



Green-synthesized Silver Nanoparticles Using *Albizia amara* Leaf Extract: Physicochemical Characterization and Colorimetric Hg²⁺ Sensing

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ABSTRACT

Sensitive and rapid determination of Hg²⁺ in water is essential because of its toxicity, bioaccumulation, and environmental persistence. In this study, a colorimetric sensor was developed using phytochemical-stabilized silver nanoparticles (AgNPs) synthesized with *Albizia amara* leaf extract. Naturally occurring phenolic constituents facilitated Ag⁺ reduction and nanoparticle stabilization while providing oxygen-containing functional groups capable of interacting with Hg²⁺. The addition of Hg²⁺ altered the AgNP surface environment and interparticle organization, producing concentration-dependent changes in localized surface plasmon resonance and a visually distinguishable colorimetric response. The calibration response was linear over 5–100 µM Hg²⁺, following $y = 0.00118x + 0.0042$, with $R^2 = 0.996$. Using $\sigma = 0.00301$ a.u. as the residual standard deviation of the regression, the calculated limit of detection (LOD) and limit of quantification (LOQ) were 8.42 and 25.51 µM, respectively. A pronounced optical response was observed within 2 min and progressively evolved over the 15 min observation period. FE-SEM analysis after Hg²⁺ exposure revealed larger associated domains with an apparent mean diameter of 104.8 ± 23.6 nm, predominantly between 80 and 130 nm. The sensing platform showed good analytical precision, with repeatability and reproducibility of 1.48–2.31% and 2.86–4.06% RSD, respectively. Interference experiments demonstrated a preferential response to Hg²⁺ in the presence of competing metal ions. Spike-recovery analysis yielded recoveries of 94.30–101.95%, while environmental water analysis showed 95.65–105.24% agreement with the reference method. These results demonstrate that *Albizia amara*-mediated AgNPs provide a precise, selective, and rapid colorimetric platform for quantitative Hg²⁺ monitoring in aqueous samples.

Keywords: Toxicity; Colorimetric sensing; Green synthesis; Plasmon resonance; Sustainability.

1. INTRODUCTION

Water contamination by toxic metal ions and other persistent pollutants remains a major environmental concern, creating a need for sensitive and reliable analytical approaches for water-quality monitoring (Amale *et al.* 2026). Conventional instrumental methods provide accurate metal-ion determination but may require specialized equipment and sample-processing procedures. In this context, nanomaterial-based optical sensing has received considerable attention because nanostructures possess high surface area, tunable optical and electronic properties, and surface-active sites that can respond sensitively to changes in the surrounding chemical environment (Mungle *et al.* 2026).

The physicochemical performance of nanomaterials is strongly governed by their morphology,

surface structure, particle size, and optical characteristics. Scanning electron microscopy (SEM) is therefore widely used to examine particle morphology, distribution, porosity, surface texture, and structural uniformity, which directly relate to adsorption and remediation efficiency (Sugumar *et al.* 2024). SEM-based surface analysis can also provide valuable information on pore development, agglomeration, surface modification, and morphological changes after functionalization, all of which influence interactions between adsorbent surfaces and contaminant species (Rao *et al.* 2025). In parallel, UV–Vis spectroscopy is routinely used to investigate the optical response of nanostructured materials. Changes in absorption intensity and wavelength provide useful information regarding electronic transitions, surface plasmon resonance, nanoparticle formation, aggregation, and interfacial interactions (Ganvir *et al.* 2024).

Among plant-derived materials, *Albizia amara* is particularly attractive as a biological precursor for nanoparticle synthesis because of its diverse phytochemical composition. Phytochemical screening of *A. amara* leaf extract has demonstrated the presence of flavonoids, alkaloids, saponins, tannins, and sterols, indicating the availability of several biomolecular constituents containing hydroxyl, carbonyl, and related functional groups capable of participating in metal-ion reduction and nanoparticle stabilization (Sedahmed *et al.* 2021). The capability of *A. amara* constituents to support nanoparticle formation has previously been demonstrated through the plant-mediated preparation of metal-oxide nanoparticles, confirming that its leaf-derived biomolecules can participate effectively in nanoparticle nucleation, growth and surface stabilization (Swathilakshmi *et al.* 2022).

Beyond nanoparticle synthesis, *A. amara* and other plant-derived materials have been explored in several agricultural applications. Pre-storage fortification of sesame seeds with plant extracts was reported to improve germination, seedling growth, vigor, dry matter production, and oil content during prolonged storage (Sathiya *et al.* 2020a). Organic seed pelleting using *Pongamia pinnata* (arappu) leaf powder also improved growth, physiological performance, pod development, and seed yield of cluster bean under saline conditions (Prakash *et al.* 2020). Similarly, sesame seeds treated with *A. amara* leaf powder in combination with fly ash and stored in polythene bags retained higher germination, vigor, and dry matter production while exhibiting reduced moisture content and electrical conductivity (Sathiya *et al.* 2019). These studies collectively demonstrate the functional versatility of plant-derived phytochemicals and reinforce their suitability as sustainable precursors for material synthesis and environmental technologies.

Recent developments in green nanotechnology have particularly emphasized silver-based and hybrid nanomaterials because of their strong optical, catalytic, sensing, and photocatalytic characteristics. Ag-Fe₃O₄ nanocomposites prepared using *Brachychiton populneus* leaf extract demonstrated excellent visible-light activity, achieving approximately 97% degradation of methylene blue and 92% degradation of Congo red within 30 min, while retaining the advantage of magnetic recoverability (El-Shishtawy *et al.* 2026). Similarly, Ag-decorated ZnO systems showed pH-dependent photo-Fenton-like degradation of 4-nitrophenol, indicating that surface charge, pollutant speciation, and reactive oxygen species play decisive roles in photocatalytic performance (Jo *et al.* 2026).

Recent studies support the applicability of biogenic AgNPs for Hg²⁺ recognition. AgNPs synthesized using *Ficus religiosa* fruit extract exhibited selective Hg²⁺ detection together with a distinct optical

response, demonstrating the suitability of plant-derived AgNP surfaces for mercury sensing (Lekshmi *et al.* 2026). Similarly, biogenic AgNPs with nanoscale dimensions were reported to provide selective Hg²⁺ sensing, further confirming that plant-mediated silver nanoparticles can serve as responsive optical probes for mercury ions (Akhe *et al.* 2025).

Hybridization and surface engineering have further improved the environmental performance of silver-based nanomaterials. Nitrogen-doped TiO₂ containing embedded AgNPs formed internal Schottky junctions that promoted visible-light absorption and suppressed electron-hole recombination, resulting in 86.6% mineralization (Salzano *et al.* 2026). Likewise, AgNPs immobilized on microcrystalline cellulose produced a stable MCC@AgNPs catalyst capable of removing 98.77% methylene blue, 95.51% methyl orange, and 94.40% Allura Red, while retaining more than 90% efficiency after six catalytic cycles (Bradai *et al.* 2026). AgNPs synthesized using *Citrus aurantium* peel extract also exhibited dual catalytic activity, with high removal efficiencies for phenol red, methylene blue, and methyl orange (Hamze *et al.* 2025). Plant-mediated and silver-based nanomaterials have also demonstrated broader environmental applications, including nanoparticle-modified electrodes for microbial electrolysis and AgNP-based catalytic systems for pollutant degradation (Aslam *et al.* 2026), (Abbas and Abas, 2025).

Although plant-mediated AgNPs have increasingly been explored for Hg²⁺ recognition, the analytical performance of *Albizia amara*-derived AgNPs for colorimetric mercury determination remains insufficiently established. Previous studies have mainly demonstrated either the phytochemical richness and nanoparticle-forming capability of *A. amara* or the Hg²⁺ sensing ability of AgNPs synthesized from other botanical sources, while a quantitatively validated *A. amara*-based sensing system is still limited. Therefore, this study develops phytochemical-stabilized AgNPs using *A. amara* leaf extract as a biogenic colorimetric platform for Hg²⁺ detection without additional surface-functionalization steps. The sensing response relies on Hg²⁺-induced changes in nanoparticle surface interactions and localized surface plasmon resonance, producing measurable spectral and visible color changes. The developed AgNPs were systematically evaluated for concentration-dependent response, calibration behavior, precision, competitive-ion interference, colloidal changes, and analytical performance in Hg²⁺-spiked environmental water samples, thereby addressing the key analytical gap associated with *A. amara*-mediated AgNP sensing.

2. MATERIALS AND METHODS

2.1 Chemicals and Plant Material

Fresh *Albizia amara* leaves were collected and thoroughly rinsed with distilled water to eliminate adhering dust and other contaminants. The cleaned leaves were air-dried before preparation of the plant extract. Silver nitrate (AgNO_3), hydrochloric acid (HCl), nitric acid (HNO_3), and sodium hydroxide (NaOH) were of analytical grade. Metal-ion standards used for selectivity experiments included Al^{3+} , Cr^{3+} , Ni^{2+} , Hg^{2+} , Cu^{2+} , Pb^{2+} , Cd^{2+} and Co^{2+} prepared from their corresponding nitrate salts. All chemicals were used as received without further purification, and solutions were prepared using ultrapure water.

2.2 Green Synthesis of AgNPs Using *Albizia amara* Leaf Extract

A. amara leaf extract was prepared by dispersing 5 g of finely powdered dried leaves in 100 mL of ultrapure water. The suspension was maintained at 60 °C for 30 min with gentle stirring, and then allowed to cool to room temperature. After extraction, the

suspension was filtered through Whatman No. 1 filter paper and centrifuged at 10,000 rpm for 15 min to remove residual plant material. The clarified extract was subsequently mixed with aqueous AgNO_3 solution, and the reaction pH was adjusted to approximately 7.0 using dilute HCl or NaOH . The mixture was maintained under continuous stirring at 50 °C until AgNP formation occurred. Before sensing measurements, the optical response of the AgNP colloid was standardized to minimize variations arising from nanoparticle concentration, and samples showing excessive absorbance were appropriately diluted to maintain comparable optical intensity. Formation of AgNPs was confirmed by the characteristic yellowish-brown to brown coloration, corresponding to the phytochemical-mediated reduction of Ag^+ to Ag^0 . Plant-derived extracts have similarly been reported to act as natural reducing and stabilizing agents during green AgNP synthesis (Davang *et al.* 2025). The overall plant-mediated AgNP preparation process is shown in Fig. 1.

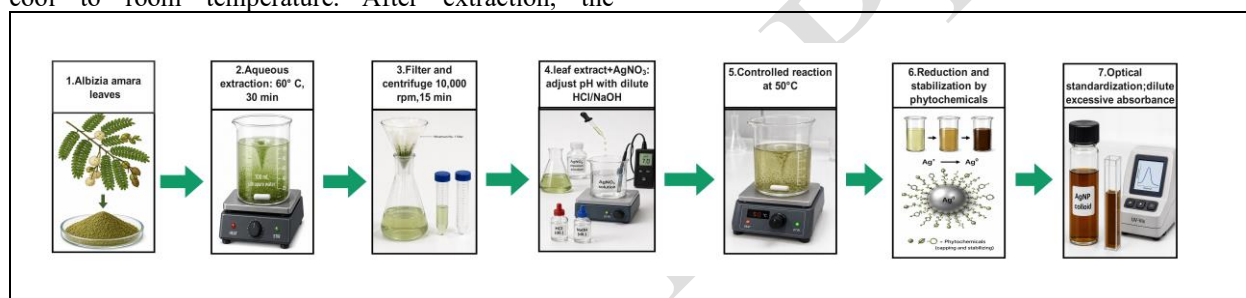


Fig. 1: Plant-mediated preparation route of AgNPs using *Albizia amara* leaf extract

2.3 Physicochemical Characterization

The optical response and surface plasmon resonance of the prepared AgNPs were recorded by a UV–Vis spectrophotometer (Shimadzu UV-1900i). Morphological observations were performed using a ZEISS field-emission scanning electron microscope (FE-SEM) equipped with an SE2 detector. For Fig. 2b, imaging was carried out at an accelerating voltage of 4.00 kV, a working distance of 14.8 mm, and a magnification of 4.00 k $\times$, with a 2 μm scale bar. For Figs. 5a and 5c, the accelerating voltage was 2.00 kV, the working distance was 4.6 mm, and the magnification was 2.00 k $\times$, with a 5 μm scale bar. FE-SEM images were used to examine the surface morphology, aggregation state, and Hg^{2+} -induced changes in the AgNP assemblies. Hydrodynamic diameter, particle-size distribution, zeta potential, and dispersion stability were determined using a Zetasizer Nano ZS (Malvern Panalytical). Hg^{2+} concentrations in environmental water samples were independently quantified using an atomic absorption spectrometer equipped with a mercury analysis system (PerkinElmer PinAAcle 900T).

2.4 Selectivity and Concentration-dependent Response

The selectivity of the sensing system was investigated against Fe^{3+} , Al^{3+} , Cr^{3+} , Ni^{2+} , Hg^{2+} , Cu^{2+} , Pb^{2+} , and Co^{2+} . For each experiment, 2 mL of an individual metal-ion solution was introduced into 1 mL of freshly prepared AgNP colloid. Sensitivity was examined using Hg^{2+} solutions covering a progressively increasing concentration range. Each Hg^{2+} solution (2 mL) was mixed separately with 1 mL of AgNP dispersion under identical conditions. All measurements were performed in triplicate to assess response consistency. Changes in surface plasmon absorption and spectral intensity produced by different ions and Hg^{2+} concentrations were monitored through UV–Vis spectroscopy.

2.5 Evaluation of Response Time

The response time of the AgNP sensing system toward Hg^{2+} was determined under ambient conditions. Fresh AgNP dispersion (1 mL) was combined with 2 mL of Hg^{2+} solution at the selected test concentration. UV–Vis spectra of the resulting mixture were collected after

2, 4, 6, 8, 10, 12, and 15 min. Variations in absorption intensity and spectral position with exposure time were used to determine the time required to obtain a stable colorimetric response.

2.6 Competitive-Ion Interference Assessment

Possible interference from coexisting metal ions was investigated to establish the specificity of Hg^{2+} recognition. 1 mL of AgNP dispersion was mixed with 1 mL of Hg^{2+} solution and an equal volume of Fe^{3+} , Al^{3+} , Cr^{3+} , Ni^{2+} , Cu^{2+} , Pb^{2+} , Cd^{2+} or Co^{2+} solution at equivalent concentrations. Each mixture was gently agitated to obtain uniform dispersion before UV–Vis measurement. The resulting optical responses were compared with the AgNP– Hg^{2+} system containing no competing ion to determine whether the additional species significantly influenced Hg^{2+} recognition.

2.7 Analytical Validation

The sensing performance was established by evaluating calibration behavior, detection capability, precision, and recovery. A calibration relationship was constructed between Hg^{2+} concentration and the corresponding change in the AgNP optical response. Calibration was based on the dual-wavelength response $\Delta A = A_{464} - A_{420}$. Hg^{2+} standards of 0, 5, 10, 20, 40, 60, 80, and 100 μM were used for linear regression, with 0 μM representing the blank; 120 μM was additionally examined spectrally. Each concentration was measured in triplicate ($n = 3$). The regression was $y = 0.00118x + 0.0042$ ($R^2 = 0.996$), with slope $S = 0.00118 \text{ a.u. } \mu\text{M}^{-1}$. Using $\sigma = 0.00301 \text{ a.u.}$ as the residual regression standard deviation, LOD = 8.42 μM and LOQ = 25.51 μM .

$$\text{LOD} = 3.3\sigma/S \quad \dots (1)$$

$$\text{LOQ} = 10\sigma/S \quad \dots (2)$$

Repeatability and reproducibility were examined through five independent Hg^{2+} measurements performed under identical analytical conditions. Method accuracy was subsequently assessed by standard addition, where known Hg^{2+} concentrations were introduced into water samples and the corresponding percentage recoveries were determined.

2.8 Evaluation of Environmental Water Samples

River water, tap water, pond water, drinking water, and domestic wastewater were collected from selected sampling sites and used to evaluate the applicability of the developed AgNP-based colorimetric method. Before analysis, each water sample was filtered through a 0.45 μm membrane to remove suspended particulate matter. For colorimetric determination, 2 mL of the prepared water sample was mixed with 1 mL of

AgNP dispersion at room temperature, and the resulting optical response was measured using UV–Vis spectroscopy. Hg^{2+} concentrations were expressed in μM . The corresponding Hg^{2+} concentrations in the environmental water samples were independently determined using atomic absorption spectroscopy and compared with the AgNP-based measurements to assess analytical reliability.

3. RESULTS AND DISCUSSION

3.1 Green Formation and Morphology of AgNPs

Fig. 2a presents the evolution of the UV–Vis spectrum during phytochemical-assisted AgNP formation using *Albizia amara* leaf extract.

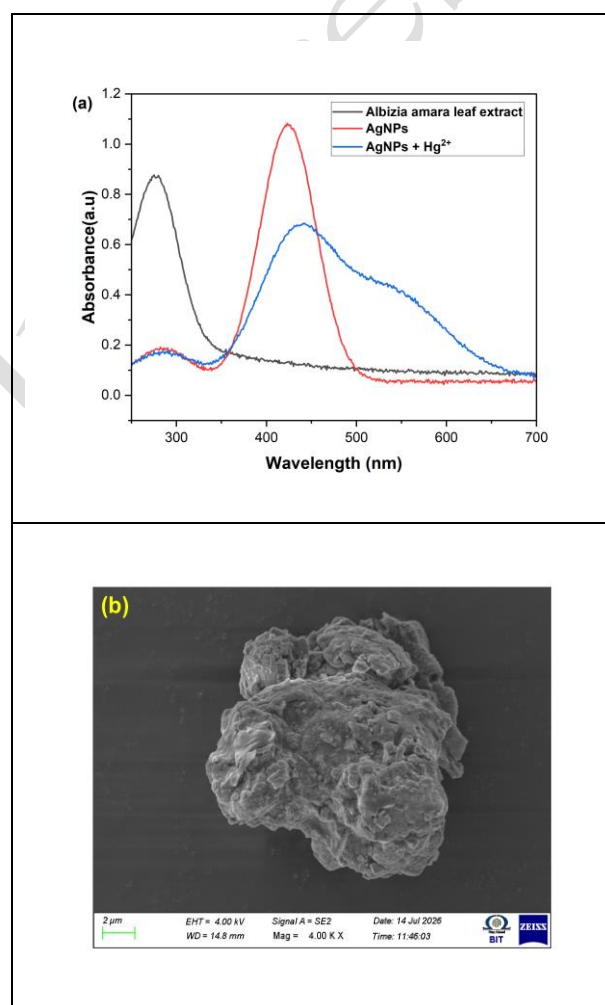


Fig. 2: (a) UV–Vis spectrum of AgNPs and (b) FE-SEM morphology of AgNP aggregates obtained at 4.00 kV, WD = 14.8 mm, and 4.00 k $\times$ magnification using an SE2 detector

Before the reaction, the AgNO_3 solution does not exhibit a characteristic surface plasmon resonance (SPR) band in the visible region, whereas *Albizia amara* leaf extract shows absorption mainly in the UV region due to its naturally occurring phytochemical constituents.

Following the reaction, a prominent absorption band develops at approximately 420 nm, providing optical evidence for AgNP formation. This characteristic SPR response originates from collective oscillations of surface conduction electrons induced by incident electromagnetic radiation. Simultaneously, the reaction medium changes from a light yellowish appearance to brown, indicating phytochemical-mediated conversion of Ag^+ into metallic Ag^0 . The comparatively defined SPR profile indicates the formation of nanoscale particles with reasonable dispersion and a moderately distributed particle size. These spectral characteristics support the dual role of leaf-derived compounds as reducing agents during nanoparticle formation and as stabilizing agents on the resulting particle surface.

Fig. 2b presents the FE-SEM morphology of the synthesized AgNPs at high magnification. The micrograph shows a large, irregular, and densely agglomerated structure with a rough, wrinkled, and porous surface. At this magnification, the primary AgNPs are not individually distinguishable, suggesting they formed secondary aggregates during sample drying. This aggregation may result from evaporation-induced particle association, interactions among surface-bound phytochemicals from *Albizia amara* leaf extract, and the high concentration of the deposited colloidal suspension. Nevertheless, the rough surface and numerous cavities indicate the presence of nanoscale substructures within the aggregate. These surface irregularities and interparticle junctions may provide accessible sites for Hg^{2+} interaction and facilitate ion-induced changes in nanoparticle coupling, thereby influencing the plasmonic absorption response. Previous studies reported SEM images of AgNPs with an average particle size of approximately 90 nm, confirming their nanoscale morphology. The micrographs provided clear evidence of particle formation and surface characteristics relevant to their filtration performance (Javed *et al.* 2026).

3.2 Selective Colorimetric Recognition of Hg^{2+}

Fig. 3a illustrates the UV–Vis response and corresponding visual changes produced after exposing the AgNP dispersion to Hg^{2+} and different competing metal ions under identical experimental conditions.

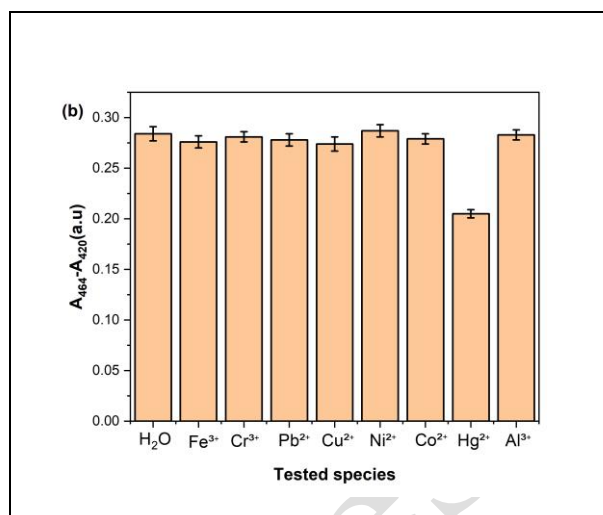
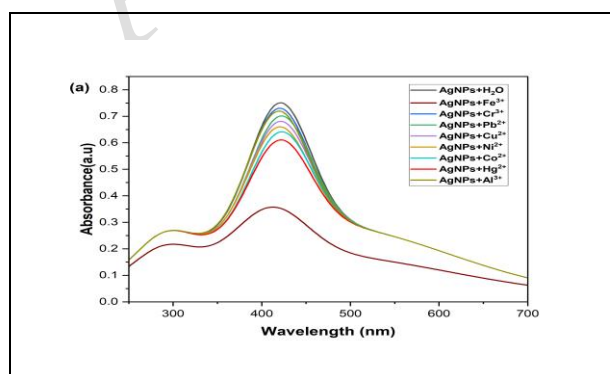


Fig. 3: (a) Hg^{2+} selectivity spectra and (b) absorbance response

The untreated AgNP dispersion shows a prominent SPR absorption band around 420 nm and a characteristic brownish-yellow color, indicating a stable colloidal system. Among the investigated ions, Hg^{2+} produces the most pronounced modification of the optical response. Exposure to Hg^{2+} substantially reduces SPR intensity and changes the absorption profile and visible solution color. In comparison, Fe^{3+} , Al^{3+} , Cr^{3+} , Ni^{2+} , Cu^{2+} , Pb^{2+} , H_2O , and Co^{2+} cause considerably smaller spectral variations under equivalent conditions, demonstrating preferential recognition of Hg^{2+} .

This distinctive response may be attributed to interactions between Hg^{2+} and the phytochemical-coated AgNP surface. Hydroxyl, carbonyl, and other oxygen-containing functionalities originating from the *A. amara* leaf extract provide potential coordination sites for metal-ion association. Hg^{2+} interaction with these surface groups can modify the interfacial environment and destabilize the original colloidal arrangement, changing interparticle interactions and consequently altering the SPR response. In addition, the strong affinity between mercury species and silver can contribute to substantial perturbation of the nanoparticle surface, making the optical response toward Hg^{2+} different from that produced by the competing ions.

Fig. 3b quantitatively compares the spectral response obtained for the investigated species using the selected absorbance variation associated with the AgNP SPR region. The blank and interfering ions show relatively small changes, whereas Hg^{2+} produces a substantially greater response. This clear separation between Hg^{2+} and the other investigated ions agrees with the spectral and visual observations in Fig. 3a. Overall, the results demonstrate the potential of phytochemical-stabilized AgNPs as a simple and selective colorimetric platform for Hg^{2+} determination in aqueous samples.

3.3 Rapid Response Evaluation

Fig. 4 shows the time-resolved UV–Vis spectral response of AgNPs after interacting with Hg^{2+} , revealing fast optical sensing behavior.

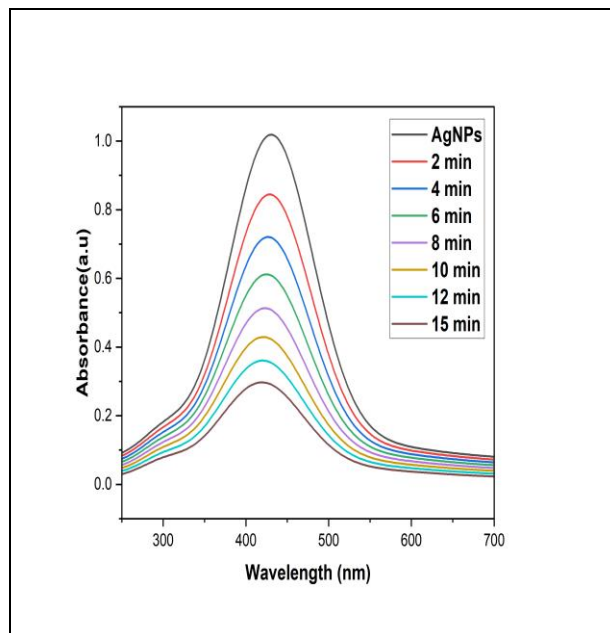


Fig. 4: Time-dependent UV–Vis response of AgNPs towards Hg^{2+} recorded at 2, 4, 6, 8, 10, 12, and 15 min

A pronounced decrease in the characteristic SPR absorption intensity occurs during the initial stage of Hg^{2+} exposure, accompanied by progressive changes in the spectral profile over 15 min. A pronounced spectral change was already evident at the first recorded time point (2 min). Therefore, 2 min was adopted as the response time for subsequent colorimetric measurements. The corresponding visual observation further shows a readily distinguishable, stable color variation, supporting rapid naked-eye recognition of Hg^{2+} . This short analytical response benefits field-based water screening and real-time environmental monitoring by reducing analysis time and requiring minimal sample processing.

3.4 Morphological and Particle-size Analysis

Fig. 5a presents the SEM morphology of the synthesized AgNP sample before Hg^{2+} treatment. At the displayed magnification, the material appears as a large, irregular, and compact aggregate with a rough, highly textured surface. Numerous smaller particulate and flake-like features are assembled throughout the structure. Therefore, this micrograph primarily represents the micron-scale organization of dried AgNP assemblies rather than clearly resolved individual nanoparticles. Such association may result from capillary forces and concentration effects occurring during deposition and solvent evaporation during SEM specimen preparation.

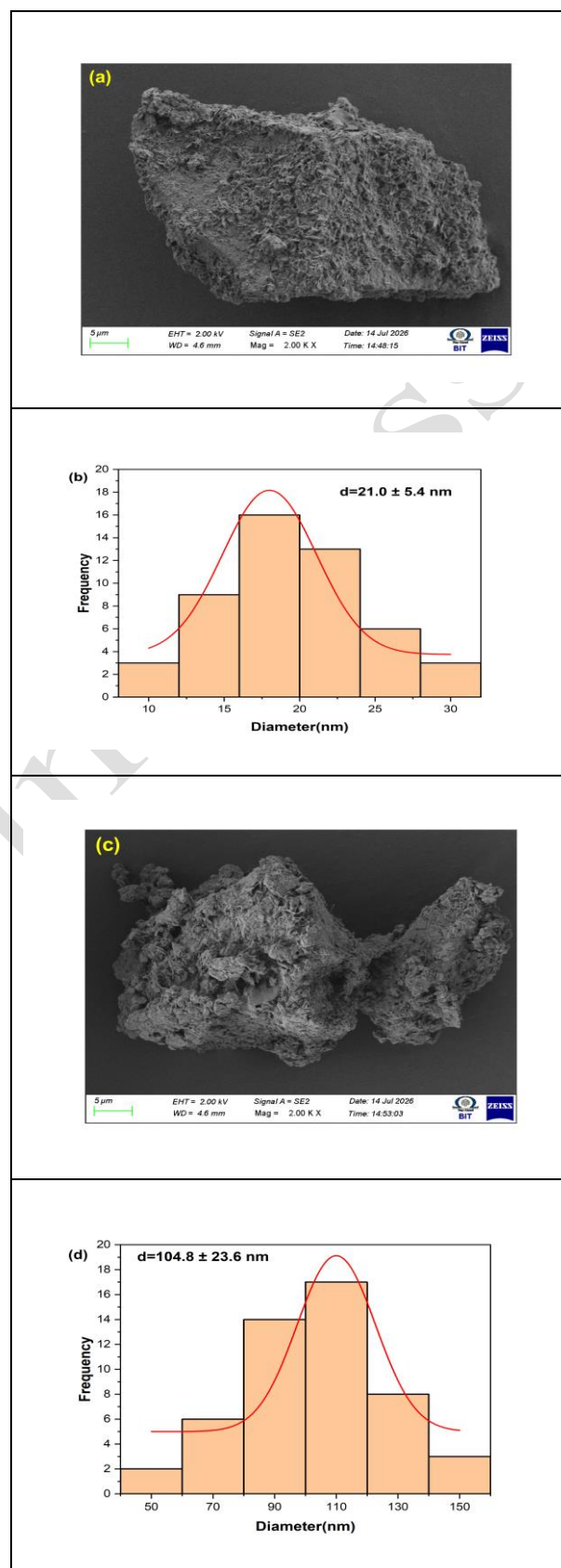


Fig. 5: AgNPs: (a) morphology before Hg^{2+} treatment; (b) size distribution; (c) morphology after Hg^{2+} treatment; and (d) aggregate distribution

The distributions in Figs. 5b and 5d represent apparent dimensions of distinguishable dried particle/aggregate domains and should not be interpreted as primary AgNP core sizes. After Hg^{2+} exposure, the associated domains showed an apparent mean diameter of 104.8 ± 23.6 nm, with most domains between approximately 80 and 130 nm.

Following Hg^{2+} treatment, Fig. 5c shows a more pronounced association of the AgNP domains, suggesting that Hg^{2+} interaction modifies the phytochemical-coated particle surface and reduces colloidal stabilization. Hg^{2+} coordination with oxygen-containing phytochemical groups, along with Ag–Hg surface interactions, decreases electrostatic repulsion and promotes closer particle association. The corresponding histogram (Fig. 5d) shows a broad apparent diameter distribution centered at 104.8 ± 23.6 nm, with most measured domains between 80 and 130 nm. This shift reflects the formation of larger associated domains rather than the growth of individual AgNP cores. The observed morphological restructuring supports Hg^{2+} -induced changes in interparticle organization that drive the accompanying SPR and colorimetric response.

Fig. 6a presents the hydrodynamic size profile of AgNPs before Hg^{2+} treatment, obtained independently by dynamic light scattering. The measured hydrodynamic diameter is expected to exceed the SEM-derived particle dimensions because DLS evaluates the solvated particle together with its surrounding phytochemical layer and any associated structures present in the colloidal medium.

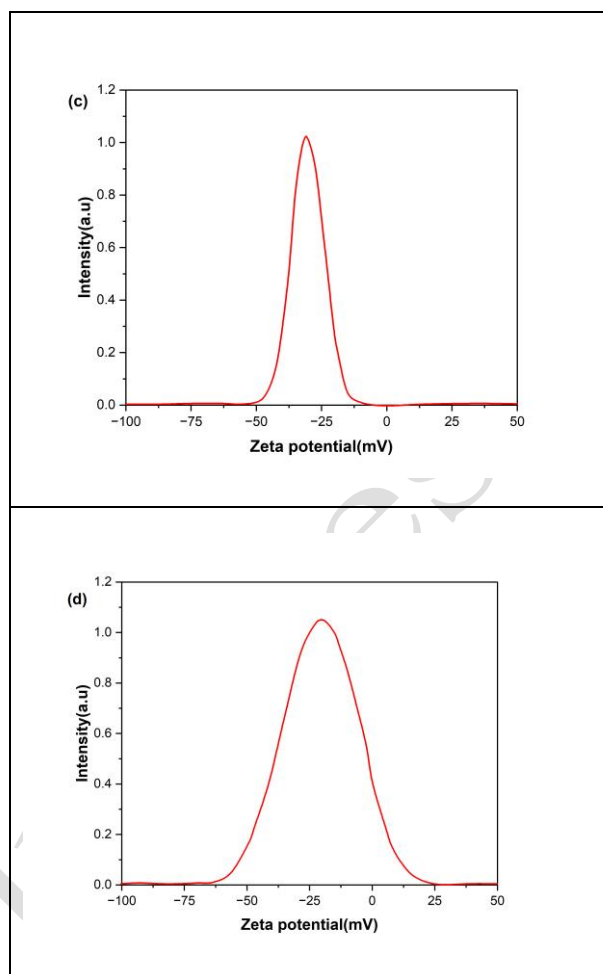
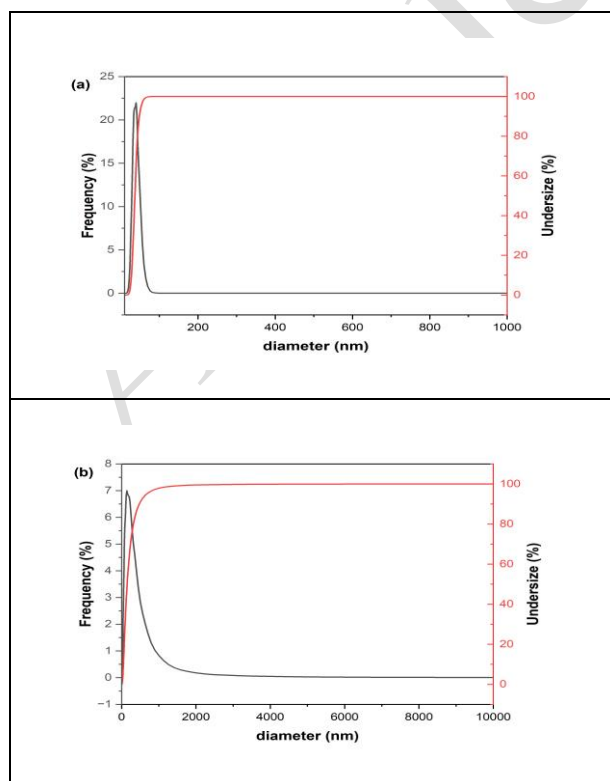


Fig. 6: (a, b) DLS distributions and (c, d) zeta potentials of AgNPs before and after Hg^{2+} treatment, respectively

Fig. 6a shows the hydrodynamic size distribution of the prepared AgNPs in aqueous suspension. A moderately distributed particle population is observed, which can be attributed to the phytochemical coating surrounding the green-synthesized nanoparticles. The DLS-derived diameter is expected to exceed the particle dimensions estimated from SEM because light-scattering measurements account for the hydrated surface layer and weakly associated particles present in solution. Following Hg^{2+} addition, the distribution shifts toward larger hydrodynamic dimensions (Fig. 6b), indicating substantial alteration of the colloidal state and increased particle association. This change agrees with the Hg^{2+} -dependent modification of the SPR profile and corresponding visual response. Prior investigations have used dynamic light scattering (DLS) to evaluate the hydrodynamic particle-size distribution and dispersion behavior of biosynthesized AgNPs in suspension. The analysis provided insight into nanoparticle uniformity, aggregation tendency, and colloidal characteristics (Heidari *et al.* 2026).

Zeta-potential measurements provide additional information on this colloidal transformation. Before Hg^{2+}

treatment (Fig. 6c), the AgNP surface exhibits a negative potential arising mainly from ionizable phytochemical groups adsorbed during green synthesis. Although electrostatic repulsion contributes to dispersion stability, steric protection from phenolic and other biomolecular constituents can further stabilize the system. After Hg^{2+} exposure (Fig. 6d), the surface potential shifts toward a less negative state, indicating a change in the nanoparticle interfacial charge. Association of Hg^{2+} with surface-active functionalities can reduce electrostatic repulsion and modify particle–particle interactions, thereby contributing to the observed changes in hydrodynamic size and plasmonic behavior. Together, the DLS and zeta-potential results support Hg^{2+} -induced restructuring of the AgNP colloidal system.

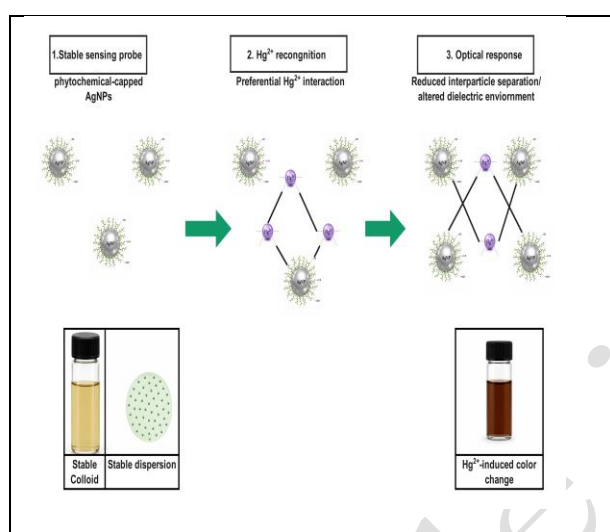


Fig. 7: Colorimetric Hg^{2+} sensing mechanism of AgNPs

Phytochemical examination of *Albizia amara* leaf extract indicates the presence of naturally occurring biomolecules capable of participating in nanoparticle formation and surface stabilization. Phenolic and flavonoid constituents containing hydroxyl, carbonyl, carboxyl, and related oxygen-bearing functionalities can contribute to Ag^+ reduction and subsequently remain associated with the AgNP surface as a stabilizing organic layer. These surface functionalities also provide chemically accessible sites capable of interacting with Hg^{2+} .

Upon Hg^{2+} introduction, interactions occur at the phytochemical-coated AgNP interface, modifying the original surface-charge environment and colloidal organization. Beyond coordinating with surface functional groups, strong interactions between mercury species and silver can perturb the AgNP surface and its electronic properties. Together, these effects alter interparticle separation and the local dielectric environment, causing measurable changes in SPR absorption and solution color. SEM observations, DLS size variation, zeta-potential changes, and UV–Vis

spectral behavior collectively support this mechanism. These observations are consistent with Hg^{2+} -induced changes in AgNP surface charge and particle association; however, they do not establish a specific molecular binding mechanism. Coordination with phytochemical functionalities and Ag–Hg interactions therefore contribute plausibly to the observed optical response. The comparatively weaker responses generated by competing ions further indicate preferential Hg^{2+} recognition under the selected experimental conditions. Thus, the phytochemical surface layer serves a dual function: maintaining AgNP stability and providing an active interface for Hg^{2+} -dependent colorimetric detection.

3.5 Analytical Performance

3.5.1 Concentration-dependent Response and Linearity

Fig. 8a illustrates the progressive UV–Vis spectral variation of AgNPs after exposure to increasing Hg^{2+} concentrations, along with the associated visual response.

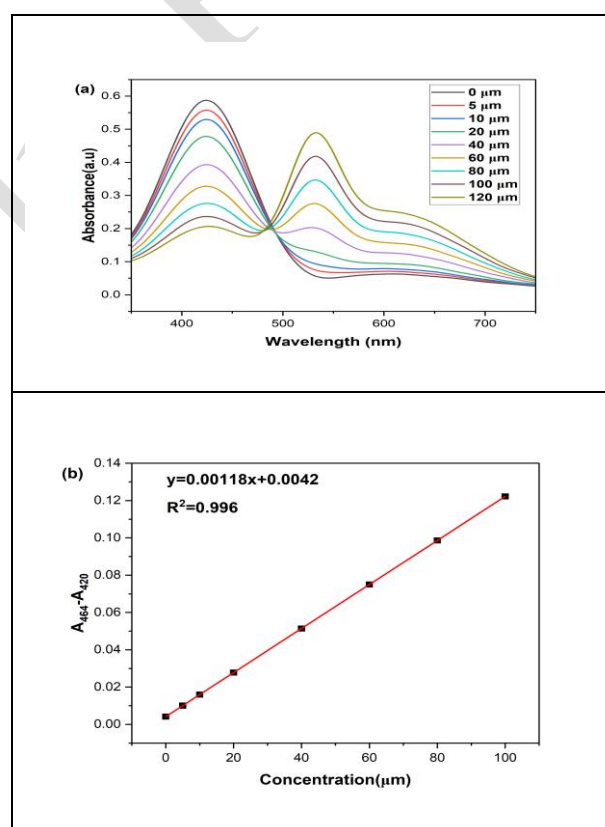


Fig. 8: (a) Hg^{2+} -dependent spectra and (b) calibration response

The untreated AgNP dispersion exhibits its characteristic SPR absorption in the visible region around 420 nm. Increasing Hg^{2+} concentration progressively modifies the intensity and shape of this band, indicating concentration-dependent perturbation of the nanoparticle surface and colloidal environment. At elevated Hg^{2+}

levels, the SPR feature shows greater attenuation and broadening, accompanied by a more pronounced change in solution color. This systematic optical evolution demonstrates that the magnitude of the AgNP response is governed by Hg^{2+} concentration and can therefore serve as the basis for quantitative determination. Previous studies have revealed that UV–Vis spectroscopy confirmed the successful formation of AgNPs through a characteristic surface plasmon resonance (SPR) absorption peak at 416 nm. This distinct absorption band indicated the development of nanoscale silver particles and their characteristic optical response (Vinayagam *et al.* 2025).

For calibration, absorbance changes at fixed wavelengths within the AgNP SPR region were selected rather than dynamically changing peak maxima. This procedure reduces uncertainty from spectral broadening and enables consistent comparisons across the investigated concentration series. Fig. 8b shows a clear concentration-dependent calibration trend between the selected absorbance response and Hg^{2+} concentration. Good linearity indicates that the optical signal varies predictably with analyte level within the working range. The corresponding regression parameters, detection limit, and quantification limit were determined from the experimental calibration data. The 5–100 μM calibration range followed $y = 0.00118x + 0.0042$ with $R^2 = 0.996$ ($n = 3$ per concentration). Using $\sigma = 0.00301$ a.u. and $S = 0.00118$ a.u. μM^{-1} , the calculated LOD and LOQ were 8.42 and 25.51 μM , respectively. Independent precision testing ($n = 5$) produced repeatability of 1.48–2.31% RSD and reproducibility of 2.86–4.06% RSD. The low detection threshold obtained from this analysis demonstrates the potential of the phytochemical-stabilized AgNP system for sensitive Hg^{2+} monitoring in aqueous media.

3.5.2 Resistance to Competing Metal Ions

Fig. 9 evaluates whether common coexisting metal ions influence the optical recognition of Hg^{2+} by the AgNP sensing system.

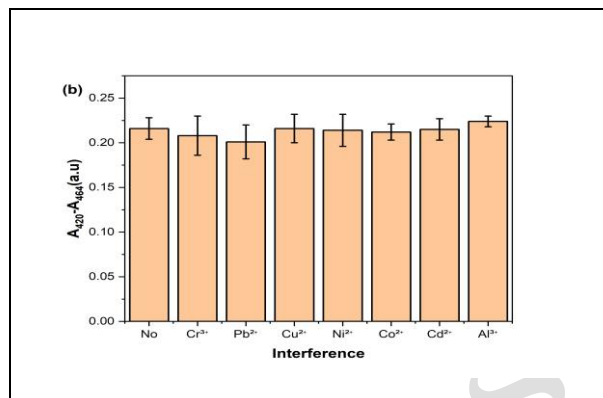
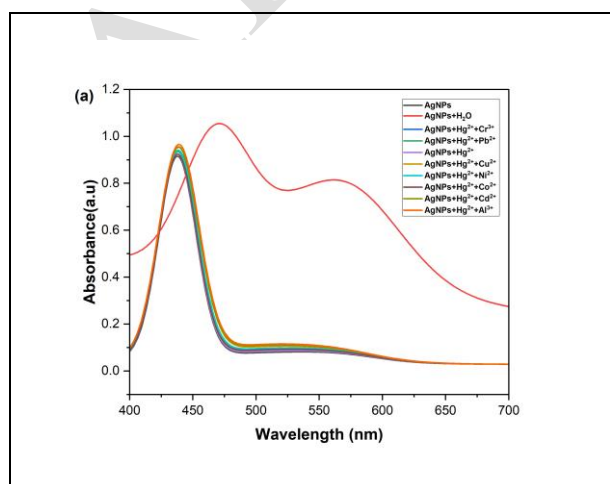


Fig. 9: (a) Hg^{2+} interference spectra in the presence of Cr^{3+} , Pb^{2+} , Cu^{2+} , Ni^{2+} , Cr^{3+} , Cd^{2+} , Al^{3+} , and Co^{2+} and (b) corresponding ΔA response comparison

As shown in Fig. 9a, addition of Cr^{3+} , Pb^{2+} , Cu^{2+} , Ni^{2+} , Cd^{2+} , Al^{3+} , and Co^{2+} causes only a limited modification of the characteristic Hg^{2+} -dependent spectral response. The principal SPR variation associated with Hg^{2+} remains evident under competitive conditions, suggesting that the presence of these foreign ions does not substantially suppress the sensing process. Although competing species can weakly interact with phytochemical functionalities surrounding the AgNP surface, their influence is insufficient to produce an optical perturbation comparable to that of Hg^{2+} . Several reports have indicated that the surface plasmon resonance (SPR) analysis was used to optimize AgNP formation by monitoring the characteristic optical response of the nanoparticles. An AgNO_3 concentration of 1.94 mM, 9.6 mL plant extract, and 43 min reaction time produced an optimum SPR response of 1.94 (Kefale and Tesfaye, 2026).

The quantitative response (Fig. 9b) further demonstrates relatively small variations after addition of the competing ions compared with the AgNP– Hg^{2+} system alone. Limited error-bar variation indicates satisfactory measurement consistency across replicate experiments. These findings demonstrate that the characteristic optical response towards Hg^{2+} can be maintained in multicomponent ionic environments and support the anti-interference capability of the proposed sensing platform.

3.6 Repeatability and Reproducibility

Precision of the AgNP-based colorimetric method was assessed through repeatability and reproducibility measurements to evaluate the consistency of Hg^{2+} determination. Repeatability was determined from five consecutive measurements at each selected Hg^{2+} concentration under identical experimental conditions, whereas reproducibility was evaluated using five independently prepared measurements from separate analytical runs.

Table 1. Precision of AgNP-based Hg²⁺ detection from five replicate measurements (n = 5)

Hg ²⁺ Concentration (μM)	Repeatability (%RSD)	Reproducibility (%RSD)
40	2.31	4.06
60	2.08	3.72
80	1.84	3.39
100	1.62	3.08
120	1.48	2.86

Repeatability ranged from 1.48 to 2.31%, while reproducibility ranged from 2.86 to 4.06%. Both parameters showed progressively lower %RSD values as Hg²⁺ concentration increased, indicating improved measurement precision at higher analyte levels. This behavior can be attributed to the stronger concentration-dependent optical response and improved signal-to-noise ratio of the AgNP sensing system. Repeatability remained below 2.5% across all investigated concentrations, demonstrating good within-run precision, whereas reproducibility remained below 4.1%, indicating satisfactory consistency between independent analytical runs. These outcomes demonstrate the AgNP-based colorimetric platform's reliable precision for quantitative Hg²⁺ determination.

3.7 Accuracy and Spike-recovery Assessment

Analytical accuracy of the AgNP-based colorimetric method for Hg²⁺ determination was assessed by spike-recovery experiments using different tap-water samples. Initial Hg²⁺ concentrations were independently determined by atomic absorption analysis before fortification. The native Hg²⁺ content was either below the detection limit or present only at trace levels, thereby minimizing its contribution to the recovery experiment.

Table 2. Hg²⁺ spike-recovery analysis in tap water

Sample	Initial Hg ²⁺ by Reference Method (μM)	Added Hg ²⁺ (μM)	Total Hg ²⁺ by AgNP Method (μM)	Recovery (%)
1	ND	40.00	37.72	94.30
2	ND	40.00	38.84	97.10
3	0.12	40.00	38.62	96.25
4	0.09	40.00	40.17	100.20
5	0.15	40.00	40.93	101.95

Following Hg²⁺ fortification, the AgNP sensing system produced recovery values ranging from 94.30 to

101.95%. The relatively narrow recovery interval indicates satisfactory agreement between the introduced and experimentally estimated Hg²⁺ concentrations. Values approaching 100% further suggest that common constituents of the tap-water matrix exert limited influence on the colorimetric response. Small positive or negative deviations may arise from variations in ionic strength, interactions between dissolved species and the phytochemical surface layer, or minor differences in nanoparticle dispersion. Overall, the recovery behavior indicates good analytical accuracy and supports the potential application of the AgNP sensing platform for Hg²⁺ determination in aqueous samples. As reported in previous studies, cold vapor atomic absorption spectrometry (CV-AAS) was used to sensitively determine Hg²⁺ in water samples after extraction. The method achieved recoveries above 95%, with an LOD of 0.06 μg/L, LOQ of 0.20 μg/L, and a linear range of 0.05–10 μg/L (Dawood *et al.* 2026).

3.8 Hg²⁺ Determination in Environmental Water Samples

The applicability of the developed sensing platform was further examined using different environmental water matrices, including river water, tap water, drinking water, pond water, and domestic wastewater. These samples represent matrices with different levels of dissolved ions, suspended constituents, and organic components, providing a broader assessment of sensing performance under realistic aqueous conditions.

Table 3. Hg²⁺ determination in environmental waters

Water Sample	Hg ²⁺ by Reference Method (μM)	Hg ²⁺ by AgNP Method (μM)	Agreement (%)
Tap water	0.184	0.176	95.65
River water	0.263	0.274	104.18
Pond water	0.317	0.329	103.79
Drinking water	ND	ND	-
Domestic wastewater	0.286	0.301	105.24

The concentrations estimated using the AgNP method show close agreement with the reference measurements for samples containing detectable Hg²⁺. Agreement values of 95.65–105.24% indicate comparatively small differences between the two analytical approaches. The slightly lower response obtained for tap water may originate from competition between dissolved inorganic species and Hg²⁺ at surface-

active phytochemical sites. Conversely, the modest positive deviations observed for river, pond, and wastewater samples may result from differences in ionic strength and interactions involving naturally occurring dissolved constituents, which can modify the AgNP surface environment and optical response. Similarly, tap and river water samples were used to evaluate the practical applicability of Hg²⁺ detection under real environmental conditions. The sensing system showed reliable performance in both matrices, achieving Hg²⁺ recoveries above 85% (Assad *et al.* 2026).

Despite differences in sample composition, the analytical response showed no substantial loss across the examined matrices. The agreement between the colorimetric and reference measurements therefore supports the feasibility of the AgNP sensing platform for rapid Hg²⁺ screening in different water matrices. Nevertheless, appropriate calibration and matrix validation remain necessary when analyzing highly complex environmental samples.

4. CONCLUSION

A plant-mediated colorimetric sensing platform based on phytochemical-stabilized AgNPs was successfully developed for rapid and selective Hg²⁺ detection. UV-Vis and FE-SEM analyses confirmed AgNP formation and showed that interactions of Hg²⁺ with the AgNPs altered the nanoparticle surface environment and colloidal organization, generating concentration-dependent changes in the SPR response. Following Hg²⁺ treatment, the associated particle domains exhibited an apparent mean diameter of 104.8 ± 23.6 nm, with most domains occurring between 80 and 130 nm, supporting Hg²⁺-induced particle association rather than the growth of individual AgNP cores. DLS and zeta-potential measurements further confirmed changes in hydrodynamic size and surface-charge characteristics after Hg²⁺ exposure.

The method exhibited good analytical precision, with repeatability values of 1.48–2.31% RSD and reproducibility values of 2.86–4.06% RSD. Repeatability remained below 2.5%, while reproducibility remained below 4.1% throughout the examined concentration range. Competitive-ion experiments demonstrated preferential Hg²⁺ recognition. Spike-recovery values ranged from 94.30 to 101.95%, confirming satisfactory accuracy, while environmental water samples showed 95.65–105.24% agreement with the reference method. Collectively, these results demonstrate that *Albizia amara*-mediated AgNPs provide a simple, precise, and rapid colorimetric platform for Hg²⁺ determination under the laboratory conditions investigated. The plant-mediated synthesis using *A. amara* leaf extract provides a green nanotechnology approach to AgNP preparation and supports their application in environmental monitoring of Hg²⁺ in aqueous matrices. The present

applicability is limited to laboratory testing of the examined tap water, river water, pond water, drinking water, and domestic wastewater matrices; broader validation across additional matrices, concentration ranges, and field conditions is required before routine environmental deployment.

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CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest.

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