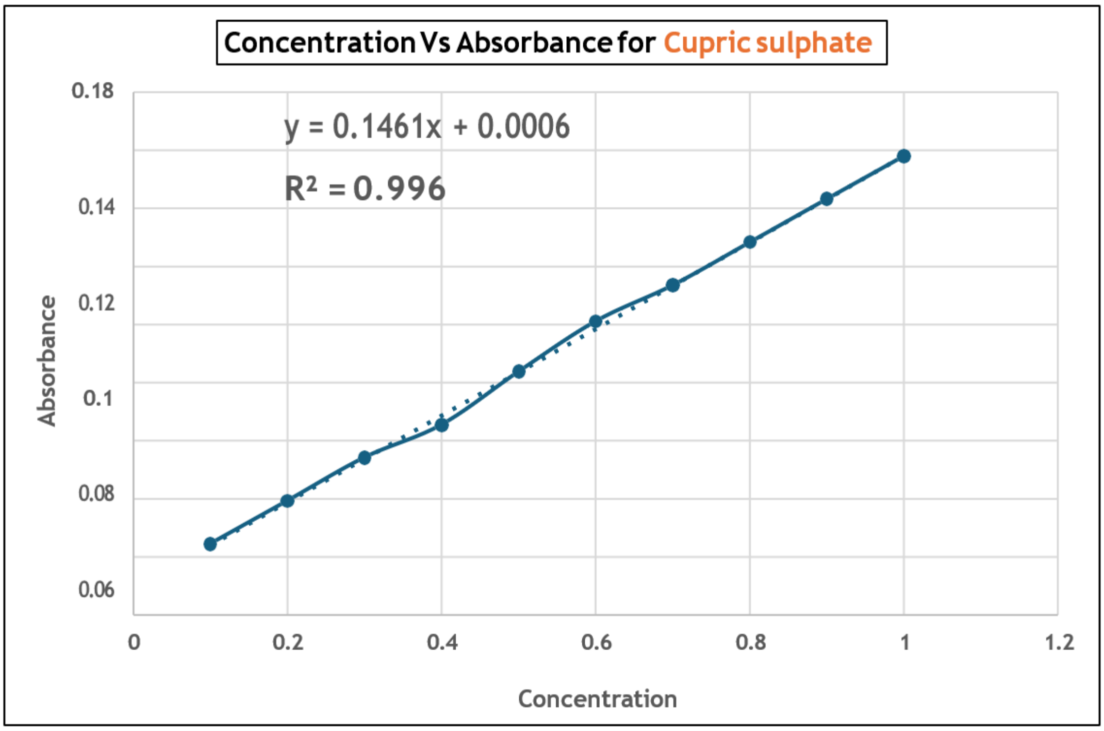

Figure 4: Standard calibration curve of cupric sulphate showing linear relationship between concentration and absorbance ($y = 0.1461x + 0.0006$; $R^2 = 0.996$)

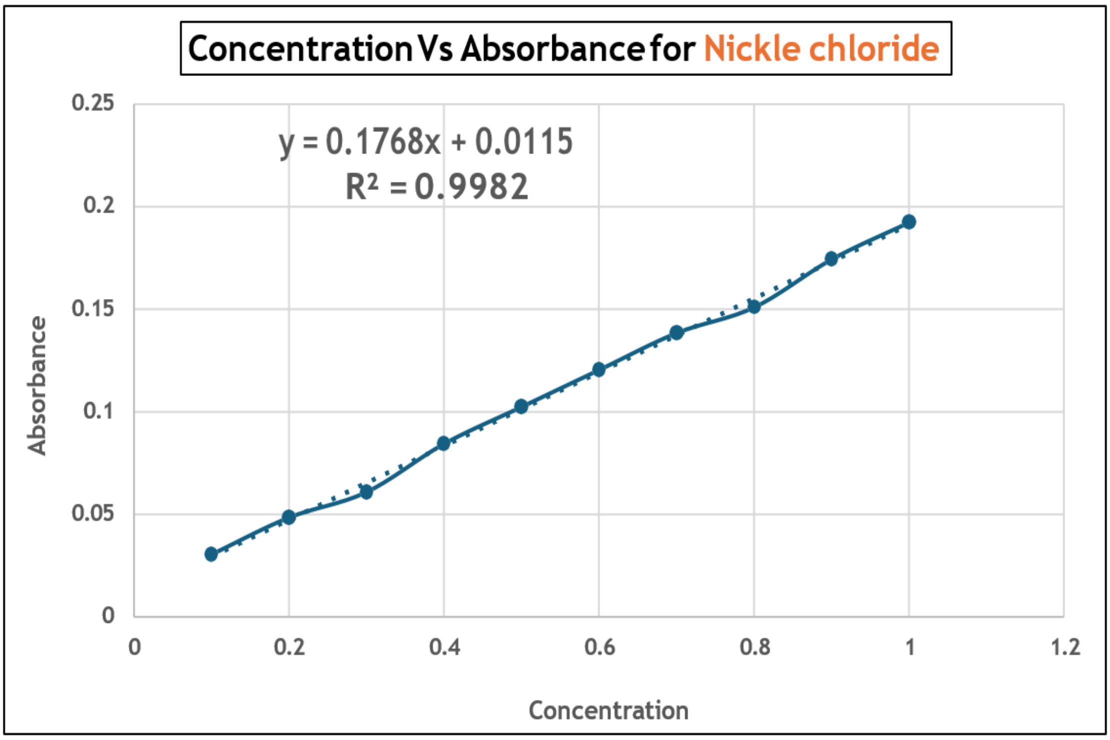

Figure 5: Standard calibration curve of nickel chloride showing linear correlation between concentration and absorbance ($y = 0.1768x + 0.0115$; $R^2 = 0.9982$)

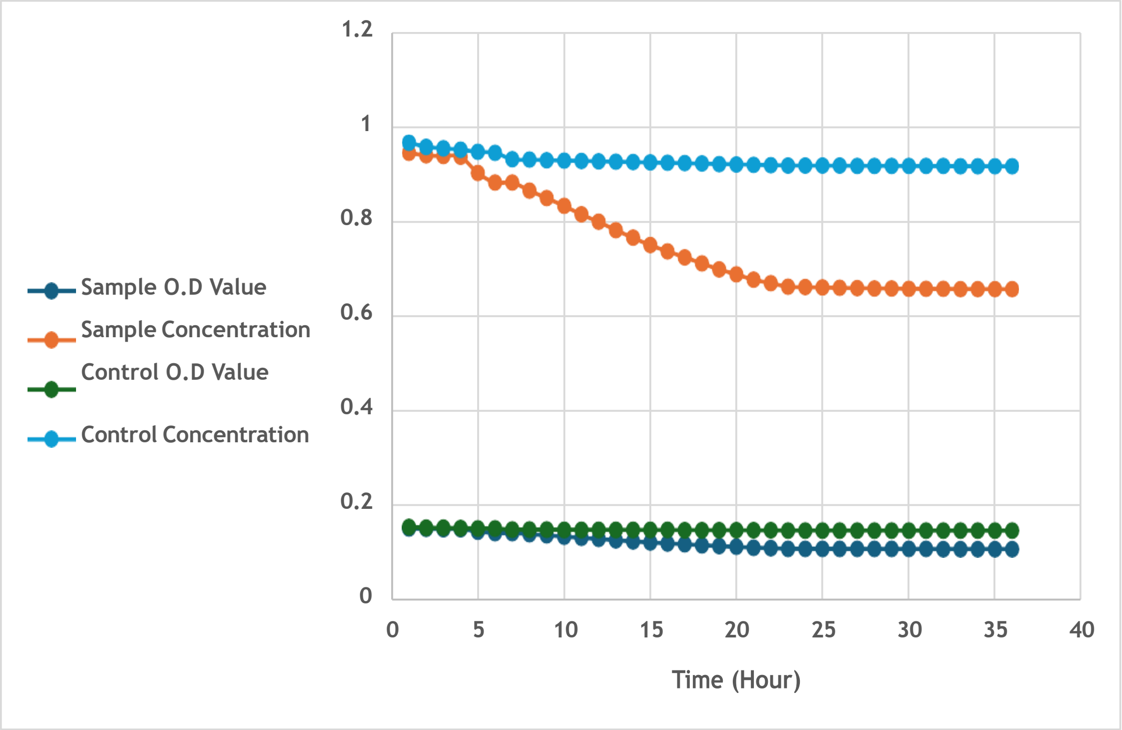

Figure 6: Time-dependent variation in optical density (OD) and metal ion concentration in sample and control groups over 36 h. The sample showed a gradual decrease in concentration with corresponding OD changes, while the control remained stable throughout the experiment

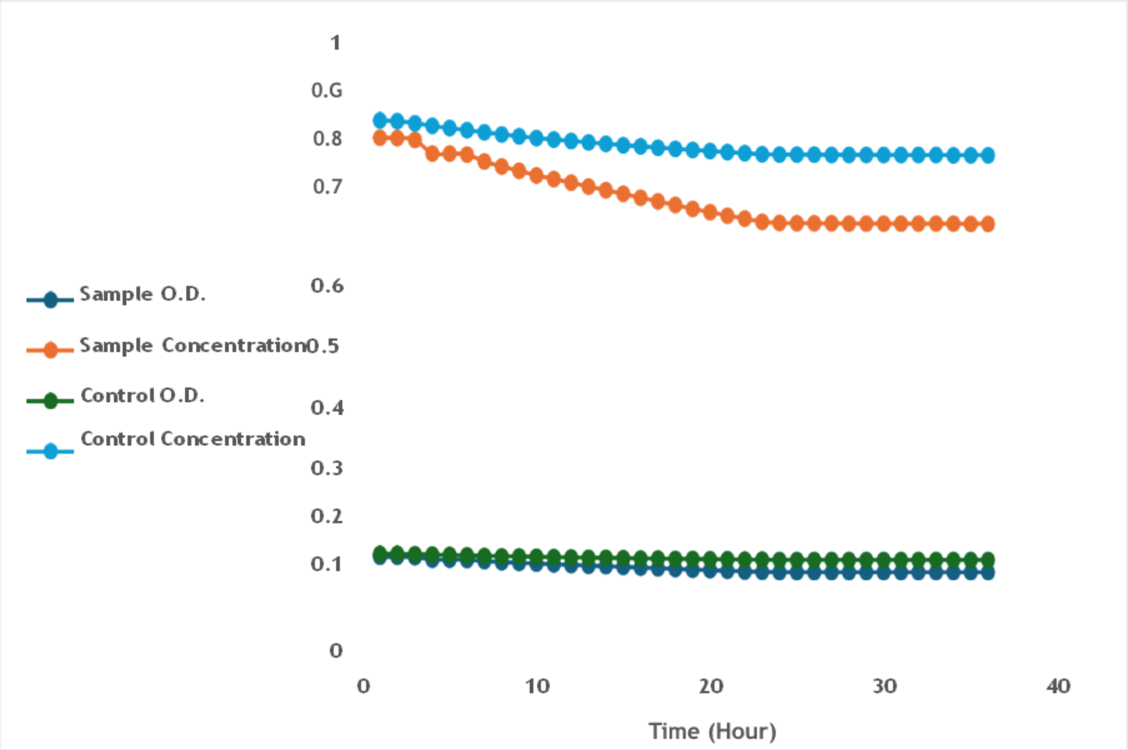

Figure 7: Time-dependent changes in optical density (OD) and metal ion concentration in sample and control groups over 36 h. The sample exhibited a gradual decline in both OD and concentration, stabilizing after 24 h, whereas the control maintained nearly constant values throughout the experimental period

DISCUSSION

Heavy metal contamination, particularly from copper and nickel, continues to pose a major environmental and public health challenge. Several studies have explored biopolymer-based hydrogels for heavy metal adsorption. For example, chitosan-gelatin composites achieved 24–28% Cu^{2+} removal [5], while lignin hydrogels showed limited Ni^{2+} adsorption (<10%) [25]. In comparison, the melanin hydrogel demonstrated superior Cu^{2+} uptake (31.24%) and competitive Ni^{2+} removal, emphasizing melanin's multifunctional chelation and antioxidant properties as a distinctive advantage. Once released into the soil and water, these metals persist in ecosystems, gradually bioaccumulating in plants, animals, and humans, where they are associated with a range of chronic health disorders. Conventional remediation strategies such as chemical precipitation, ion exchange, or membrane filtration are often costly, energy-intensive, and inefficient for long-term sustainability [26]. Industrial activities including metallurgical processes, chemical manufacturing, and plastic synthesis remain the primary contributors to copper and nickel pollution, causing significant ecological degradation and human health risks [27]. This highlights the urgent need for eco-friendly and cost-effective alternatives. In this context, melanin has recently emerged as a promising biomaterial for heavy metal remediation owing to its unique structural and functional properties. Its abundant functional groups (carboxyl, hydroxyl, and amine groups) endow it with strong chelation capacity, while its intrinsic antioxidant potential further enhances environmental resilience. Previous studies have demonstrated that fungal melanin, including that derived from *Thermothelomyces hinnules* SP1, can effectively bind metal ions, thereby contributing to detoxification systems [24]. Moreover, advanced characterization techniques such as SEM, UV–visible, and FTIR spectroscopy confirm the porous structure, surface roughness, and chemical groups responsible for its strong interactions with metal ions, underscoring its bioremediation potential. The development of melanin-based hydrogels represents a significant advancement in this field. Our study showed that the melanin hydrogel exhibited a swelling ratio of 77%, indicating excellent water retention and high ion absorption capacity. More importantly, in adsorption experiments with 1 ppm solutions, melanin hydrogels demonstrated superior removal efficiency compared to controls: 31.24% adsorption for Cu^{2+} (versus 5.66% in agarose-only) and 17.65% for Ni^{2+} (versus 7.59% in agarose-only). The adsorption was not only higher but also faster, with maximum uptake observed around 13 h for Cu^{2+} and 17 h for Ni^{2+}. These findings highlight melanin's strong affinity toward copper, with significant though comparatively lower activity for nickel. The stability of melanin–metal complexes is largely attributed to chelation through carboxyl and indolic groups. While this study maintained a neutral pH, no desorption of Cu^{2+} or Ni^{2+} was observed during equilibrium. However, environmental pH fluctuations may influence ion release. Future tests under variable pH (4–9) and salinity conditions are planned to determine leaching resistance and binding stability under realistic field environments. Adsorption performance is also influenced by metal concentration and pH equilibrium. Subsequent studies will analyse adsorption isotherms (Langmuir/Freundlich models) using concentrations ranging from 0.1–10 ppm at uniform pH values to model capacity and competitive adsorption behaviour. Integrating melanin into a hydrogel matrix provides additional benefits by improving solubility, enhancing mechanical stability, and prolonging its functional lifespan. The hydrogel structure supports melanin's active groups while maintaining flexibility and reusability, making it a practical material for continuous environmental applications [28]. Overall, our results confirm that melanin-based hydrogels not only combine the eco-friendly nature of bio-based materials with the functional efficiency of advanced adsorbents but also represent a sustainable and scalable solution for heavy metal remediation in polluted environments. The scalability is supported by the simple fabrication process, use of readily available, low-cost materials, and compatibility with standard hydrogel production methods.

Reusability and regeneration are essential for sustainable applications. Although not tested in this study, melanin hydrogels can be regenerated via mild acid or chelating agents such as EDTA. Future work will involve cyclic adsorption–desorption trials to assess capacity retention and material stability across multiple use cycles, enabling economic feasibility evaluation for large-scale applications. When compared to other natural and synthetic adsorbents, melanin-based hydrogels demonstrate competitive efficiency. For instance, chitosan-based hydrogels have been reported to achieve ~20–25% Cu^{2+} removal at similar concentrations, while plant-derived biochar typically shows lower efficiency for Ni^{2+} under comparable conditions. Activated carbon, though effective, often requires costly regeneration processes, limiting its long-term sustainability. In contrast, melanin hydrogels not only achieve higher copper adsorption (over 30% in our study) but also offer the advantage of a bio-derived, renewable, and less toxic material. This positions melanin hydrogels as a superior alternative for eco-friendly bioremediation strategies, with scope for further optimization in adsorption kinetics and reusability. It is worth noting that swelling behavior is expected to vary with temperature and water ionic composition. Future experiments will assess swelling indices using distilled, saline, and hard water at variable temperatures (20–50 °C) to understand thermal responsiveness and environmental adaptability of the hydrogel.

Mechanical strength testing (e.g., tensile and compression analyses) was not performed in this phase. Evaluating mechanical resilience under soil compaction and hydration–dehydration cycles will be critical for validating field durability and is planned for future optimization.

CONCLUSION

This study explores a sustainable approach to heavy metal remediation using melanin-integrated agarose hydrogels for the adsorption of Cu^{2+} and Ni^{2+} ions from water. Melanin extracted from *Thermothelomyces hinnuleus* SP1 was incorporated into an agarose matrix to combine the natural chelating ability of melanin with the structural stability of a biopolymer. The resulting hydrogel exhibited a high swelling ratio (77%), supporting efficient ion uptake. Adsorption efficiency significantly increased with melanin incorporation 31.24% for Cu^{2+} and 17.65% for Ni^{2+} compared to 5.66% and 7.59% in controls, respectively, with maximum adsorption observed at approximately 13 h for Cu^{2+} and 17 h for Ni^{2+}. These findings demonstrate the strong potential of fungal melanin hydrogels as eco-friendly, cost-effective adsorbents for water purification, though further statistical validation, kinetic and isotherm modeling, and detailed methodological optimization are needed to fully establish their scalability and practical applicability.

Acknowledgements

We would like to acknowledge Amity Institute of Biotechnology, Amity University Chhattisgarh for giving us laboratory Infrastructure and necessary chemicals to conduct successful experiment.

Conflict of interest

The authors declared no conflict of interest.

Financial Support

None declared.

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HOW TO CITE THIS ARTICLE

Pandey Shalini, Waghaye Rahul, Shrivastava Pragya, Agrawal Megha, Sur Arunima. Development and characterization of a fungal melanin hydrogel with potential in environmental bioremediation. J Phytopharmacol 2026; 15(2):126-134. doi: 10.31254/phyto.2026.15202