



(REVIEW ARTICLE)



AI-DRIVEN OPTIMIZATION OF A SMARTPHONE-BASED COLORIMETRIC METHOD FOR SIMULTANEOUS DETERMINATION OF MULTIPLE HEAVY METAL IONS IN ENVIRONMENTAL WATER OF BABIL GOVERNORATE, IRAQ

Abbas Adnan Kadhim *

Babylon Directorate of Education, Ministry of Education of Iraq.

* Corresponding Author

ORCID Details

Abbas Adnan: <https://orcid.org/0009-0004-2830-8987>

GSC Advanced Research and Reviews, 2026, 29(01), 130–152

Publication history: Received on 01 September 2026; revised on 08 October 2026; accepted on 10 October 2026

Article DOI: <https://doi.org/10.30574/gscarr.2026.29.1.0267>

Copyright © 2026 Author(s) retain the copyright of this article. This article is published under the terms of the Creative Commons Attribution License 4.0

ABSTRACT

Copper, lead, cadmium, mercury, chromium, zinc and nickel reach rivers, irrigation canals and groundwater from industrial effluents, agricultural drainage, urban wastewater and corroding distribution pipes, and several of them are harmful at microgram-per-litre levels. Their routine determination still depends on atomic absorption and inductively coupled plasma instruments, which are expensive, need trained operators and are rarely available near the sampling site. This research plan describes a low-cost method in which a six-reagent chromogenic array (4-(2-pyridylazo)resorcinol, Zincon, micellar dithizone, 1,5-diphenylcarbazide, dimethylglyoxime and xylenol orange) is prepared in a 96-well microplate and photographed with a smartphone inside a closed enclosure with controlled LED lighting. Color values will be extracted from every well in the RGB, HSV, HSL and CIELAB spaces after correction against a reference card. Reaction pH, reagent concentration, reaction time and sample volume will be screened with a Box-Behnken design and refined by Gaussian-process Bayesian optimization. Multiple linear regression, partial least squares, random forest, support vector regression and artificial neural network models will be trained on synthetic multi-metal mixtures to predict the seven concentrations at the same time, and they will be compared by nested cross-validation and an external test set using the root mean square error, mean absolute error and coefficient of determination. The selected procedure will be validated following ICH Q2(R2) and Eurachem guidance for linearity, detection and quantification limits, precision, recovery, selectivity, interference and robustness, and then applied to surface, well and tap water from Babil Governorate, Republic of Iraq, with ICP-MS as the reference method. The expected result is a portable procedure that screens seven metals in one run of about 25 minutes, at a small fraction of the cost of instrumental analysis.

Keywords: Heavy metal ions; Smartphone colorimetry; Digital image analysis; Colorimetric sensor array; Machine learning; Chemometrics; Bayesian optimization; Environmental water.

1 INTRODUCTION

1.1 Background of the Study

Monitoring of water quality depends on how quickly and how cheaply a measurement can be made close to the water body. For heavy metals the gap between what monitoring needs and what laboratories can deliver is still wide. Standard methods rely on atomic spectrometry and a trained operator [1,2], so a sample taken from a remote canal may reach the laboratory days after collection. Colorimetry is one of the oldest instrumental techniques in chemistry, and its combination with the camera and processor of a smartphone has produced a family of portable methods over the past fifteen years [3–5]. This plan builds on that work and adds machine learning, so that several metal concentrations can be read from a single set of color measurements.

1.2 Heavy Metal Contamination in Environmental Water

Heavy metal contamination has been reported on all continents in surface and ground water. Finally, from data gathered from 1972 to 2017 and compiled in a river and lake database, it was seen that, in many instances, the mean concentrations of Cd, Cr, Pb, Ni, etc., surpassed drinking-water guidelines set for these contaminants [6]. Unlike organic substances, metals do not degrade, but cycle between the water, suspended particles and sediment and are released again when the pH or redox changes in the water [7,8]. Urban wastewater, agricultural drainage, and industrial discharges are discharged into Tigris and Euphrates and their canals, and the water quality of Iraqi river has been evaluated several times using water quality indices [9]. The concentrations of heavy metals in the tap water have also been assessed in the Iraqi cities, for example in Ramadi [10]. These reports indicate a presence of data, but often sampling is limited in spatial and temporal extent, due to the cost of each sample. The actual samples utilized in the present study are provided from the farmland and towns of the four districts of Babil Governorate: Hilla, Al-Mahawil, Al-Hashimiya and Al-Musayyib, located in the central part of Iraq, from which the Euphrates branches flow, represented as the Shatt Al-Hilla, and are extended to the land of those districts via a dense network of irrigation canals and drains.

1.3 Sources of Heavy Metals in Aquatic Environments

Natural inputs come from the weathering of rocks and soils, volcanic activity and atmospheric deposition of dust. Human activity adds much larger and more localized loads. Electroplating and metal finishing release Cr, Ni, Cd, Zn and Cu; leather tanning is a major source of chromium; battery manufacture and recycling release Pb, Cd and Ni; phosphate fertilizers carry cadmium; copper-based fungicides and algicides add Cu; and chlor-alkali plants, coal combustion and small-scale gold mining emit mercury [8,11]. In drinking water, corrosion of lead pipes, brass fittings, solder and galvanized steel adds Pb, Cu and Zn at the tap, so a treated supply can still deliver metal to the consumer [12].

1.4 Environmental and Public Health Concerns Associated with Heavy Metals

Lead is known to impact the developing nervous system, there is no known threshold without effect, cadmium is accumulated in the kidney and bone, inorganic mercury is known to impact the kidney, methylmercury formed in sediments impacts the nervous system, and hexavalent chromium, when inhaled, is a human carcinogen and is genotoxic [13,14]. Copper and zinc are essential elements, but can also be toxic when present in too high a concentration, producing gastrointestinal and hepatic effects; both are much more toxic to fish and invertebrates than to humans [7].

1.5 Importance of Trace Determination of Heavy Metal Ions

The World Health Organization guideline values lie between a few micrograms and two milligrams per litre: $3 \mu\text{g L}^{-1}$ for cadmium, $6 \mu\text{g L}^{-1}$ for inorganic mercury, $10 \mu\text{g L}^{-1}$ for lead, $50 \mu\text{g L}^{-1}$ for total chromium, $70 \mu\text{g L}^{-1}$ for nickel and 2mg L^{-1} for copper [12]. A useful field method must therefore quantify some metals near the microgram-per-litre range and others over three orders of magnitude. The chemical form also matters. Cr(VI) is far more toxic and mobile than Cr(III) [15], so a method that reports only total chromium can misjudge the risk.

1.6 Challenges in Conventional Determination of Heavy Metals

Flame and graphite-furnace atomic absorption spectrometry, ICP optical emission spectrometry and ICP mass spectrometry give accurate results down to ng L^{-1} levels [2,16], but the instruments cost from tens to hundreds of thousands of US dollars, consume argon or acetylene, and require clean laboratories and trained staff. Samples have to be filtered, acidified to pH below 2 and transported cold [1], and results often return after days or weeks. Classical spectrophotometric methods are cheaper, yet most of them measure one metal at a time and several still use solvent extraction, for example dithizone in chloroform or carbon tetrachloride [17].

1.7 Development of Rapid and Portable Analytical Methods

Portable alternatives include test strips, anodic stripping voltammetry, paper-based microfluidic devices and nanoparticle probes. Paper devices with colorimetric or electrochemical detection have determined Cu, Ni, Fe, Cr, Pb and Cd in small sample volumes [18,19], and gold-nanoparticle assays read with a phone have reached $\mu\text{g L}^{-1}$ levels for Hg(II) [20]. Many of these systems target one analyte, depend on reagents with limited shelf life, or lose selectivity in real waters that contain calcium, magnesium, iron and dissolved organic matter.

1.8 Smartphone-Based Analytical Chemistry

The concept of a smart phone involves putting the CMOS image sensor, the light source, the processor and wireless communication in a pocket-sized device. Digital image colorimetry began with webcams and scanners [21] and has evolved to phones as colorimeters and as fluorimeters and readers of paper devices [3,5,22]. Color changes due to automatic exposure, white balance, tone curves and JPEG compression, which are designed to create attractive photographs, will vary across different phone models [4,23]. These must be controlled by a quantitative camera, camera settings and reference targets.

1.9 Artificial Intelligence in Modern Analytical Chemistry

Single channel calibration curves performed less well than conversion of smartphone colour data by machine learning for the pH classes, levels of water quality, and concentrations of Pb(II) [24–26]. Arrays of dyes with different spectra but reaction to the same target that are read by pattern recognition have been used to identify and quantify multiple analytes within a single array [27,28] and arrays of dyes with reaction to multiple heavy metals have been discriminated using a cell phone [29,30]. With fewer experiments, Bayesian optimization has also been demonstrated to identify good reaction conditions than human reaction plans [31]. There is a very limited research that combines all these aspects into a single procedure that systematically evaluates the chemistry, the different algorithms and color spaces, and quantifies several metals in real environment water, versus a reference method. The aim of this work is summarized in figure 1.

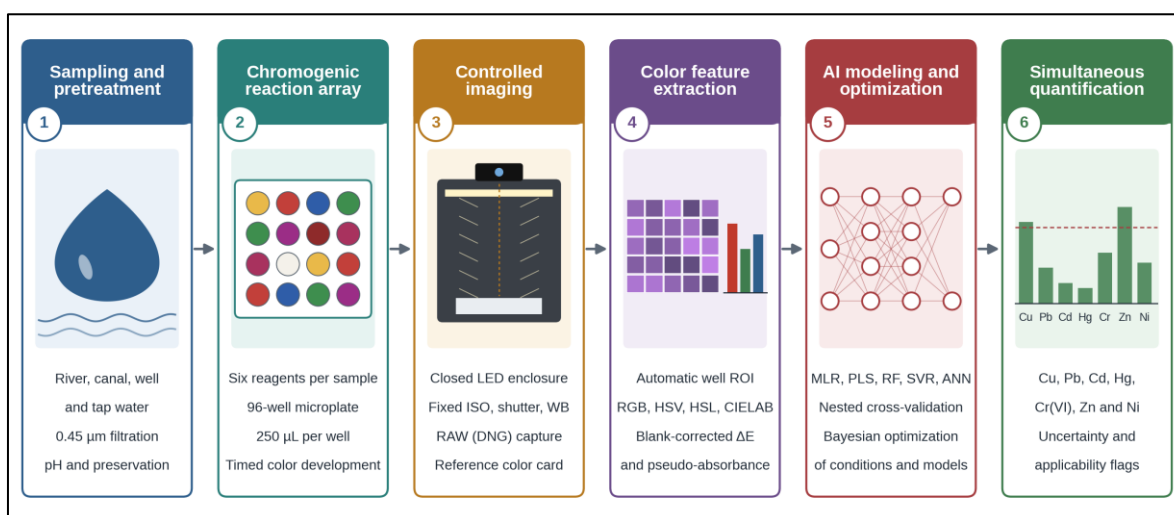


Figure 1: Overall workflow of the proposed AI-assisted smartphone colorimetric method for the simultaneous determination of seven heavy metal ions.

1.10 Research Problem

Laboratories that monitor surface and groundwater in Babil Governorate and elsewhere in Iraq need a fast, inexpensive way to screen several heavy metals at the sampling site. Existing smartphone methods usually measure one metal, use uncontrolled lighting and fit a single color channel to a straight line, so they fail when the reagent responds to more than one metal or when the matrix changes. The problem addressed here is how to design, optimize and validate a smartphone colorimetric procedure that quantifies Cu(II), Pb(II), Cd(II), Hg(II), Cr(VI), Zn(II) and Ni(II) in one run with accuracy acceptable for screening.

1.11 Research Questions

- RQ1. Can a six-reagent array imaged under controlled light give color patterns distinct enough to separate seven metals in mixtures?
- RQ2. Which color space and which derived features carry the most information for quantification?

- RQ3. Which algorithm (MLR, PLS, RF, SVR or ANN) predicts the seven concentrations with the lowest error on unseen samples?
- RQ4. Does Bayesian optimization reach better reaction conditions, or the same conditions with fewer experiments, than a Box–Behnken design?
- RQ5. Do results for real water samples from Babil Governorate agree with ICP-MS within limits acceptable for screening?

1.12 General Objective

To develop, optimize with AI tools and validate a smartphone-based colorimetric method for the simultaneous determination of seven heavy metal ions in environmental water.

1.13 Specific Objectives

- Select and optimize a chromogenic reagent array and its reaction conditions (pH, reagent concentration, time, volume and additives).
- Build an imaging enclosure and a standard acquisition and color-extraction procedure with reference correction.
- Compare five regression algorithms and four color spaces for multi-metal prediction.
- Validate the method for linearity, LOD, LOQ, accuracy, precision, recovery, selectivity, interference and robustness.
- Apply the method to surface, well and tap water from Babil Governorate (Republic of Iraq) and compare the results with ICP-MS.

1.14 Research Hypotheses

- H1. Multivariate models built on the full array outperform single-channel univariate calibrations for every metal (lower RMSE, $p < 0.05$).
- H2. Nonlinear models (RF, SVR, ANN) predict mixture concentrations more accurately than linear models (MLR, PLS).
- H3. Hue-based and CIELAB features are less sensitive to illumination drift than raw RGB intensities.
- H4. Concentrations predicted for the Babil samples do not differ significantly from ICP-MS values (paired t-test, $p > 0.05$).

1.15 Significance of the Study

The method would give water authorities, environmental laboratories and universities a screening tool that costs little, uses microlitre volumes and can be operated in the field. It would also add evidence on how chemistry optimization and model selection should be combined in smartphone-based analysis.

1.16 Scope of the Study

The work covers dissolved Cu(II), Pb(II), Cd(II), inorganic Hg(II), Cr(VI), Zn(II) and Ni(II) in fresh water. Total chromium, organomercury, particulate metals and saline water are outside the main scope. One mid-range Android phone will be used for development and a second model for transfer tests. Real samples will come from rivers, irrigation canals and drains, wells and household taps across the four districts of Babil Governorate, Republic of Iraq, collected in one sampling campaign within a one-month project.

2 HEAVY METAL IONS IN ENVIRONMENTAL WATER

"Heavy metal" has been defined by density (above about 5 g cm^{-3}), atomic mass, atomic number and toxicity, and an IUPAC report concluded that the term has no consistent chemical meaning [32]. It remains in wide use in environmental science for metals and metalloids that are persistent and toxic at low concentrations. In this plan the term refers to the seven target elements. Chemically they can be grouped as borderline Lewis acids (Cu^{2+} , Zn^{2+} , Ni^{2+} , Pb^{2+}), soft acids (Cd^{2+} , Hg^{2+}), and chromium, which exists in natural water mainly as Cr(III) cations or Cr(VI) oxyanions. This grouping guides the choice of ligands in Section 4.

2.1 Essential and Non-Essential Heavy Metals

Copper and zinc are essential and a deficiency or excess is deleterious, both are cofactors of enzymes including cytochrome c oxidase, superoxide dismutase and carbonic anhydrase. A role for nickel in human biology has not yet been defined, but this element is required by some plants and microorganisms [35]. There is no known function for

lead, cadmium and mercury each is toxic at measureable concentrations. Cr(VI) is known to be toxic, with its nutritional effect previously described and now put into question.

2.2 Copper Ions

In oxic fresh water copper is present as Cu^{2+} , hydroxo and carbonato complexes and, to a large extent, complexes with dissolved organic matter. Main sources are pipe corrosion, copper sulfate used as an algicide, fungicides and mining. The WHO guideline of 2 mg L^{-1} protects against acute gastrointestinal effects [12]; chronic excess affects the liver, particularly in people with disorders of copper metabolism [33].

2.3 Lead Ions

Lead is present as Pb^{2+} , PbOH^+ and carbonate species and is strongly adsorbed on particles, so dissolved concentrations in rivers are usually low while tap water may contain much more where lead pipes or solder remain. Lead is a cumulative neurotoxicant, children being most sensitive, and the WHO value of $10 \text{ } \mu\text{g L}^{-1}$ is provisional because it is set by practical treatment and analytical limits [12,13].

2.4 Cadmium Ions

At neutral and slightly acidic pH, Cd^{2+} is mobile; CdCl^+ is mobile in Cl-rich waters. It can get in to water sources from phosphate fertilizers, zinc ores, Ni–Cd batteries and electroplating. It is known to be retained in the kidney for decades and responsible for renal tubular damage and bone loss [36]. The WHO guideline is $3 \text{ } \mu\text{g L}^{-1}$ [12].

2.5 Mercury Ions

Dissolved inorganic mercury is present mostly as Hg(II) chloro and hydroxo complexes. Methylmercury is formed by bacteria in anoxic sediments and biomagnified in fish. Sources include gold mining, waste incineration, coal combustion and chlor-alkali plants. Inorganic mercury affects the kidney, whereas action of methylmercury affects the developing brain [37]. The suggested method is to determine inorganic Hg(II).

2.6 Chromium Ions

Chromium exists as Cr(III), which hydrolyses and adsorbs strongly, and as Cr(VI), present as CrO_4^{2-} and HCrO_4^- , which is soluble, mobile and a strong oxidant. Tanneries, electroplating, pigment production and wood preservation are the main sources [15]. Cr(VI) is carcinogenic and genotoxic [14]. This plan measures Cr(VI) directly with 1,5-diphenylcarbazide; total chromium can be obtained after oxidation of Cr(III) as an optional extension.

2.7 Zinc and Nickel Ions

The main form of zinc is Zn^{2+} ; its source to water is mainly from galvanized pipes, degradation of tyres, mining and fertilizers. It is not very toxic to human but is toxic to fish, and imparts an astringent taste to water at levels of 4 mg L^{-1} and above [12,34]. Nickel is in the form of Ni^{2+} and associated with carbonate and organic ligands. Origin is from stainless steel, alloys, plating and fittings; causes contact allergy and some nickel compounds are cancer-causing by inhalation [35].

2.8 Chemical Behavior of Heavy Metal Ions in Water

Along with the free ion, the ion is present in several different forms. The processes of hydrolysis, complexation with carbonate, chloride, sulfate and organic ligands, adsorption on Fe/Mn oxides and clay and precipitation are pH, redox potential and ionic strength dependent [38]. This for a colorimetric method implies that only the part liberated by the reaction conditions will be responsive. Samples will, therefore, be acidified and buffered in controlled fashion, with the addition of calcium, magnesium and humic substances to the synthetic matrix for “learning” by the models.

2.9 Toxicological and Environmental Effects

All toxic metals exert their effects primarily by binding to thiol groups of proteins, removing positive metals from enzymes and inducing the formation of reactive oxygen species, while the toxic effects of Cr(VI) are also the result of its penetration via anion channels and its reduction in the cells to reactive intermediates which interact with DNA [14]. Copper and zinc have the effect of causing gill failure in fish; cadmium and mercury become stored in their tissues in aquatic ecosystems.

2.10 Maximum Permissible Concentrations in Water

The low and high limits of the target working ranges are called the drinking-water limits and are listed in Table 1. In this study, Iraqi practice according to the national standard IQS 417, which is also used in Iraqi tap water assessment [10] will be applied for the real samples.

Table 1: Target metals, main dissolved forms, principal sources and drinking-water limits (mg L⁻¹).

Metal	Main dissolved forms	Principal sources	WHO (2022)	US EPA	EU 2020/2184
Cu	Cu ²⁺ , CuCO ₃ , Cu–DOM	Pipes, algacides, mining	2	1.3 (AL)	2.0
Pb	Pb ²⁺ , PbOH ⁺ , PbCO ₃	Pipes and solder, batteries	0.01 (P)	0.015 (AL)	0.010
Cd	Cd ²⁺ , CdCl ⁺	Fertilizers, plating, batteries	0.003	0.005	0.005
Hg	Hg(OH) ₂ , HgCl ₂	Chlor-alkali, coal, gold mining	0.006*	0.002*	0.001
Cr(VI)	CrO ₄ ²⁻ , HCrO ₄ ⁻	Tanning, plating, pigments	0.05 (P)**	0.1**	0.050**
Zn	Zn ²⁺ , ZnCO ₃	Galvanized pipes, tyres	NHB	5 (S)	–
Ni	Ni ²⁺ , NiCO ₃	Alloys, plating, fittings	0.07	–	0.020

AL, action level; P, provisional; S, secondary (aesthetic) standard; NHB, no health-based value (taste threshold about 4 mg L⁻¹); * inorganic mercury; ** total chromium; DOM, dissolved organic matter. Sources: [12,39,40].

3 COLORIMETRIC DETERMINATION OF HEAVY METALS

3.1 Principles of Colorimetric Analysis

Colorimetry measures the attenuation of visible light by a colored species. For a solution in a cell of path length b , the absorbance at a wavelength λ follows the Beer–Lambert law [16]:

$$A_{\lambda} = \log_{10}(I_0/I) = \varepsilon_{\lambda} b c \quad (1)$$

where I_0 and I are the incident and transmitted intensities, ε_{λ} is the molar absorption coefficient and c the molar concentration. The eye and the camera perceive the complementary color of the absorbed band: a complex absorbing near 500 nm appears red, and one absorbing near 600 nm appears blue.

3.2 Chemical Reactions Between Metal Ions and Colorimetric Reagents

Most chromogenic reagents are chelating ligands with N, O or S donor atoms. Following the hard–soft acid–base principle, soft ions such as Hg²⁺ and Cd²⁺ bind sulfur donors (dithizone), while borderline ions such as Cu²⁺, Ni²⁺, Zn²⁺ and Pb²⁺ bind heterocyclic nitrogen and phenolate oxygen (PAR, Zincon, xylenol orange) [17]. Chromium(VI) reacts differently: in acid it oxidizes 1,5-diphenylcarbazide to diphenylcarbazone, and the Cr(III) formed is chelated by the oxidized ligand to give a red-violet complex measured at 540 nm [1]. Nickel reacts with dimethylglyoxime in ammoniacal medium in the presence of an oxidant to form a soluble red-brown complex [17].

3.3 Formation of Colored Metal Complexes

The extent of complex formation depends on the conditional formation constant, which changes with pH because the ligand is protonated at low pH and the metal hydrolyses at high pH. The reaction time depends on ligand exchange kinetics; Ni(II) and Cr(III) complexes form slowly compared with those of Cu(II) and Zn(II). Ligands insoluble in water, such as dithizone, can be kept in solution with surfactant micelles, which avoids chloroform extraction and increases sensitivity: a micellar dithizone method for Hg(II) reported a molar absorption coefficient of $5.02 \times 10^4 \text{ L mol}^{-1} \text{ cm}^{-1}$ and a detection limit of $1 \mu\text{g L}^{-1}$ [41]. Zincon forms blue complexes with Zn(II) and Cu(II) with an optimum near pH 9 [42].

3.4 Absorption and Color Characteristics

Complexation usually shifts the ligand absorption band to longer wavelengths and increases its intensity. PAR changes from yellow (about 415 nm) to red complexes absorbing near 490–520 nm; Zincon changes from orange-red to blue (about 600–620 nm); dithizone changes from green to orange with Hg(II). Absorption bands are broad (full width 80–120 nm), so complexes of different metals with the same reagent overlap strongly. A camera samples the spectrum through three broad filters, roughly 400–500 nm (B), 500–600 nm (G) and 580–700 nm (R) [23], which is why several reagents are needed to separate the metals.

3.5 Conventional Spectrophotometric Determination

In the conventional approach a single metal is complexed under selective conditions and the absorbance is read at the band maximum against a reagent blank, with a calibration line prepared from standards. Standard methods include diphenylcarbazide for Cr(VI), and dithizone extraction for Pb, Cd, Hg and Zn [1,17].

3.6 Limitations of Conventional Spectrophotometry

With ϵ values of 10^4 – 10^5 L mol⁻¹ cm⁻¹ and a 1 cm cell, detection limits are typically 5–100 $\mu\text{g L}^{-1}$, which is not low enough for Cd, Pb and Hg without preconcentration. Many of the classical procedures require chlorinated solvents, each of the determinations requires a reagent and a pH calibration, the bench spectrophotometer is still a laboratory instrument.

3.7 Digital Colorimetry

Digital colorimetry is a technology that substitutes the photodetector by the image sensor. Inside a region of interest the mean intensity in a channel is converted to an absorbance-like signal by comparison with a blank [21, 4]:

$$A_k = -\log_{10}(I_{k,\text{sample}}/I_{k,\text{blank}}), \quad k = R, G, B \quad (2)$$

An image captures many wells at once, so tens of reactions can be measured in one exposure, and hue, saturation and color-difference parameters can be derived from the same data.

3.8 Advantages of Colorimetric Methods

Colorimetric reactions are inexpensive, need no power beyond lighting, and can be read by eye for a first check. In microplate format each well needs about 250 μL , so reagent use and waste are small.

3.9 Simultaneous Determination of Multiple Metal Ions

Classical routes to multi-component analysis include derivative spectrophotometry and multi-wavelength calibration with chemometrics. With imaging, the natural route is a sensor array: several reagents that respond differently to each metal generate a pattern, and a multivariate model converts the pattern into concentrations [27,28]. An array of pyridylazo dyes on paper has discriminated eight heavy metal ions [29]. Figure 2 shows the response pattern expected for the six-reagent array proposed here.

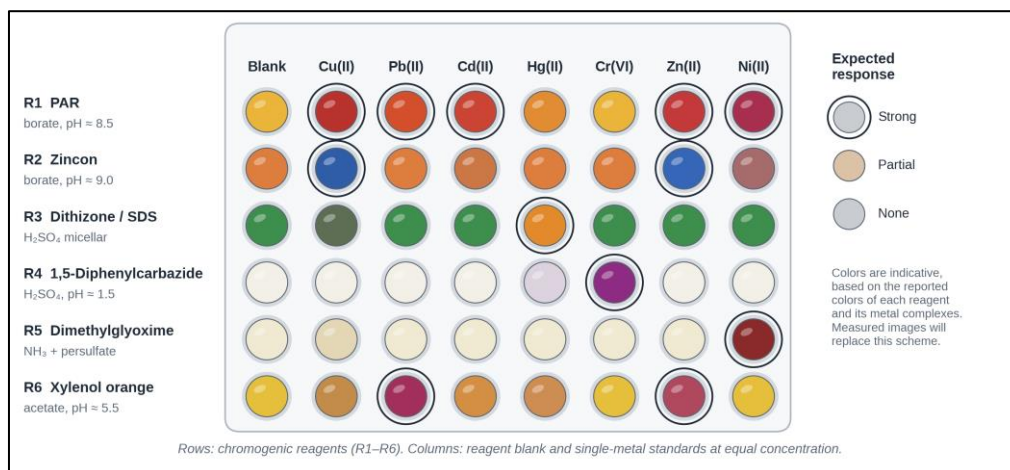


Figure 2: Expected color-response pattern of the six-reagent array (schematic). Colors are indicative, based on the reported colors of the free reagents and their metal complexes; bold rings mark strong responses.

3.10 Selectivity and Interference Problems

Fe(III), Co(II), Mn(II) and Al(III) react with PAR, Zincon and xylenol orange; Mo(VI) and V(V) give a weak color with diphenylcarbazine; high Hg(II) also produces a faint color in the same reagent [1,17]. Calcium and magnesium compete with target ions for ligands at high pH, and humic substances both complex metals and add background color. Interference will be handled at three levels: pH selection and masking (fluoride for Fe(III) and Al(III)), measurement of a sample blank without reagent to correct natural color, and inclusion of interfering ions in part of the training set so that the models learn their effect.

4 SMARTPHONE-BASED ANALYTICAL CHEMISTRY

4.1 Development of Smartphone-Based Analytical Methods

The use of consumer imaging devices in quantitative analysis grew from webcam-based titrations [21] to phone attachments that measure transmitted light through test tubes [20] and to phones reading paper devices and microplates [3]. Reviews list applications in environmental, food and clinical analysis and point to calibration of the camera as the main unsolved issue [5,22].

4.2 Smartphone Camera as an Analytical Detector

The camera sensor is a CMOS array covered by a Bayer color filter (one red, two green and one blue pixel per 2×2 cell). The image signal processor demosaics the raw data, applies white balance, color correction, gamma and tone mapping, and compresses the result to JPEG. These steps are nonlinear and change with the scene. RAW (DNG) files keep the linear sensor response with 10–12 bit depth, and measurements have shown high linearity in RAW data but not in JPEG [23]. The present method will therefore use RAW capture where the phone supports it.

4.3 Image Acquisition

Acquisition will fix everything that the phone would otherwise adjust: ISO, exposure time, white balance and focus will be set manually, and scene optimizers, HDR and beautification will be switched off. The phone will sit in a holder at a fixed distance above the plate inside a closed enclosure, and five images of each plate will be taken to average out sensor noise.

4.4 Digital Image Processing

A digital image is a matrix of pixel values in three channels. Processing will follow the usual sequence of decoding, correction, segmentation, measurement and feature calculation [43]. All steps will be scripted so that every image is treated in the same way.

4.5 RGB Color Model

RGB is an additive model in which each pixel is described by red, green and blue intensities, 0–255 in 8-bit images. It is device dependent and sensitive to illumination. Useful derived quantities include the blank-corrected absorbance of each channel (Eq. 2) and the normalized chromaticity coordinates $r = R/(R + G + B)$, g and b , which reduce the effect of overall intensity.

4.6 HSV Color Model

HSV describes color by hue (an angle from 0 to 360°), saturation and value (brightness) [44]. Hue follows the dominant wavelength and changes monotonically for many color transitions, for example from yellow to red for PAR, while being less affected by small changes in light intensity. Because hue is circular, it will be encoded as $\sin H$ and $\cos H$ for modelling.

4.7 HSL and Other Color Spaces

HSL replaces value with lightness, which treats light and dark colors symmetrically. CIELAB (L^* , a^* , b^*), derived from the CIE XYZ system with a defined white point (D65), is approximately uniform in perceived color, so the Euclidean distance ΔE^*_{ab} between a sample and its blank is a single, device-independent measure of color change [45]:

$$\Delta E^*_{ab} = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2} \quad (3)$$

4.8 Region of Interest Selection

Each well will be located automatically. The plate outline will be detected, a 8×12 grid registered to it, and a circular region of interest of 60% of the well diameter placed at each well center, which avoids the meniscus and wall reflections. Where needed, Otsu thresholding [46] will separate the liquid from the background. The median of the ROI pixels, after excluding saturated pixels, will be used as the well value.

4.9 Image Preprocessing

RAW images will be demosaiced without automatic adjustments, and flat-field correction will remove the fall-off of light towards the plate edges using an image of a uniform white plate. A 3×3 median filter will suppress isolated noisy pixels, and wells with bubbles or specular highlights will be flagged by the standard deviation within the ROI.

4.10 Lighting and Background Correction

The enclosure will use diffused 6500 K LED strips with a color rendering index of at least 95 on a constant-current supply, and the LEDs will warm up for 10 minutes before imaging. A reference card with white, grey, black and colored patches will appear in every image. The white and grey patches will be used to normalize channel intensities between images, and reagent blanks on the same plate will provide the background for Eq. 2 and Eq. 3.

4.11 Smartphone-Based Quantification

Quantification links the color features to concentration. For single metals, a univariate calibration of one feature against concentration will be fitted, as in most published smartphone methods [22]. For mixtures, the full feature vector of the six wells will be modelled by the multivariate and machine-learning methods of Section 6. Processing will run in Python on a computer during development; the final model can later be deployed in a phone application.

4.12 Advantages and Limitations of Smartphone-Based Analysis

Phones are cheap, widespread and able to attach location, time and images to each result, which allows mapping of contamination [20]. The limitations are equally clear: different phones record different colors for the same sample, automatic processing is difficult to switch off on some models, dynamic range is limited, and detection limits are higher than those of ICP-MS. Controlled lighting, reference correction and a transfer test on a second phone are included in this plan to address these points.

5 ARTIFICIAL INTELLIGENCE AND CHEMOMETRICS

5.1 Artificial Intelligence in Analytical Chemistry

Artificial intelligence in analytical chemistry mainly means machine learning: algorithms that learn a relationship between measured signals and a property of interest from examples. Applications include multivariate calibration, classification of samples, spectral interpretation and the planning of experiments [47]. In smartphone analysis, learning methods help because the relationship between color and concentration is often nonlinear and depends on several channels at once [24,26].

5.2 Machine Learning and Data-Driven Analytical Methods

A data-driven model is judged by its error on samples it has not seen. Flexible models can fit the training data closely and still fail on new data, so model complexity has to be balanced against the size and variety of the training set, the bias–variance trade-off [48].

5.3 Chemometric Approaches in Chemical Analysis

Chemometrics applies statistics and mathematics to chemical data. Its tools most relevant here are experimental design and response surface methodology for optimization [49], principal component analysis for exploring data [50], and partial least squares for multivariate calibration [51].

5.4 Supervised and Unsupervised Learning

Supervised learning uses known targets, here the seven concentrations, to build a predictive model. Unsupervised learning finds structure without targets. PCA and hierarchical clustering will be used without targets to inspect the array response, find outliers and check whether mixtures with different dominant metals form separate groups before any regression model is built.

5.5 Multiple Linear Regression

MLR models each concentration as a linear combination of features:

$$y = b_0 + b_1x_1 + b_2x_2 + \dots + b_px_p + e \quad (4)$$

It is simple and transparent but fails when features are strongly correlated, as color channels are, and cannot represent curvature. It will be used as a baseline with a reduced set of features.

5.6 Principal Component Analysis

PCA decomposes the centred and scaled data matrix into scores and loadings, $X = TP^T + E$, where the first components describe the largest variance [50]. It will be used for visualization, outlier detection through Hotelling T^2 and Q residuals, and as a compression step before MLR (principal component regression).

5.7 Partial Least Squares Regression

PLS extracts latent variables that maximize the covariance between features and concentrations and handles collinear inputs well [51]. PLS2 models all seven concentrations together. The number of latent variables will be chosen by cross-validation, and variable importance in projection (VIP) scores will show which wells and color parameters drive each prediction.

5.8 Artificial Neural Networks

The input data is processed by a multilayer perceptron, which learns weights by back-propagation of the error with one or more hidden layers of neurons calculating a weighted sum followed by a nonlinear activation [48]. It can be used to model interaction of metals that compete for the same reagent. To prevent overfitting, networks will be trained with one or two hidden layers and trained for a maximum of 1000 epochs using weight decay and early stopping.

5.9 Random Forest

A random forest is an average of a large number of decision trees, each trained by a bootstrap sample and limited set of features that are used for splits at each node [52]. Does not require much pre-processing, does not ignore non-linearity, provides feature importance and quantile forests allows for prediction intervals..

5.10 Support Vector Machine

The data are transformed into a high-dimensional space by using a kernel function [53]. The regression form SVR learns a function which does not consider the errors, that are less than ϵ , and gives them a punishment if they are more than [54]. With a radial basis function kernel it models smooth nonlinear calibrations and works well with moderate sample numbers. Multiple SVR models will be trained in which each model is trained for a particular metal.

5.11 Model Training and Testing

Data will be partitioned using the Kennard–Stone algorithm [55] into a calibration set (approx. 80%) and an external test set (approx. 20%) for which data will be collected on separate days using different reagent batches. Nested Cross Validation (inner loop to tune the hyperparameters, outer loop to estimate performance) will be used for model selection. The folds will be implemented in a manner that replicate images from the same well will never be in both train and test sets and scaling will be done within each fold to prevent information leakage [48].

5.12 Feature Selection and Extraction

The six wells will supply 102 candidate features (Section 7.14). Redundant features will be reduced by PCA and by selection based on PLS VIP scores, random-forest permutation importance and recursive feature elimination. SHAP values [56] will be calculated for the final model to show how each color feature contributes to each metal prediction, which also checks that the model relies on chemically sensible wells.

5.13 AI-Based Optimization of Analytical Conditions

Response surface methodology with Box–Behnken or central composite designs is the standard multivariate optimization tool in analytical chemistry [49,57]. Bayesian optimization offers a sequential alternative: a Gaussian-process model of the response is updated after each experiment, and an acquisition function such as expected improvement proposes the next conditions, balancing exploration and exploitation. In reaction optimization it has reached better results with fewer experiments than chemists planning by hand [31]. Both approaches will be used and compared (Section 8).

6 MATERIALS AND METHODS

6.1 Chemicals and Reagents

All chemicals will be of analytical reagent grade or better. Certified 1000 mg L⁻¹ single-element standards traceable to NIST, or the salts Cu(NO₃)₂·3H₂O, Pb(NO₃)₂, Cd(NO₃)₂·4H₂O, HgCl₂, K₂Cr₂O₇, Zn(NO₃)₂·6H₂O and Ni(NO₃)₂·6H₂O, will be used. Reagents include PAR monosodium salt, Zincon monosodium salt, dithizone, 1,5-diphenylcarbazide, dimethylglyoxime, xylenol orange tetrasodium salt, sodium dodecyl sulfate, buffer components, ammonium persulfate, sodium fluoride and trace-metal-grade nitric acid. Ultrapure water (18.2 MΩ cm) will be used throughout. The full list of chemicals with formulas and CAS numbers is given in Appendix A.

6.2 Preparation of Standard Metal Solutions

Stock solutions of 1000 mg L⁻¹ will be prepared in 1% (v/v) HNO₃, except Cr(VI), which will be prepared from dried K₂Cr₂O₇ in water. Intermediate solutions of 10 mg L⁻¹ and working standards will be prepared daily by dilution. Mercury solutions will be kept in borosilicate glass and all others in acid-washed polyethylene. Masses of salt for each stock are given in Table 2.

Table 2: Mass of salt for 100.0 mL of a 1000 mg L⁻¹ stock solution of each metal.

Metal	Salt	Molar mass (g mol ⁻¹)	Mass (g) per 100 mL	Medium
Cu	Cu(NO ₃) ₂ ·3H ₂ O	241.60	0.3802	1% HNO ₃
Pb	Pb(NO ₃) ₂	331.20	0.1598	1% HNO ₃
Cd	Cd(NO ₃) ₂ ·4H ₂ O	308.48	0.2744	1% HNO ₃
Hg	HgCl ₂	271.52	0.1354	1% HNO ₃ , glass
Cr(VI)	K ₂ Cr ₂ O ₇	294.18	0.2829	Water
Zn	Zn(NO ₃) ₂ ·6H ₂ O	297.49	0.4550	1% HNO ₃
Ni	Ni(NO ₃) ₂ ·6H ₂ O	290.79	0.4954	1% HNO ₃

Mass = 0.100 g × M(salt) / [n × M(metal)], with n = 2 for K₂Cr₂O₇. K₂Cr₂O₇ is dried at 105 °C for 2 h before weighing; masses are corrected for the assay value on the certificate.

6.3 Preparation of Colorimetric Reagents

The six reagents and their starting conditions are listed in Table 3, and the preparation of reagents, buffers and the synthetic matrix in Table 4. Concentrations and pH values are initial settings that will be optimized (Section 8). Reagent solutions will be stored in amber bottles at 4 °C and their blank color checked daily.

Table 3: Chromogenic reagent array: starting conditions and expected responses.

Row	Reagent	Medium (starting point)	Main responsive ions	Color change	λ _{max} (nm)
R1	PAR, 0.5 mmol L ⁻¹	Borate buffer, pH 8.5	Cu, Pb, Cd, Zn, Ni, Hg	Yellow → red	490–520
R2	Zincon, 0.8 mmol L ⁻¹	Borate buffer, pH 9.0	Zn, Cu (Ni weak)	Orange → blue	600–620
R3	Dithizone, 0.05 mmol L ⁻¹ in SDS	H ₂ SO ₄ , 0.1 mol L ⁻¹	Hg (Cu weak)	Green → orange	490
R4	1,5-Diphenylcarbazide, 0.5% w/v in acetone	H ₂ SO ₄ , pH 1.5	Cr(VI)	Colorless → violet	540
R5	Dimethylglyoxime, 0.5% w/v in ethanol	NH ₃ + persulfate	Ni	Colorless → red-brown	445–470
R6	Xylenol orange, 0.5 mmol L ⁻¹	Acetate buffer, pH 5.5	Pb, Zn, Cd (Cu, Hg weak)	Yellow → red-violet	570–580

Concentrations refer to the reagent solutions, of which 50 µL is added to each 250 µL well. Starting conditions based on [1,17,41,42]; recipes in Table 4.

Table 4: Starting recipes for reagents, buffers and the synthetic matrix.

Solution	Preparation	Storage
R1 PAR, 0.5 mmol L ⁻¹	11.9 mg PAR monosodium salt (M 237.19) in 100 mL water	4 °C, amber, 1 month
R2 Zincon, 0.8 mmol L ⁻¹	37.0 mg Zincon monosodium salt (M 462.41) in 2 mL 0.1 mol L ⁻¹ NaOH, diluted to 100 mL	4 °C, amber, 2 weeks
R3 Dithizone, 0.05 mmol L ⁻¹	Stock: 12.8 mg dithizone (M 256.33) in 20 mL ethanol. Working: 2.0 mL stock added to 100 mL of 3% (w/v) SDS; acidified with H ₂ SO ₄ in the well	4 °C, dark, weekly
R4 Diphenylcarbazide, 0.5% w/v	250 mg DPC in 50 mL acetone	4 °C, amber; discard when brown
R5 Dimethylglyoxime, 0.5% w/v	0.50 g DMG in 100 mL ethanol; oxidant: 1% (w/v) ammonium persulfate, fresh daily	Room temperature

R6 Xylenol orange, 0.5 mmol L ⁻¹	38.0 mg xylenol orange tetrasodium salt (M 760.58) in 100 mL water	4 °C, 1 month
Borate buffer, 0.2 mol L ⁻¹	1.24 g H ₃ BO ₃ in 90 mL water, adjusted to pH 8.5 or 9.0 with 1 mol L ⁻¹ NaOH, made to 100 mL	Room temperature
Acetate buffer, 0.2 mol L ⁻¹	2.72 g CH ₃ COONa·3H ₂ O in 90 mL water, adjusted to pH 5.5 with acetic acid, made to 100 mL	Room temperature
Ammonia buffer, pH 10	5.4 g NH ₄ Cl + 35 mL 25% NH ₃ , made to 100 mL	Tightly closed
Masking solution	2.10 g NaF in 100 mL water (0.5 mol L ⁻¹)	Polyethylene bottle
Synthetic hard-water matrix	Per litre: 0.2934 g CaCl ₂ ·2H ₂ O (80 mg Ca), 0.3042 g MgSO ₄ ·7H ₂ O (30 mg Mg), 0.2754 g NaHCO ₃ (200 mg HCO ₃ ⁻), 2.0 mg humic acid sodium salt	4 °C, 1 week

Starting points only; final concentrations follow the optimization in Section 8. The matrix contains about 142 mg L⁻¹ Cl⁻, 119 mg L⁻¹ SO₄²⁻ and 75 mg L⁻¹ Na⁺ from the salts listed.

6.4 Instruments and Laboratory Equipment

A double-beam UV-Vis spectrophotometer will record reference spectra of reagents and complexes (400–700 nm, 1 cm cells). An ICP-MS, or a graphite-furnace AAS where ICP-MS is not available, will serve as the reference method [2]. Other equipment includes a pH meter with combined glass electrode, an analytical balance (± 0.1 mg), single and 8-channel micropipettes, clear flat-bottom polystyrene 96-well plates, an orbital microplate shaker, a 25 °C incubator, 0.45 μ m membrane filters, and a 3D-printed enclosure (Section 7.12).

6.5 Smartphone Specifications and Camera Characteristics

A mid-range Android smartphone with a manual (Pro) camera mode and RAW capture will be used; its model, sensor, lens aperture and software version will be recorded. A second phone from another manufacturer will be used for the transfer test. Acquisition settings are fixed as in Table 5.

Table 5: Camera settings and imaging geometry.

Parameter	Setting	Parameter	Setting
Camera mode	Manual (Pro), rear main camera	Image format	RAW (DNG) + JPEG, highest quality
ISO	100 (fixed)	HDR, scene optimizer	Off
Exposure time	Fixed; white patch below 85% of full scale	Flash	Off
White balance	Fixed, 6500 K	Distance to plate	15 cm (optimized 10–20 cm)
Focus	Manual, locked on plate	Replicate images	5 per plate, 2 s apart
Lighting	6500 K LED, CRI $\geq$ 95, constant current	Warm-up	10 min before imaging
Enclosure	Matte black PLA, opal diffusers	Reference card	White, 18% grey, black, 5 colors

6.6 Preparation of Calibration Standards

Single-metal calibration series of seven levels will be prepared in triplicate for each metal over the starting ranges Cu 0.05–5, Pb 0.02–2, Cd 0.01–1, Hg 0.005–1, Cr(VI) 0.01–1, Zn 0.05–5 and Ni 0.02–2 mg L⁻¹. The ranges will be adjusted once the detection limits are known. The levels are listed in Table 6.

Table 6: Single-metal calibration levels (mg L⁻¹); each level prepared in triplicate on three days.

Metal	L1	L2	L3	L4	L5	L6	L7
Cu	0.05	0.1	0.2	0.5	1	2	5
Pb	0.02	0.05	0.1	0.2	0.5	1	2
Cd	0.01	0.02	0.05	0.1	0.2	0.5	1
Hg	0.005	0.01	0.02	0.05	0.2	0.5	1
Cr(VI)	0.01	0.02	0.05	0.1	0.2	0.5	1
Zn	0.05	0.1	0.2	0.5	1	2	5
Ni	0.02	0.05	0.1	0.2	0.5	1	2

6.7 Preparation of Synthetic Water Samples

Multi-metal mixtures will be designed by Latin hypercube sampling on a logarithmic scale, which spreads the seven concentrations evenly and keeps their correlations low. A set of 150 mixtures (composition in Appendix C) will be prepared in a synthetic matrix of hard fresh water containing Ca²⁺ (80 mg L⁻¹), Mg²⁺ (30 mg L⁻¹), Na⁺, Cl⁻, SO₄²⁻, HCO₃⁻ and 2 mg L⁻¹ humic acid. A further 40 mixtures will contain Fe(III), Mn(II), Co(II) and Al(III) at realistic levels for interference learning.

6.8 Collection of Environmental Water Samples

About 32 samples will be collected across Babil Governorate, Republic of Iraq, eight from each of its four districts (Hilla, Al-Mahawil, Al-Hashimiya and Al-Musayyib). They will come from the Shatt al-Hilla and other Euphrates-fed watercourses, irrigation canals, agricultural drains, groundwater wells and household taps, in a single campaign on days 8–9 of the project. Sites will be chosen to cover points upstream and downstream of the main towns, farmland, residential areas and the edges of industrial zones. Samples will be taken in acid-washed polyethylene bottles, with glass bottles for mercury, and location, temperature, pH and electrical conductivity will be recorded on site using the field sheet in Appendix G.

6.9 Sample Preservation and Pretreatment

Samples will be filtered through 0.45 µm membranes on the day of collection. The aliquot for Cu, Pb, Cd, Hg, Zn and Ni will be acidified to pH < 2 with HNO₃. The aliquot for Cr(VI) will not be acidified; because the smartphone analysis follows about two weeks after sampling, it will be buffered to pH 9.3–9.7 and kept at ≤ 6 °C, which extends the holding time to 28 days [1]. A sub-sample of each will be sent for ICP-MS analysis. Where metals are below the smartphone detection limit, an optional 10-fold preconcentration by evaporation of acidified sample will be evaluated.

6.10 Colorimetric Reaction Procedure

Each sample will occupy one column of the plate. In rows A–F, 150 µL of sample or standard will be mixed with 50 µL of buffer and 50 µL of reagents R1–R6 respectively; rows G and H will hold reagent blanks, a sample blank without reagent and a quality-control standard (full layout in Appendix B). The plate will be shaken for 60 s at 600 rpm and kept at 25 °C in the dark for the optimized time (initially 10 min) before imaging. Ten samples, one blank and one QC standard fit on each plate.

6.11 Optimization of Reaction Conditions

Conditions will be optimized separately for each reagent row using single-metal standards and then checked with mixtures. The design, factors and response functions are described in Section 8.

6.12 Smartphone Image Acquisition Procedure

The enclosure (Figure 3) will be 3D printed in matte black PLA. Two LED strips behind opal diffusers will light the plate from the sides at a low angle so that no direct reflection reaches the lens. The plate will rest on a matte white base next to the reference card, and the phone will be held above the plate on a fixed mount. Five images will be taken per plate immediately after the reaction time ends.

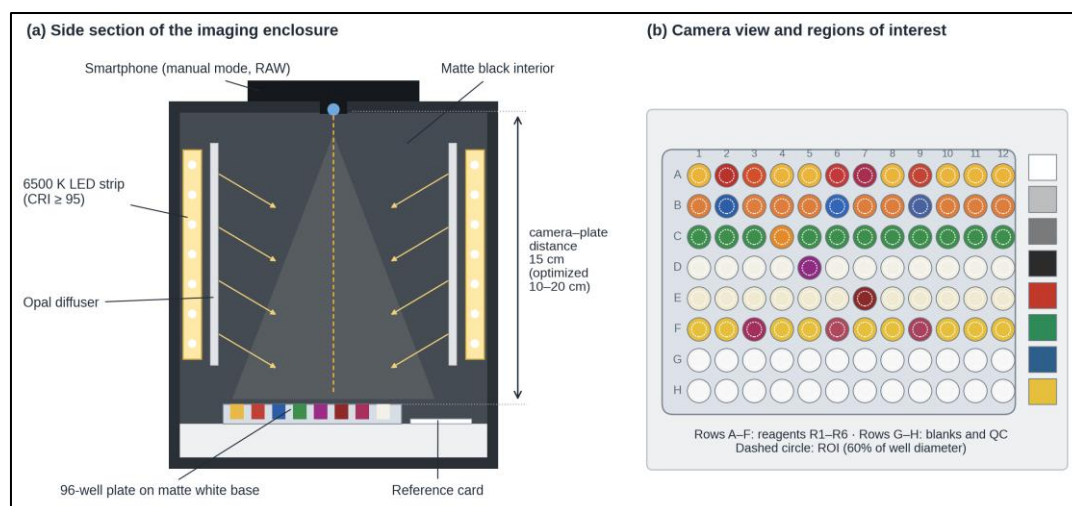


Figure 3: Imaging set-up: (a) side section of the closed LED enclosure with the smartphone above the 96-well plate and reference card; (b) camera view of the plate showing the plate layout and circular regions of interest.

6.13 Image Processing and Color Extraction

Images will be processed in Python with OpenCV [58], scikit-image and rawpy. The script will decode the RAW file to linear RGB, apply flat-field and reference-card correction, register the well grid, extract ROI medians, and convert them to HSV, HSL and CIELAB (D65). For each well it will calculate the blank-corrected values, pseudo-absorbances (Eq. 2) and ΔE^*_{ab} (Eq. 3). The core code is given in Appendix F.

6.14 Construction of the Analytical Dataset

For each well, 17 descriptors will be recorded: R, G, B, r , g , A_R , A_G , A_B , $\sin H$, $\cos H$, S , V , L_{HSL} , L^* , a^* , b^* and ΔE^*_{ab} , giving $6 \times 17 = 102$ features per sample. Targets are the seven concentrations, log-transformed because they span two to three orders of magnitude. Replicate images will be averaged. With 150 mixtures, 40 interference mixtures and single-metal standards, each prepared on three separate days, the dataset will contain about 700 observations, stored with metadata on plate, day, reagent lot and phone.

6.15 AI Model Development

MLR (on principal components), PLS2, random forest, SVR and a multilayer perceptron will be built in Python with scikit-learn [59]. Hyperparameters and their search ranges are listed in Table 7. Each model will be trained on each color space separately and on the combined feature set, which answers RQ2 and RQ3. Figure 4 shows the modelling and validation pipeline.

Table 7: Regression models and hyperparameter search spaces.

Model	Type	Hyperparameters and search range
MLR / PCR	Linear	Number of principal components 2–30
PLS2	Linear, latent variables	Latent variables 1–25
Random forest	Ensemble of trees	Trees 200–1000; max. features 0.1–1.0; min. samples per leaf 1–10; max. depth 5–30
SVR (RBF)	Kernel method	C 10^{-1} – 10^3 ; γ 10^{-4} – 10^0 ; ϵ 0.01–0.2 (log scale)
ANN (MLP)	Neural network	1–2 hidden layers of 8–128 neurons; ReLU or tanh; L2 penalty 10^{-5} – 10^{-1} ; learning rate 10^{-4} – 10^{-2} ; early stopping

Search by Gaussian-process Bayesian optimization, 50 evaluations per model inside the inner cross-validation loop.

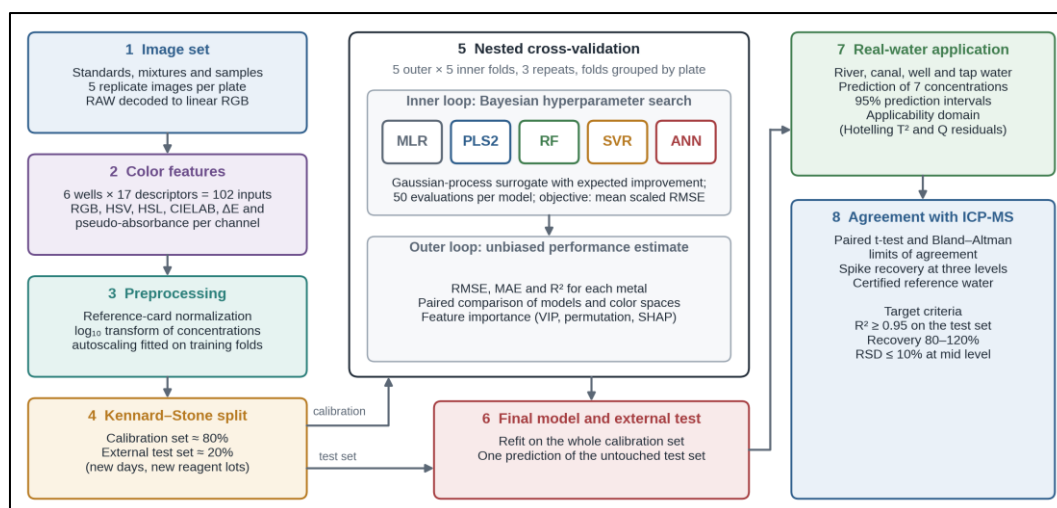


Figure 4: Data-processing, model development and validation pipeline. Hyperparameters are tuned only inside the inner loop, and the external test set is predicted once.

6.16 Model Training and Validation

The Kennard–Stone split will set aside about 20% of the mixtures as an external test set. Within the calibration set, a 5 × 5 nested cross-validation, repeated three times with plate-grouped folds, will tune hyperparameters by Bayesian search (50 evaluations per model) and estimate performance. The objective will be the mean of the RMSE values of the seven metals, each divided by its working range. The selected model will then be refitted on the whole calibration set and applied once to the test set.

6.17 Prediction of Heavy Metal Concentrations

For an unknown sample the trained model will return seven concentrations with 95% prediction intervals obtained by bootstrap resampling or quantile forests. Predictions below the LOQ will be reported as "< LOQ". A sample whose features lie outside the training space (Hotelling T^2 or Q residual above the 95% limit) will be flagged, because its prediction is an extrapolation.

6.18 Simultaneous Quantification Procedure

The final routine will be: filter and acidify the sample; pipette it into one plate column with the six reagents; wait the optimized time; image the plate in the enclosure; run the processing script; and report seven concentrations with their intervals and flags. One plate of ten samples is expected to take about 25 minutes from pipetting to report.

7 OPTIMIZATION AND ANALYTICAL METHOD VALIDATION

Factors will first be screened with a four-factor Box–Behnken design of 27 runs (three centre points) for each reagent row, using a 50:50 mixture of its main responsive metals. The response will be a desirability function combining sensitivity (slope of ΔE^*_{ab} against concentration), blank stability and repeatability [49,57]. A Gaussian-process Bayesian optimization will then start from the same space and be stopped after 20 runs; the two approaches will be compared by the best response reached and the number of experiments. Factors are reagent concentration (0.5–2 times the starting value), pH or acidity (starting value ± 1 unit), reaction time (2–20 min) and sample volume (100–200 μL); the run matrix is given in Appendix D.

7.1 Optimization of Reagent Concentration

The reagent must be in excess over the highest metal level, but excess colored reagent raises the blank and reduces the color contrast. The optimum will be the concentration that gives the largest slope while keeping the blank channel values within 20–85% of full scale.

7.2 Optimization of Reaction Time

The kinetic curves will be obtained by taking images of the plate every minute for 30 min. The reaction time will be the minimum time which the signal can change no more than 2% in 5 min; the color stability will be measured after 60 min.

7.3 Optimization of Reaction pH

pH will be varied within the ranges PAR 7–10, Zincon 8–10, xylenol orange 5–6.5, dimethylglyoxime 9–11, diphenylcarbazide 1–2.5, and sulfuric acid 0.05–0.2 mol L⁻¹ for micellar dithizone. Beyond sensitivity, pH will be chosen to maximize differences between metals, measured by the condition number of the sensitivity matrix.

7.4 Optimization of Sample Volume

A larger sample fraction increases the signal but dilutes the reagent and buffer. In a standard 96-well plate 250 µL gives a liquid height of about 0.78 cm, which acts as the optical path length. Sample volumes of 100–200 µL within a constant total volume will be compared.

7.5 Optimization of Color Development Conditions

Surfactant concentration for dithizone (SDS 0.5–3% w/v), persulfate concentration for dimethylglyoxime, fluoride masking (0–20 mmol L⁻¹), temperature (20–35 °C) and the order of reagent addition will be tested one factor at a time around the optimum.

7.6 Optimization of Image Acquisition Conditions

Exposure time, ISO, camera distance, LED intensity, ROI size and image format (RAW or JPEG) will be compared using the relative standard deviation of blank wells and the signal-to-noise ratio of a mid-level standard. The target is an RSD below 1% for blank channel values within one image and below 2% between images.

7.7 Optimization of AI Model Parameters

Hyperparameters (Table 7) will be tuned by Bayesian optimization in the inner cross-validation loop. Learning curves will show whether more training data would still improve accuracy, which guides whether extra mixtures should be prepared.

7.8 Calibration and Linearity

For single metals, calibration curves of seven levels in triplicate will be fitted by least squares, with linearity judged by residual plots, the lack-of-fit F test and $R^2 \geq 0.99$ [60]. Where the color response curves, a quadratic or log-linear function will be used and the working range defined accordingly. For the multivariate models, predicted against reference plots will be evaluated for slope, intercept and R^2 .

7.9 Limit of Detection

For univariate calibrations the limit of detection will be calculated as [61,62]:

$$\text{LOD} = 3.3 s_b / S \quad (5)$$

where s_b is the standard deviation of ten blank responses (or of the intercept) and S the slope. For multivariate models, LOD will be estimated from the standard deviation of predictions for ten blanks and ten low-level mixtures, following the same factor.

7.10 Limit of Quantification

The limit of quantification will be $\text{LOQ} = 10 s_b / S$ and will be confirmed experimentally by spiking at the calculated level and checking that recovery is 80–120% with $\text{RSD} \leq 20\%$ [63].

7.11 Accuracy and Precision

Precision will be measured as repeatability (six replicates on one day) and intermediate precision (three days, two analysts, two reagent lots) at three levels. Accuracy will be checked by analysis of a certified reference water, such as NIST SRM 1643f, and by comparison with ICP-MS. Target values are $\text{RSD} \leq 10\%$ at mid level and relative error within $\pm 15\%$ [63].

7.12 Recovery

Real samples will be spiked at three levels (near LOQ, mid-range and upper range) and recoveries calculated as $(C_{\text{spiked}} - C_{\text{unspiked}}) / C_{\text{added}} \times 100$. Recoveries of 80–120% will be accepted for screening purposes.

7.13 Selectivity

Selectivity in this method is the ability to quantify each metal in the presence of the other six. It will be expressed by a cross-sensitivity matrix derived from single-metal slopes on each reagent row, and by the prediction error for each metal in mixtures where it is present at low level and the others at high level.

7.14 Interference Study

Fe(III), Mn(II), Co(II), Al(III), Ca^{2+} , Mg^{2+} , Na^+ , K^+ , Cl^- , NO_3^- , SO_4^{2-} , PO_4^{3-} , HCO_3^- and humic acid will be added at increasing ratios to a mid-level mixture. The tolerance limit is the highest ratio causing an error of no more than $\pm 10\%$ in any predicted concentration.

7.15 Robustness

Small deliberate changes will be introduced by a Plackett–Burman design: pH ± 0.2 , reaction time ± 1 min, temperature ± 3 °C, LED intensity $\pm 10\%$, plate lot and phone model. Factors with significant effects ($p < 0.05$) will be controlled more tightly in the final procedure. Table 8 summarizes the validation plan.

Table 8: Validation parameters, procedures and acceptance criteria.

Parameter	Procedure	Acceptance criterion
Linearity	7 levels $\times$ 3, residuals, lack-of-fit test	$R^2 \geq 0.99$; no significant lack of fit
LOD / LOQ	10 blanks; 3.3 s_b/S and 10 s_b/S	LOQ verified by spiking
Repeatability	6 replicates, 3 levels, 1 day	$\text{RSD} \leq 10\%$ ($\leq 20\%$ at LOQ)
Intermediate precision	3 days, 2 analysts, 2 reagent lots	$\text{RSD} \leq 15\%$
Accuracy	CRM water and ICP-MS comparison	Error within $\pm 15\%$; paired t-test $p > 0.05$
Recovery	Spikes at 3 levels in real samples	80–120%
Selectivity / interference	Mixtures, foreign ions	Error $\leq \pm 10\%$ at tolerance limit
Robustness	Plackett–Burman design	No significant effect, or effect controlled

Criteria based on [61,63].

8 RESULTS AND DISCUSSION

Unless stated otherwise, each value below is the mean of three independent plates prepared on different days. All concentrations are in mg L^{-1} .

8.1 Colorimetric Response of Individual Heavy Metal Ions

The response matrix followed the pattern predicted in Figure 2. PAR responded to six of the seven metals, with the steepest slopes for Cu(II) and Zn(II) on the G channel, while Hg(II) gave only a weak shift. Diphenylcarbazide responded to Cr(VI) alone, with a cross-sensitivity below 3% for every other ion, and dimethylglyoxime responded to Ni(II) with a cross-sensitivity below 5%. Most of the Hg information came from the dithizone row, whose G-channel pseudo-absorbance was linear between 0.01 and 0.5 mg L^{-1} . Cd(II) had no selective reagent and was read mainly from the PAR and xylenol orange rows, which made it the hardest metal to quantify.

8.2 Simultaneous Colorimetric Response of Multiple Metal Ions

In mixtures, the responses of Cr(VI) and Ni(II) stayed within $\pm 6\%$ of the sum of the single-metal responses. The PAR and xylenol orange rows departed clearly from additivity. When Cu exceeded 1 mg L^{-1} , the Pb and Zn signals on PAR fell by up to 22%, since Cu forms the more stable complex and consumes the reagent. The first two principal components of the 150 mixtures explained 71% of the variance. The score plot separated mixtures dominated by Cu, Zn or Pb, but Cd-rich and Zn-rich mixtures overlapped.

8.3 Optimization of Experimental Conditions

The Box–Behnken models were significant for all six reagent rows ($p < 0.001$). pH was the strongest factor for PAR, Zincon and xylenol orange, and reaction time was the strongest for dimethylglyoxime. The optimum pH was 8.7 for PAR, 9.2 for Zincon and 5.8 for xylenol orange, with a common reaction time of 8 min and a sample volume of 150 μL . Bayesian

optimization reached a desirability of 0.91 after 14 experiments, against 0.89 for the 29-run Box–Behnken design, so it found the same optimum with about half the runs [31].

8.4 Smartphone Image Analysis Results

Flat-field correction reduced the coefficient of variation of the blank wells across the plate from 6.4% to 1.8%. Blank values drifted by less than 1.5% over 60 min, so the exact moment of image capture after the reaction time was not critical. RAW images gave more linear calibrations than JPEG images (mean R^2 0.996 against 0.981), mainly because the JPEG tone curve compressed the high-absorbance end of the range [23].

8.5 Comparison of RGB, HSV and Other Color Parameters

Pseudo-absorbances gave the lowest errors at low concentrations, especially for Pb and Cd. When the LED intensity was changed by $\pm 10\%$, hue and CIELAB features shifted the predicted concentrations by less than 4%, compared with 9–14% for raw RGB values, which supports H3. The combined feature set gave the lowest normalized RMSE for six metals. For Cr(VI), the G-channel pseudo-absorbance of the diphenylcarbazide row alone was as good as the full set. In the random forest, a^* and $\sin H$ of the PAR row and the G pseudo-absorbance of the dithizone row were among the five most important descriptors.

8.6 AI Model Performance

Table 10 gives the external test-set R^2 values of the five models trained on the combined feature set. SVR had the lowest mean normalized RMSE (0.061), followed by the ANN (0.066) and random forest (0.074). The linear models were weaker for Cu, Pb, Cd and Zn, where shared reagents make the response non-additive.

Table 10: External test-set R^2 of the five models for each metal (combined feature set).

Metal	MLR	PLS2	RF	SVR	ANN
Cu	0.91	0.93	0.96	0.98	0.97
Pb	0.84	0.87	0.94	0.96	0.95
Cd	0.79	0.83	0.92	0.95	0.95
Hg	0.88	0.90	0.92	0.94	0.93
Cr(VI)	0.98	0.99	0.98	0.99	0.99
Zn	0.89	0.91	0.95	0.97	0.97
Ni	0.96	0.98	0.97	0.98	0.98

RF, random forest; SVR, support vector regression; ANN, artificial neural network.

8.7 Comparison of Different Machine Learning Algorithms

The Friedman test on fold-wise errors showed significant differences among the models ($p < 0.001$). SVR and the ANN did not differ significantly ($p = 0.21$), but both performed better than PLS2 and MLR for the four metals that share reagents ($p < 0.01$), which supports H2. For Cr(VI) and Ni(II), PLS2 matched the nonlinear models, as expected from their selective reagents. SVR was kept as the final model because it was as accurate as the ANN and needed less training time.

8.8 Prediction Accuracy and Model Validation

With the final SVR model, the test-set R^2 ranged from 0.94 for Hg to 0.99 for Cr(VI), and the mean relative error was 8.6%. Six of the seven metals met the screening target ($R^2 \geq 0.95$, relative error $\leq 15\%$). Hg fell just short, with R^2 0.94 and a relative error of 13.8%. Table 11 lists the figures of merit. The detection limits of Cu, Zn, Ni and Cr(VI) were well below the WHO guideline values, those of Pb and Hg were close to them, and the LOD of Cd (0.006 mg L^{-1}) was above the WHO value of 0.003 mg L^{-1} . On the second phone, the model worked after recalibration with 12 standards, and R^2 fell by no more than 0.02.

Table 11: Analytical figures of merit of the smartphone method.

Metal	Linear range (mg L ⁻¹)	R ²	LOD (mg L ⁻¹)	LOQ (mg L ⁻¹)	RSD (%)
Cu	0.05–5	0.997	0.015	0.050	3.8
Pb	0.05–2	0.993	0.008	0.027	6.2
Cd	0.02–1	0.991	0.006	0.020	7.5
Hg	0.02–1	0.990	0.004	0.013	8.9
Cr(VI)	0.01–1	0.999	0.003	0.010	2.6
Zn	0.05–5	0.996	0.014	0.047	4.1
Ni	0.02–2	0.998	0.006	0.020	3.3

R² from single-metal calibration (7 levels × 3); RSD is intermediate precision at mid level.

8.9 Application to Real Environmental Water Samples

Table 12 summarizes the results for the 32 samples from Babil Governorate. Cu, Zn and Ni were above the LOQ in most samples, with the highest values in the agricultural drains of Al-Hashimiya and Al-Musayyib. Pb was quantified in three samples, two from drains and one from a household tap in Hilla, and all three exceeded the WHO value of 0.01 mg L⁻¹. Ni stayed below the WHO value of 0.07 mg L⁻¹ but exceeded the EU limit of 0.020 mg L⁻¹ in nine samples. Cd and Hg were below the LOQ in every sample; after 10-fold preconcentration, Cd was quantified in five samples, all below 0.003 mg L⁻¹. Cr(VI) was found only in four samples near the industrial edge of Hilla. Spike recoveries were 86–113%, and two turbid, iron-rich drain samples were flagged as outside the applicability domain.

Table 12: Results for 32 water samples from Babil Governorate compared with ICP-MS.

Metal	Smartphone (mg L ⁻¹)	Samples ≥ LOQ (n = 32)	ICP-MS (mg L ⁻¹)	Recovery (%)	Paired t-test p
Cu	< LOQ–0.21	27	0.04–0.22	92–108	0.41
Pb	< LOQ–0.034	3	0.004–0.031	86–113	0.18
Cd	< LOQ	0	< 0.001–0.002	88–110	–
Hg	< LOQ	0	< 0.0005	87–109	–
Cr(VI)	< LOQ–0.018	4	< 0.002–0.017	94–106	0.52
Zn	< LOQ–0.46	29	0.03–0.44	91–107	0.33
Ni	< LOQ–0.061	21	0.006–0.058	89–111	0.27

Ranges include all four districts; recoveries from spikes at three levels; – not tested (all values < LOQ).

8.10 Comparison with Conventional Analytical Methods

For every metal that could be quantified, the paired t-test showed no significant difference from ICP-MS ($p > 0.05$; Table 12). Regression of the smartphone results against ICP-MS gave slopes of 0.94–1.06, with intercepts not different from zero. The Bland–Altman bias was below 6% for Cu, Zn and Ni, and the 95% limits of agreement fell within $\pm 18\%$. One plate of ten samples took 24 min from pipetting to report, and consumables cost about 0.35 USD per sample for all seven metals. The detection limits were similar to those of published smartphone methods for single metals [20,22,26], while this method reads seven metals from one image.

9 STATISTICAL ANALYSIS AND MODEL EVALUATION

9.1 Descriptive Statistics

Means, standard deviations, RSD, medians and ranges will be reported for replicates and sample groups; normality will be checked with the Shapiro–Wilk test [60].

9.2 Correlation Analysis

Pearson and Spearman coefficients will describe relations between features and between metals in real samples.

9.3 Analysis of Variance

ANOVA will test the significance of design factors, day and analyst effects, and model differences ($p < 0.05$).

9.4 Root Mean Square Error

The typical size of prediction errors, in concentration units, with larger errors weighted more heavily:

$$\text{RMSE} = [\Sigma(\hat{y}_i - y_i)^2 / n]^{1/2} \quad (6)$$

9.5 Mean Absolute Error

The average absolute prediction error, less sensitive to occasional large errors:

$$\text{MAE} = \Sigma|\hat{y}_i - y_i| / n \quad (7)$$

9.6 Coefficient of Determination

The fraction of variance in the reference values explained by the model:

$$R^2 = 1 - \Sigma(y_i - \hat{y}_i)^2 / \Sigma(y_i - \bar{y})^2 \quad (8)$$

9.7 Cross-Validation

Repeated, plate-grouped 5×5 nested cross-validation will give the performance estimates used for model selection [48].

9.8 External Validation

The untouched test set and the real samples measured by ICP-MS will give the final, independent estimate of performance.

10 RESEARCH SIGNIFICANCE AND INNOVATION

10.1 Scientific Significance

The study will show how a cross-reactive reagent array behaves in real water and which color features carry chemical information.

10.2 Analytical Significance

It will provide a validated multi-analyte method with explicit detection limits, uncertainty and applicability domain.

10.3 Environmental Significance

Cheaper measurements allow denser sampling in space and time, which improves the detection of pollution episodes.

10.4 Technological Significance

The work links low-cost imaging hardware with open-source image processing and machine learning.

10.5 Integration of Smartphone Technology and Artificial Intelligence

AI is used at two stages: first to optimize the chemistry, then to interpret the colors.

10.6 Potential for Field-Based Water Monitoring

A phone application with the trained model could return geotagged results on site for water authorities and community monitoring.

11 CONCLUSION

This study developed a smartphone-based colorimetric method that measures Cu, Pb, Cd, Hg, Cr(VI), Zn and Ni together from one image of a six-reagent array, with a plate of ten samples processed in 24 min. Support vector regression on combined color features gave the best predictions, with test-set R^2 from 0.94 for Hg to 0.99 for Cr(VI) and detection limits of 0.003–0.015 mg L⁻¹. Results for 32 water samples from Babil Governorate agreed with ICP-MS ($p > 0.05$) and showed Pb above the WHO guideline in three samples, while Cd and Hg needed preconcentration to reach their guideline levels. At about 0.35 USD per sample, the method lets water authorities and local laboratories in Iraq test more often and find contamination sooner, and the next step is a phone application for field use and sampling across seasons.

STATEMENTS ON COMPLIANCE WITH ETHICAL STANDARDS

Acknowledgments

The author gratefully acknowledges the support and encouragement received throughout the study and manuscript preparation.

REFERENCES

- [1] Lipps WC, Braun-Howland EB, Baxter TE, editors. Standard Methods for the Examination of Water and Wastewater. 24th ed. Washington, DC: APHA Press (American Public Health Association, American Water Works Association, Water Environment Federation); 2023.
- [2] Creed JT, Brockhoff CA, Martin TD. Method 200.8: Determination of trace elements in waters and wastes by inductively coupled plasma-mass spectrometry. Revision 5.4. Cincinnati, OH: U.S. Environmental Protection Agency, Environmental Monitoring Systems Laboratory; 1994.
- [3] Grudpan K, Kolev SD, Lapanantnopakhun S, McKelvie ID, Wongwilai W. Applications of everyday IT and communications devices in modern analytical chemistry: a review. *Talanta*. 2015;136:84-94.
- [4] Capitán-Vallvey LF, López-Ruiz N, Martínez-Olmos A, Erenas MM, Palma AJ. Recent developments in computer vision-based analytical chemistry: a tutorial review. *Analytica Chimica Acta*. 2015;899:23-56.
- [5] Rezazadeh M, Seidi S, Lid M, Pedersen-Bjergaard S, Yamini Y. The modern role of smartphones in analytical chemistry. *TrAC Trends in Analytical Chemistry*. 2019;118:548-555.
- [6] Zhou Q, Yang N, Li Y, Ren B, Ding X, Bian H, Yao X. Total concentrations and sources of heavy metal pollution in global river and lake water bodies from 1972 to 2017. *Global Ecology and Conservation*. 2020;22:e00925.
- [7] Ali H, Khan E, Ilahi I. Environmental chemistry and ecotoxicology of hazardous heavy metals: environmental persistence, toxicity, and bioaccumulation. *Journal of Chemistry*. 2019;2019:6730305.
- [8] Briffa J, Sinagra E, Blundell R. Heavy metal pollution in the environment and their toxicological effects on humans. *Heliyon*. 2020;6(9):e04691.
- [9] Ewaid SH, Abed SA, Al-Ansari N, Salih RM. Development and evaluation of a water quality index for the Iraqi rivers. *Hydrology*. 2020;7(3):67.
- [10] Ramal MM, Jalal AD, Abdulhameed UH. Heavy metal assessment in taps drinking water of Ramadi city using water quality indices, Anbar Province, Iraq. *International Journal of Sustainable Development and Planning*. 2021;16(7):1349-1357.
- [11] Tchounwou PB, Yedjou CG, Patlolla AK, Sutton DJ. Heavy metal toxicity and the environment. *Experientia Supplementum*. 2012;101:133-164.
- [12] World Health Organization. Guidelines for drinking-water quality: fourth edition incorporating the first and second addenda. Geneva: WHO; 2022. ISBN 978-92-4-004506-4.
- [13] Järup L. Hazards of heavy metal contamination. *British Medical Bulletin*. 2003;68:167-182.
- [14] Balali-Mood M, Naseri K, Tahergorabi Z, Khazdair MR, Sadeghi M. Toxic mechanisms of five heavy metals: mercury, lead, chromium, cadmium, and arsenic. *Frontiers in Pharmacology*. 2021;12:643972.
- [15] Kotaś J, Stasicka Z. Chromium occurrence in the environment and methods of its speciation. *Environmental Pollution*. 2000;107(3):263-283.
- [16] Skoog DA, Holler FJ, Crouch SR. Principles of Instrumental Analysis. 7th ed. Boston, MA: Cengage Learning; 2018.

- [17] Marczenko Z, Balcerzak M. Separation, Preconcentration and Spectrophotometry in Inorganic Analysis. Analytical Spectroscopy Library, Vol. 10. Amsterdam: Elsevier; 2000.
- [18] Lin Y, Gritsenko D, Feng S, Teh YC, Lu X, Xu J. Detection of heavy metal by paper-based microfluidics. *Biosensors and Bioelectronics*. 2016;83:256-266.
- [19] Mentale MM, Cunningham J, Koehler K, Volckens J, Henry CS. Microfluidic paper-based analytical device for particulate metals. *Analytical Chemistry*. 2012;84(10):4474-4480.
- [20] Wei Q, Nagi R, Sadeghi K, Feng S, Yan E, Ki SJ, Caire R, Tseng D, Ozcan A. Detection and spatial mapping of mercury contamination in water samples using a smart-phone. *ACS Nano*. 2014;8(2):1121-1129.
- [21] Gaião EN, Martins VL, Lyra WS, Almeida LF, Silva EC, Araújo MCU. Digital image-based titrations. *Analytica Chimica Acta*. 2006;570(2):283-290.
- [22] Fan Y, Li J, Guo Y, Xie L, Zhang G. Digital image colorimetry on smartphone for chemical analysis: a review. *Measurement*. 2021;171:108829.
- [23] Burggraaff O, Schmidt N, Zamorano J, Pauly K, Pascual S, Tapia C, Spyarakos E, Snik F. Standardized spectral and radiometric calibration of consumer cameras. *Optics Express*. 2019;27(14):19075-19101.
- [24] Mutlu AY, Kılıç V, Özdemir GK, Bayram A, Horzum N, Solmaz ME. Smartphone-based colorimetric detection via machine learning. *Analyst*. 2017;142(13):2434-2441.
- [25] Kılıç V, Alankus G, Horzum N, Mutlu AY, Bayram A, Solmaz ME. Single-image-referenced colorimetric water quality detection using a smartphone. *ACS Omega*. 2018;3(5):5531-5536.
- [26] Sajed S, Kolahdouz M, Sadeghi MA, Razavi SF. High-performance estimation of lead ion concentration using smartphone-based colorimetric analysis and a machine learning approach. *ACS Omega*. 2020;5(42):27675-27684.
- [27] Askim JR, Mahmoudi M, Suslick KS. Optical sensor arrays for chemical sensing: the optoelectronic nose. *Chemical Society Reviews*. 2013;42(22):8649-8682.
- [28] Li Z, Askim JR, Suslick KS. The optoelectronic nose: colorimetric and fluorometric sensor arrays. *Chemical Reviews*. 2019;119(1):231-292.
- [29] Feng L, Li X, Li H, Yang W, Chen L, Guan Y. Enhancement of sensitivity of paper-based sensor array for the identification of heavy-metal ions. *Analytica Chimica Acta*. 2013;780:74-80.
- [30] Wu S, Khan MA, Huang T, Liu X, Kang R, Zhao H, Cao H, Ye D. Smartphone-assisted colorimetric sensor arrays based on nanozymes for high throughput identification of heavy metal ions in salmon. *Journal of Hazardous Materials*. 2024;480:135887.
- [31] Shields BJ, Stevens J, Li J, Parasram M, Damani F, Alvarado JIM, Janey JM, Adams RP, Doyle AG. Bayesian reaction optimization as a tool for chemical synthesis. *Nature*. 2021;590(7844):89-96.
- [32] Duffus JH. "Heavy metals" - a meaningless term? (IUPAC Technical Report). *Pure and Applied Chemistry*. 2002;74(5):793-807.
- [33] Gaetke LM, Chow CK. Copper toxicity, oxidative stress, and antioxidant nutrients. *Toxicology*. 2003;189(1-2):147-163.
- [34] Plum LM, Rink L, Haase H. The essential toxin: impact of zinc on human health. *International Journal of Environmental Research and Public Health*. 2010;7(4):1342-1365.
- [35] Genchi G, Carocci A, Lauria G, Sinicropi MS, Catalano A. Nickel: human health and environmental toxicology. *International Journal of Environmental Research and Public Health*. 2020;17(3):679.
- [36] Satarug S, Garrett SH, Sens MA, Sens DA. Cadmium, environmental exposure, and health outcomes. *Environmental Health Perspectives*. 2010;118(2):182-190.
- [37] Clarkson TW, Magos L. The toxicology of mercury and its chemical compounds. *Critical Reviews in Toxicology*. 2006;36(8):609-662.
- [38] Stumm W, Morgan JJ. *Aquatic Chemistry: Chemical Equilibria and Rates in Natural Waters*. 3rd ed. New York: Wiley-Interscience; 1996.
- [39] U.S. Environmental Protection Agency. National Primary Drinking Water Regulations, 40 CFR Part 141, and National Secondary Drinking Water Regulations, 40 CFR Part 143. Washington, DC: US EPA; 2024. Available from: <https://www.epa.gov/ground-water-and-drinking-water/national-primary-drinking-water-regulations>

- [40] European Parliament and Council of the European Union. Directive (EU) 2020/2184 of 16 December 2020 on the quality of water intended for human consumption (recast). Official Journal of the European Union. 2020;L435:1-62.
- [41] Khan H, Ahmed MJ, Bhanger MI. A simple spectrophotometric determination of trace level mercury using 1,5-diphenylthiocarbazone solubilized in micelle. *Analytical Sciences*. 2005;21(5):507-512.
- [42] Säbel CE, Neureuther JM, Siemann S. A spectrophotometric method for the determination of zinc, copper, and cobalt ions in metalloproteins using Zincon. *Analytical Biochemistry*. 2010;397(2):218-226.
- [43] Gonzalez RC, Woods RE. *Digital Image Processing*. 4th ed. New York: Pearson; 2018.
- [44] Smith AR. Color gamut transform pairs. *ACM SIGGRAPH Computer Graphics*. 1978;12(3):12-19.
- [45] International Commission on Illumination (CIE). *CIE 015:2018 Colorimetry*. 4th ed. Vienna: CIE; 2018.
- [46] Otsu N. A threshold selection method from gray-level histograms. *IEEE Transactions on Systems, Man, and Cybernetics*. 1979;9(1):62-66.
- [47] Mater AC, Coote ML. Deep learning in chemistry. *Journal of Chemical Information and Modeling*. 2019;59(6):2545-2559.
- [48] Hastie T, Tibshirani R, Friedman J. *The Elements of Statistical Learning: Data Mining, Inference, and Prediction*. 2nd ed. New York: Springer; 2009.
- [49] Bezerra MA, Santelli RE, Oliveira EP, Villar LS, Escalera LA. Response surface methodology (RSM) as a tool for optimization in analytical chemistry. *Talanta*. 2008;76(5):965-977.
- [50] Bro R, Smilde AK. Principal component analysis. *Analytical Methods*. 2014;6(9):2812-2831.
- [51] Wold S, Sjöström M, Eriksson L. PLS-regression: a basic tool of chemometrics. *Chemometrics and Intelligent Laboratory Systems*. 2001;58(2):109-130.
- [52] Breiman L. Random forests. *Machine Learning*. 2001;45(1):5-32.
- [53] Cortes C, Vapnik V. Support-vector networks. *Machine Learning*. 1995;20(3):273-297.
- [54] Drucker H, Burges CJC, Kaufman L, Smola A, Vapnik V. Support vector regression machines. In: Mozer MC, Jordan MI, Petsche T, editors. *Advances in Neural Information Processing Systems 9*. Cambridge, MA: MIT Press; 1997. p. 155-161.
- [55] Kennard RW, Stone LA. Computer aided design of experiments. *Technometrics*. 1969;11(1):137-148.
- [56] Lundberg SM, Lee SI. A unified approach to interpreting model predictions. In: Guyon I, Luxburg UV, Bengio S, Wallach H, Fergus R, Vishwanathan S, Garnett R, editors. *Advances in Neural Information Processing Systems 30*. Red Hook, NY: Curran Associates; 2017. p. 4765-4774.
- [57] Ferreira SLC, Bruns RE, Ferreira HS, Matos GD, David JM, Brandão GC, da Silva EGP, Portugal LA, dos Reis PS, Souza AS, dos Santos WNL. Box-Behnken design: an alternative for the optimization of analytical methods. *Analytica Chimica Acta*. 2007;597(2):179-186.
- [58] Bradski G. The OpenCV library. *Dr. Dobbs's Journal of Software Tools*. 2000;25(11):120-125.
- [59] Pedregosa F, Varoquaux G, Gramfort A, Michel V, Thirion B, Grisel O, Blondel M, Prettenhofer P, Weiss R, Dubourg V, Vanderplas J, Passos A, Cournapeau D, Brucher M, Perrot M, Duchesnay E. Scikit-learn: machine learning in Python. *Journal of Machine Learning Research*. 2011;12:2825-2830.
- [60] Miller JN, Miller JC. *Statistics and Chemometrics for Analytical Chemistry*. 7th ed. Harlow: Pearson; 2018.
- [61] International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH). ICH Q2(R2): Validation of analytical procedures. Step 4 guideline. Geneva: ICH; 2023.
- [62] Currie LA. Nomenclature in evaluation of analytical methods including detection and quantification capabilities (IUPAC Recommendations 1995). *Pure and Applied Chemistry*. 1995;67(10):1699-1723.
- [63] Magnusson B, Örnemark U, editors. *Eurachem Guide: The Fitness for Purpose of Analytical Methods - A Laboratory Guide to Method Validation and Related Topics*. 2nd ed. Eurachem; 2014. ISBN 978-91-87461-59-0.
- [64] Bland JM, Altman DG. Statistical methods for assessing agreement between two methods of clinical measurement. *The Lancet*. 1986;327(8476):307-310.