AB Journals

African Journal of Environment and Natural Science Research

ISSN 2689-9434 • Volume 9, Issue 4, 2026 • pp. 1–17

RESEARCH ARTICLE Received: 10 Jul 2026, Accepted: 4 Aug 2026, Published: 3 Sep 2026

Spectrophotometric Determination of Trace Heavy Metals in Groundwater with Novel Chelating Reagents

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Abstract

For quantification of trace heavy metals in groundwater samples employing a new set of chelating agents obtained through Schiff bases and azo compounds . Heavy metals such as lead (Pb^{2+}), cadmium (Cd^{2+}), nickel (Ni^{2+}), chromium (Cr^{3+}), and copper (Cu^{2+}) were examined in this study, and complex formation properties of these heavy metals with synthesized ligands have been analyzed under optimized parameters such as pH, metal-ligand ratio, and absorbance wavelength (λmax).

Physicochemical properties of 35 ground water samples collected at chosen sampling sites were determined. Parameters such as pH, electrical conductivity (EC), total dissolved solids (TDS) and total hardness of the sample were considered along with the heavy metals. All spectrophotometric studies were carried out with the help of a UV-VIS Spectrophotometer.

It was observed that stable and highly colored metal–ligand complexes were obtained, with maximum molar absorptivity and distinct absorption maxima corresponding to each metal ion. The new method proved to be linear ($R^2 > 0.997$), with low detection limits and good recovery values (97.6 to 99.5%) with acceptable precision. It was observed that copper possessed the highest stability constant, whereas cadmium had the least stable complex.

Use of the method on actual groundwater samples showed the presence of trace amounts of other heavy metals. Correlation analysis demonstrated positive relationships between electrical conductivity (EC), total dissolved solids (TDS), and the concentrations of lead (Pb), cadmium (Cd), nickel (Ni), chromium (Cr), and copper (Cu), indicating that higher EC and TDS values were associated with increased heavy metal concentrations in the groundwater samples.

Keywords: Groundwater; Heavy metals; Metal–ligand complexes; Spectrophotometry; Trace metal determination; Water quality analysis.

How to Cite: Israa Nibras Qader (2026), Spectrophotometric Determination of Trace Heavy Metals in Groundwater with Novel Chelating Reagents. African Journal of Environment and Natural Science Research 9(4), 1-17. DOI: 10.52589/AJENSR-RUZ9IGHN

INTRODUCTION

Groundwater is one of the most important resources of freshwater for many domestic, agricultural, and industrial uses, especially in arid and semi-arid areas (Bouchaou et al., 2024). Because of growing anthropogenic activities, including the discharge from industries, run-off water from agriculture, and urban development, there is continuous contamination of groundwater with various pollutants, including trace heavy metals(Alemu, 2025). Such metals are environmentally hazardous due to their toxic properties and persistence as well as due to their potential for accumulation in living matter in tiny amounts(Edo et al., 2024).

Examples of heavy metals include lead (Pb^{2+}), cadmium (Cd^{2+}), nickel (Ni^{2+}), chromium (Cr^{3+}), and copper (Cu^{2+}), which may be present in groundwater due to natural or anthropogenic activity (Dahdah et al., 2025). These metals, once released in the aquatic medium, can go through a variety of chemical reactions, which will depend on conditions like pH, ionic strength, organic matter, and minerals present in the groundwater aquifer (Gantayat & Elumalai, 2024). Long-term exposure to high amounts of heavy metals in the water can cause a number of serious diseases, including nervous system disorders, kidney damage, cancer, and metabolic disruptions(Fatima et al., 2026).

Thus, precise, selective, and fast measurement of heavy metal ions present in groundwater is necessary in order to protect the environment and the public from any harm (Vinay et al., 2026). Several methods have been used to detect heavy metal ions in groundwater samples, such as atomic absorption spectroscopy (AAS), inductively coupled plasma mass spectrometry (ICP-MS), and electrochemical techniques (Bacon et al., 2024). Although these are precise, they are also very costly and time-consuming (Ahmed et al., 2024).

In this scenario, there are spectrophotometric approaches that provide a relatively inexpensive and easy technique for the determination of trace metals, especially when used along with selective chromogenic reagents(BK et al., 2026). Schiff base and azo complexes have been extensively studied as they possess strong chelating properties , relative ease of preparation, and chromogenicity(Uddin et al., 2025). The purpose of this research was to characterize new ligands before use. Ligands can form stable complexes with transition and heavy metals;, hence, their quantification using UV-Visible spectrophotometry (Omar et al., 2025).

This study introduces an innovative spectrophotometric technique for the detection of heavy metals at trace levels in groundwater samples, using newly synthesized chelating agents made of Schiff base and azo groups. Other important features of the study include analysis of physical and chemical properties of the groundwater samples, metal ion coordination characteristics in varied experimental conditions, and analysis performance of the method.

In addition, this research intends to use the above-described technique on actual groundwater samples to analyze how contaminated the water is by heavy metals, as well as whether there is any relationship between metal concentrations and water parameters. This suggested technique should offer an effective means of analyzing the state of water samples.

METHODOLOGY

Study Area and Sample Collection

Total number of 35 groundwater samples were collected from seven sampling stations situated in the study area. These sampling stations have been chosen to include diverse groundwater conditions and sources of contamination like agricultural, residential and others. Samples were collected from March to May, 2025. Water samples were collected from different wells using pre-cleaned 1-liter polyethylene bottles which were properly cleaned with 10% nitric acid and rinsed with deionized water. Collected water samples were immediately brought into the laboratory where they were stored in the refrigerator at 4°C until further analysis. For determining heavy metals, a part of the sample was acidified with concentrated nitric acid to keep the pH of sample less than 2.

Chemicals and Reagents

Pure analytical reagents were used in all experiments. Stock standard solutions of **lead (Pb^{2+}), cadmium (Cd^{2+}), nickel (Ni^{2+}), chromium (Cr^{3+}), and copper (Cu^{2+})** were prepared from corresponding metal salts solutions with concentration of **1000 mg/L**. Schiff base and azo group based novel chelating ligands were synthesized via organic chemistry methods known from literature (Zhao et al., 2020). Prepared chelating ligands were purified and then characterized with the help of Fourier Transform Infrared (FTIR) and UV–Vis Spectroscopy techniques in order to confirm the presence of required functional groups suitable for coordination reactions with metal ions. Reaction solvents like ethanol, methanol and dimethylformamide (DMF) were used in some of the cases, whereas buffer solutions with different pH were also prepared for further optimization of coordination reaction conditions. Synthesized chelating ligands were used for selective complex formation and detection of **lead (Pb^{2+}), cadmium (Cd^{2+}), nickel (Ni^{2+}), chromium (Cr^{3+}), and copper (Cu^{2+})** ions with spectrophotometry method.

Instrumentation

The spectrophotometric experiments were conducted using a UV/Vis spectrophotometer fitted with 1 cm path length quartz cells under optimum conditions for analysis (Asfaram et al., 2016). The absorbance values obtained were corrected against suitable reagent blanks to ensure that any interference caused by solvents, reagents, and the matrix was minimized.

Preparation of Standard Solutions

Standard solutions of each metallic ion were prepared through proper dilution of the stock solutions so that the concentration varied between 0.1 to 10 mg/L. The solutions of chelating agent were prepared at approximately 1×10^{-3} M concentration and kept in amber colored bottles (Hubicki & Kołodyńska, 2012).

Complex Formation Studies

Aliquots of solutions containing various metal ions were mixed with the prepared chelating agents under controlled laboratory conditions. Various parameters such as pH, time of reaction, temperature, and mole ratios of metal ion to ligand were optimized for the formation of complexes. Absorption spectra were then obtained from 200 to 800 nm to determine the λmax of all metal-ligand complexes.

Determination of Stoichiometry and Stability Constants

Job's Method and Mole Ratio Method were employed to ascertain the stoichiometry of the complexes. The stability constants (Kst) of the complexes were determined from the spectrophotometric results obtained using equations in coordination chemistry (Elsherif et al., 2020).

Calibration and Quantitative Analysis

The calibration graphs were established by plotting absorbance values against the concentration of metal ions under optimized condition of experiment. Linear regression analysis was used to obtain the calibration equations and correlation coefficients (R^2)(Pourebrahim et al., 2017). The proposed technique was used to measure trace amounts of heavy metals in groundwaters.

Evaluation of Analytical Performance

Method validation was done using the International Council for Harmonization (ICH) Q2 (R1) guidelines. The validation was carried out with respect to linearity, accuracy, precision, LOD, and LOQ, thereby establishing the validity of the method.

Possible interferences by some commonly existing groundwater ions such as Ca^{2+}, Mg^{2+}, Na^{+}, K^{+}, Cl^{-}, and SO_4^{2-} were tested using the optimized experimental protocol. Each of these ions was tested at concentrations as high as 100 times that of the analyte concentration to test for the possible presence of any matrix effect. However, no interference was observed, as deviations in absorbance were less than 5%.

Physicochemical Analysis of Groundwater

Standard analysis techniques for the determination of physicochemical parameters such as pH, electrical conductivity (EC), total dissolved solids (TDS), and total hardness have been utilized in order to study their impact on the behavior of metal ions and their complexes.

Statistical Analysis

The data analyses were done using Statistical Package for Social Science (SPSS) software version 26.0. Normal distribution of the data was determined by the Shapiro-Wilk test, and parametric statistics were then used. The outcomes were reported as mean ± SD, whereas 95% confidence intervals were computed where necessary. Differences in means were determined using one-way analysis of variance (ANOVA), while physicochemical parameter-metal relationship was analyzed using Pearson's correlation coefficient test. $P < 0.05$ was considered significant.

RESULTS

Physicochemical Characteristics of Groundwater Samples

Physicochemical parameters for the various groundwater samples are presented in Table 1. The groundwater samples were found to have neutral to alkaline conditions and considerable variation in their electrical conductivity, total dissolved solids, and hardness.

Table 1: Physicochemical characteristics of groundwater samples

Parameter	Minimum	Maximum	Mean ± SD
pH	6.8	8.1	7.42 ± 0.34
EC (µS/cm)	720	1650	1125 ± 265
TDS (mg/L)	480	1080	735 ± 145
Total Hardness (mg/L)	210	520	356 ± 82

According to the analysis from Table 1, there were variations in the quality of groundwater across different sample sites. However, the values of pH maintained a suitable range for the formation of metal complexes. Higher values of EC and TDS imply higher degrees of mineralization and the presence of more dissolved ions. Total hardness could be attributed to variations in rock formations in different regions. This distribution is shown in Figure 1.

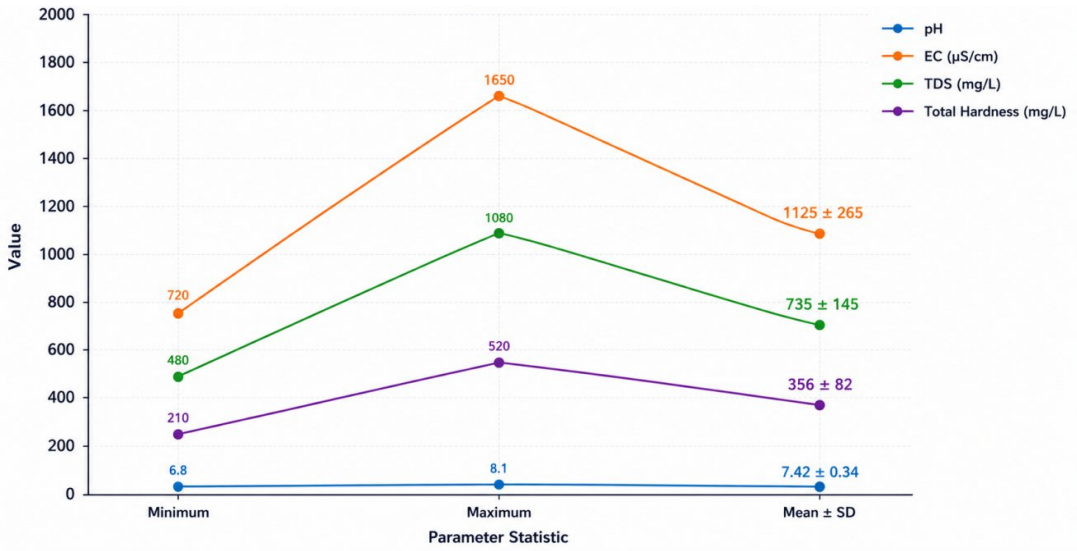

Figure 1: Distribution of pH, EC, TDS, and total hardness values in groundwater samples.

Spectral Characteristics of the Synthesized Chelating Reagents

The synthesized Schiff bases and azo compounds were spectroscopically characterized before using them for heavy metal determination. Different absorption peaks were recorded, suggesting the involvement of different active chromophores that would be involved in complex formation reactions.

Table 2: Spectral properties of synthesized ligands

Ligand	λmax (nm)	Absorbance
Schiff Base (L1)	342	0.824
Azo Ligand (L2)	386	0.911

It can be observed from Table 2 below that the prepared ligands have very good absorption properties in the UV-visible wavelength range. It was found that the absorbance of the azo ligand was higher than the Schiff base ligand, showing better chromophoric behavior.

Optimization of pH for Metal Complex Formation

The effect of pH on metal-ligand complex formation was studied to establish the best possible conditions to ensure maximum sensitivity.

Table 3: Optimum pH values for metal complex formation

Metal Ion	Optimum pH
Pb^{2+}	6.5
Cd^{2+}	6.0
Ni^{2+}	7.0
Cr^{3+}	6.5
Cu^{2+}	7.5

As seen from Table 3, the optimum pH ranges between 6.0 and 7.5, which depended on the particular metal ion used. In acidic solution, the presence of hydrogen ions inhibited the efficiency of metal ion binding, while in an alkaline medium, some of the metals underwent hydrolysis reactions. Hence, neutral pH resulted in the maximum absorption readings.

Stoichiometric Composition of Metal Complexes

The composition of the synthesized complexes was determined using Job's method and the mole-ratio method.

Table 4: Stoichiometric ratios of metal complexes

Metal Ion	Metal-: Ligand Ratio
Pb^{2+}	1-: 2
Cd^{2+}	1-: 2
Ni^{2+}	1-: 2
Cr^{3+}	1 : 3
Cu^{2+}	1-: 2

From the data presented in Table 4, most metal ions had complexes with two ligands. However, chromium exhibited a unique coordination behavior, coordinating with three ligands to form a complex with a 1:3 stoichiometry.

Spectral and Stability Parameters of Metal Complexes

The spectral properties and the thermodynamic stability of the metal complexes formed are shown in Table 5 below.

Table 5: Spectral and stability characteristics of metal complexes

Metal Ion	λmax (nm)	Molar Absorptivity (×10⁴ L mol⁻¹ cm⁻¹)	Stability Constant (×10⁵)
Pb^{2+}	462	3.92	5.41
Cd^{2+}	448	3.15	4.62
Ni^{2+}	510	4.36	6.12
Cr^{3+}	538	4.81	7.25
Cu^{2+}	486	5.12	8.43

These findings clearly show significant variations in the stability of the complex compounds with regard to the metals studied. The compound with the highest stability constants is that formed by copper. In addition, cadmium was found to form the least stable complex, while chromium and nickel were highly stable and can be used for spectrophotometry. Comparison data are shown in Figure 2.

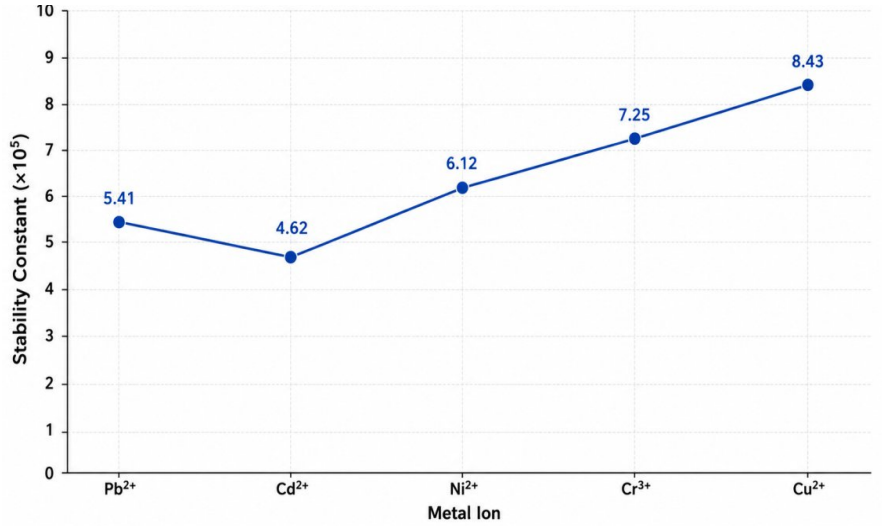

Figure 2: Comparison of molar absorptivity and stability constants of metal complexes.

Calibration Characteristics and Linearity

Calibration experiments were conducted under optimized experimental conditions for the evaluation of the quantitative behavior of the analytical method.

Table 6: Calibration equations and correlation coefficients

Metal Ion	Regression Equation	R^2
Pb^{2+}	A = 0.098C + 0.004	0.9987
Cd^{2+}	A = 0.086C + 0.003	0.9979

Ni^{2+}	A = 0.112C + 0.005	0.9991
Cr^{3+}	A = 0.119C + 0.006	0.9985
Cu^{2+}	A = 0.127C + 0.004	0.9994

Linearity of Table 6 is very good across the examined concentration range. The correlation coefficient values were higher than 0.997 in all cases, indicating the high reliability of the Beer-Lambert equation and proving the suitability of the suggested analytical method. Graphical representations are illustrated in Figure 3.

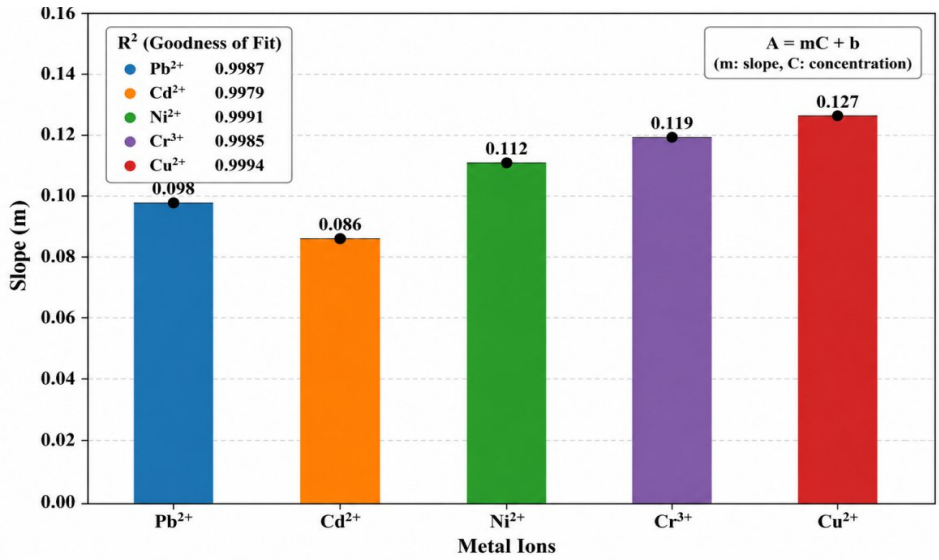

Figure 3: Calibration curves of heavy metal ions under optimized conditios.

Analytical Validation of the Proposed Method

The analytical performance parameters are summarized in Table 7.

Table 7: Validation parameters of the developed method

Metal Ion	LOD (mg/L)	LOQ (mg/L)	Recovery (%)	RSD (%)
Pb^{2+}	0.021	0.070	98.4	2.3
Cd^{2+}	0.018	0.060	97.6	2.8
Ni^{2+}	0.015	0.050	99.1	1.9
Cr^{3+}	0.017	0.056	98.8	2.1
Cu^{2+}	0.012	0.040	99.5	1.7

Analytical performance can be assessed from the validation study. High sensitivity is represented by the low limits of detection, while recovery rates around 100% guarantee high accuracy. Moreover, the very low RSD values obtained (<3%) suggest that good precision was achieved for the proposed analytical technique.

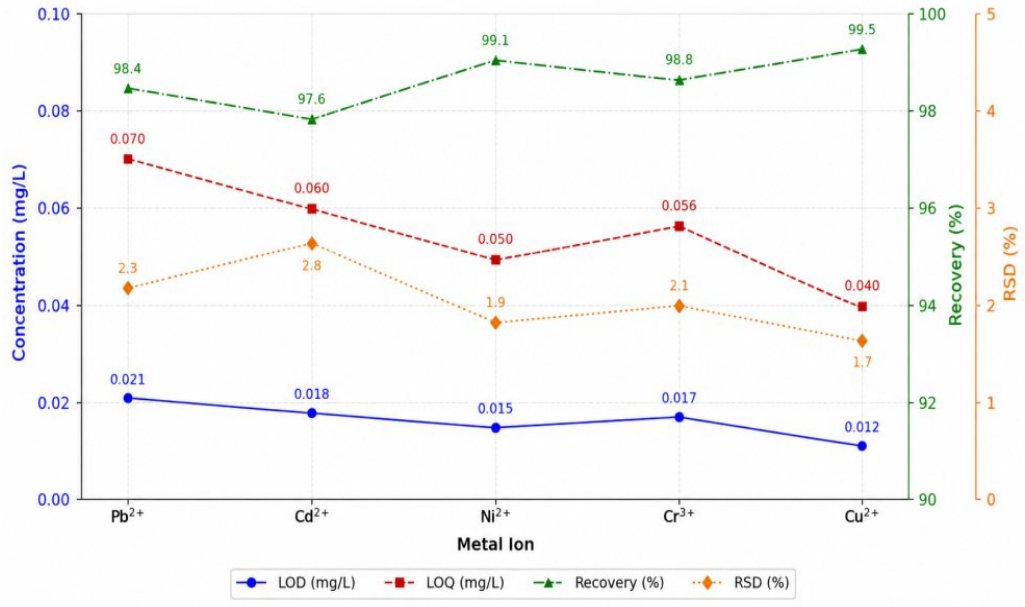

Figure 4 : Analytical Figures of Merit for Pb^{2+}, Cd^{2+}, Ni^{2+}, Cr^{3+}, and Cu^{2+} Determination

Heavy Metal Concentrations in Groundwater Samples

The spectrophotometric method developed was used for the determination of trace heavy metals in groundwater samples.

Table 8: Heavy metal concentrations in groundwater samples

Metal Ion	Range (mg/L)	Mean ± SD (mg/L)	WHO Guideline (mg/L)*
Pb^{2+}	0.008–0.042	0.024 ± 0.009	0.01
Cd^{2+}	0.002–0.011	0.006 ± 0.003	0.003
Ni^{2+}	0.015–0.067	0.036 ± 0.014	0.07
Cr^{3+}	0.011–0.053	0.029 ± 0.010	0.05
Cu^{2+}	0.009–0.058	0.031 ± 0.012	2.0

Nickel and copper proved to be the most abundant metals present in groundwater, while cadmium was found in the lowest levels. The varying metal concentrations may have resulted from various factors such as geological make-up, human actions, and groundwater flow mechanisms. Graphical presentation of the metal concentrations is depicted in Figure 7.

Analysis showed that most heavy metals were still within allowable limits as compared to WHO's drinking water guidelines. Despite this, however, some samples of Ni^{2+} and Cu^{2+} were found to be close to the guideline limits, indicating a greater geological and/or anthropogenic impact on the site. Though an exceedance did not occur immediately, this could imply a future problem.

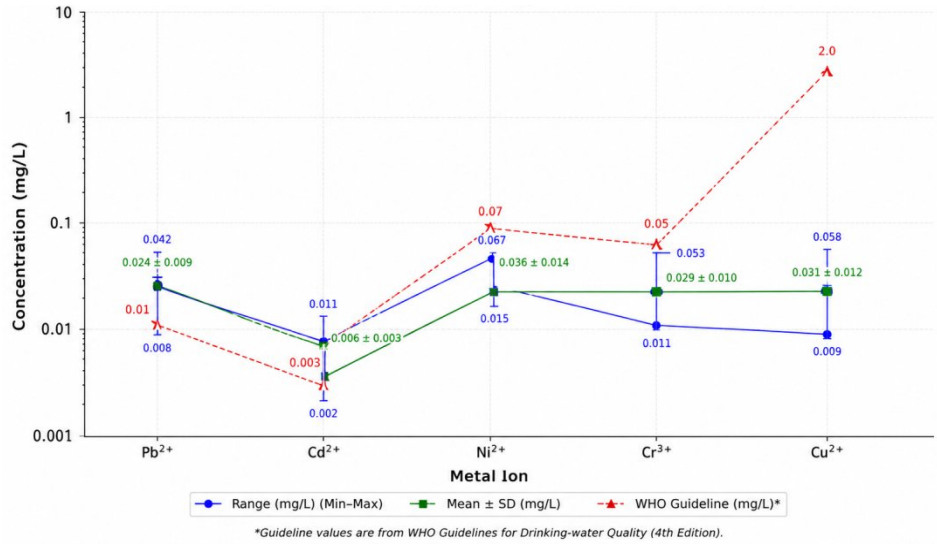

Figure 5: Mean concentrations of heavy metals detected in groundwater samples.

Correlation Analysis

Pearson correlation analysis was done to determine any relationships among physicochemical parameters and metal concentrations in groundwater .

Table 9: Pearson correlation coefficients between physicochemical parameters and heavy metal concentrations

Parameter	Pb^{2+}	Cd^{2+}	Ni^{2+}	Cr^{3+}	Cu^{2+}
pH	-0.42	-0.35	-0.48	-0.29	-0.45
EC	+0.73*	+0.52*	+0.78*	+0.61*	+0.75*
TDS	+0.69*	+0.49*	+0.74*	+0.57*	+0.71*
Total Hardness	+0.55*	+0.43	+0.63*	+0.48*	+0.60*

* Significant at $P < 0.05$.

From the correlation matrix, there were very high positive correlations among EC, TDS, and most heavy metals. The highest positive correlation coefficient value was found between EC and nickel (r=0.78), implying that ionic concentration is vital in controlling the concentrations of the metals in the groundwater. On the other hand, pH had low negative correlations due to decreased metal mobility in slightly alkaline conditions. Correlation matrices are depicted in Fig. 8.

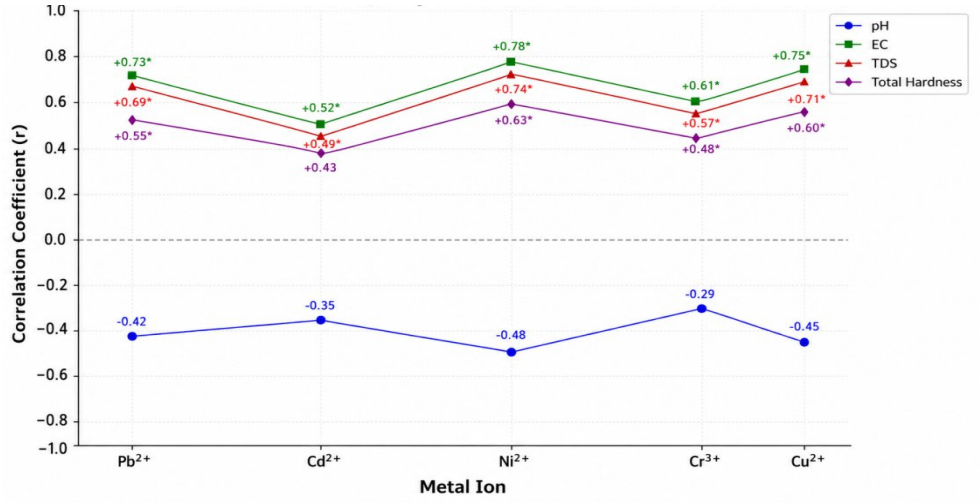

Figure 6: Heatmap showing correlations between groundwater quality parameters and heavy metal concentrations

DISCUSSION

Physicochemical Characteristics of Groundwater

The physicochemical data showed that the groundwater in the region studied was mostly neutral to slightly alkaline, which is characteristic of water in groundwater systems affected by carbonate and silicate mineral weathering(Bakshe et al., 2024). The differences in EC and TDS values between the samples imply heterogeneity in the hydrogeological environment and the extent of water-rock interaction(Akhtar et al., 2025). High levels of EC and TDS in some areas suggest more dissolution of mineral salts or introduction by anthropogenic sources such as farm drainage and human waste(Boci et al., 2024). Total hardness also indicates geologic formations rich in Ca++ and Mg++, greatly impacting groundwater quality (Davraz & Aksever, 2025).

Formation and Stability of Metal–Ligand Complexes

Successful synthesis of colored complexes through complexation of the Schiff base and azo ligands with metal ions proves that the Schiff bases are potent chelators(Elbadawy et al., 2025). The existence of donor atoms like nitrogen and oxygen increases electron donation to the metal center, hence making the coordination complexes more stable(Aziz et al., 2025). The differences in stability constants of various metal ions arise from differences in ion charge densities and electronic configuration (Chen et al., 2024).

The stability constant of copper was found to be the highest among all the metal ions studied, and this could be due to a number of factors that influence the coordination chemistry of copper(Kubicek et al., 2018). These factors include the ligand field stabilization energy, high covalency between Cu and N/O, and the Jahn–Teller effect, which causes further stabilization due to the energy reduction (Ali et al., 2026). These effects contribute towards enhancing the stability of metal-ligand complex formation through Cu^{2+}(Riaz et al., 2025).

Effect of pH on Complex Formation

These results support the coordination chemistry concepts(Bernhardt & Lawrance, 2025). In the case of low pH, the protonation of the donor atoms of the ligands reduces the ability of those atoms to coordinate with metal ions, therefore, reducing absorption(Boguta & Sokołowska, 2020). With high pH, hydrolysis of metal ions interferes with the binding of the ligand, hence reducing absorption(Song et al., 2019).

An optimum pH range (6.0-7.5) has been achieved through a compromise between the degree of deprotonation of the ligand and stability of metal ions in solution(Wang et al., 2020). It is also an environmental pH range, hence its suitability for the determination of trace metal content in natural water samples (Morel et al., 2025).

Stoichiometry of Metal Complexes

The predominance of a 1:2 molar ratio metal: ligand among the studied ions suggests that the two ligands surround each metal ion. Hence, the formation of a chelate ring structure is possible(Elshewemy et al., 2026). This is common for Schiff bases and azo ligands with several donor atoms (Kahraman et al., 2025).

The anomaly seen with chromium, where it has a ratio of 1:3, could be explained by its high coordination ability because of its small ionic radius and hence higher charge density(Dang et al., 2023). As such, chromium can coordinate more ligands than other metal ions (Bala et al., 2020).

Analytical Performance of the Proposed Method

The developed spectrophotometric method exhibited excellent sensitivity, which is confirmed by the low detection limits and high molar absorptivities (Dong et al., 2024). The good linearity ($R^2 > 0.997$) proves the feasibility of applying Beer's law at the considered concentrations (Pradhan et al., 2025).

The high recovery values (97.6% to 99.5%) show the accuracy and freedom from any systematic errors in the method(Yu et al., 2025). Low RSD values are evidence of precision and repeatability(Kotani et al., 2024). Analytical properties of the proposed method can be used for the routine analysis of trace heavy metal content in water (Zhang, 2024).

Heavy Metal Distribution in Groundwater

Heavy metals in detected concentrations were observed to exhibit significant spatial differences from sampling point to point(Liu et al., 2022). Nickel and copper were observed to exist in relatively higher quantities than other metals, and this could be due to geological sources as well as human-related factors like discharges from industries and fertilizers (Mishra & De, 2024).

Cadmium demonstrated the lowest concentration, implying minimal sources of contamination or efficient geochemical sequestration of the pollutant in the aquifer(Fan et al., 2026). Despite most concentrations being within the acceptable range, the detection of concentrations of such hazardous metals raises health concerns, should contamination continue unabated (Abdelmonem et al., 2025).

Correlation Between Water Quality Parameters and Metals

While the positive correlations among EC, TDS, and concentrations of metals indicate a link between ions and the presence of metals, it is worth noting that there is no causal relationship established in this study(Shakeri et al., 2025).

Of all the correlations, the strongest positive correlation was obtained between EC and the concentration of Nickel ion (Ni^{2+}) (r=0.78) (Bouziane et al., 2026).

Method Evaluation and Environmental Applicability

Generally speaking, the suggested spectrophotometric technique employing Schiff base and azo-chelating agents appears to be a cheap, easy, and sensitive tool for metal analysis(Alahmady et al., 2026). In contrast to traditional instrumental approaches, the proposed method does not need complicated equipment and can be successfully applied in laboratory or field environmental analysis (Durmishi et al., 2025).

The capability to form such complexes, along with being highly selective and having low detection limits, ensures that there is a real possibility for applying these reagents in practical terms for groundwater quality testing (Suqi et al., 2024).

Overall Interpretation

The combination of physicochemical parameters, spectrophotometric determination, and statistics helps understand the state of groundwater pollution (Debassi et al., 2025). This research proves that it is possible to detect low-level heavy metal pollution by applying the proposed methodology and thus makes it helpful for environmental monitoring purposes (Sharafi & Salehi, 2025).

CONCLUSION

A simple and sensitive spectrophotometric technique was devised using Schiff base and azo chelating reagents for estimating trace heavy metal ions from groundwater samples. The developed analytical technique showed good linear response, low detection limits, excellent recoveries, and acceptable precision for the determination of Pb^{2+}, Cd^{2+}, Ni^{2+}, Cr^{3+}, and Cu^{2+}.

The experiments confirmed that metal-ligand complexation occurred effectively with optimized pH values, displaying a 1:2 stoichiometric relationship mostly. The application of the experiment in real groundwater samples found the presence of heavy metals; however, nickel and copper exhibited comparatively higher concentrations than other tested ions.

In summary, the suggested methodology offers an efficient and inexpensive means for detecting heavy metals in the environment and may potentially be used as an alternative technique for such applications

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