



## Rapid and cost-effective detection of perchlorate in water using paper-based analytical devices

Praveen Kumar<sup>a</sup>, Ashish Kapoor<sup>b</sup>, MuthuKumar Raghunathan<sup>a,\*</sup>

<sup>a</sup>Department of Biotechnology, School of Bioengineering, SRM Institute of Science and Technology, Kattankulathur, Chennai-603203, Tamil Nadu, India, emails: muthukur@srmist.edu.in (M. Raghunathan), praveenatrish@gmail.com (P. Kumar)

<sup>b</sup>Department of Chemical Engineering, Harcourt Butler Technical University, Kanpur, Uttar Pradesh-208002, India, email: ashishkapoorchem@gmail.com (A. Kapoor)

Received 25 May 2023; Accepted 29 October 2023

---

### ABSTRACT

Perchlorate, a hazardous pollutant, is mainly found in untreated wastewater from urban and industrial sites and unregulated surface and groundwater sources. Effective monitoring of perchlorate in water is essential to mitigate its potential harmful effects. Microfluidic systems are evolving as promising technologies for detecting chemical contaminants in water due to their ability to enable rapid analysis with minimal consumption of reagents and samples. The integration of paper-based microfluidic devices with digital imaging has garnered enormous attention from the perspective of developing portable analytical techniques. Nevertheless, there is a need for further exploration to fully realize the potential of these systems. This study aimed to develop and evaluate the performance of a microfluidic paper-based device for measuring perchlorate levels in water samples. Smartphone-based digital imaging was integrated with microfluidic paper-based analytical device to establish a reliable colorimetric method for detecting perchlorate contamination. The results demonstrated successful quantitative estimation of perchlorate levels in water samples using a colorimetric assay based on the methylene blue-perchlorate reaction. Real-time, on-site colorimetric data were collected using a digital smartphone, and image processing methods were used to detect the occurrence of perchlorate in water samples from digital images. The developed approach yielded a broad linear response ranging from 4 to 12 µg/L ( $R^2 = 0.97$ ) for perchlorate detection, with a limit of detection of 3.41 µg/L and a limit of quantification of 10.34 µg/L. The findings underscore the effectiveness of colorimetric analysis and digital imaging for paper-based analytical devices. The limitations of this method include the capability to detect only a single analyte and the requirement for additional steps in image processing to obtain analytical results. Future developments should focus on designing devices for simultaneous detection of multiple contaminants and exploring automated methods of image analysis.

*Keywords:* Microfluidics; Perchlorate; Digital image analysis; Paper-based analytical device

---

### 1. Introduction

Growing industry and urbanisation have had the most negative influence on water supplies. Pollutants from industrial and residential sources pollute water bodies and present a grave threat to the health of living

organisms. In this context, an adequate supply of clean drinking water has become a distant dream for a large population. Detection of chemical contaminants in the water is necessary to develop effective anti-pollution measures. Conventional techniques for species estimation include atomic absorption spectroscopy [1], inductively coupled

---

\* Corresponding author.

plasma mass spectrometry [2], electrochemical impedance spectroscopy and voltammetry [3–5]. Most of these techniques need access to specialised equipment and are typically cumbersome, costly, and time-consuming. This equipment is housed in centralised laboratory facilities and is operated by qualified technicians. Samples are gathered from many locations handled with care and transported to laboratories for analysis which adds to the expense. Environmental monitoring applications, portable analytical techniques can solve several constraints and supplement conventional laboratory-based technology [6].

Introducing lab-on-a-chip devices based on microfluidics is a potential step toward portable analytical instruments. There have been successful demonstrations of microfluidic devices for numerous sensing applications, including water quality measurement, biological diagnostics, and food toxicity testing [7,8]. Using paper as a substrate has increased the availability and functionality of these devices [9]. The cellulosic fibre structure of paper provides natural microscale spaces that facilitate the capillary action of fluids, minimising the need for external pumping systems to propel the fluids. Miniaturized paper-based analytical instruments are simple to manufacture and need small quantities of samples and reagents, significantly reducing operational logistics and costs. The functions and applications of paper-based analytical instruments have progressed swiftly over the years [10]. Digital imaging and analysis tools have permitted the capture of data on-site for quick identification of target analytes. For water quality monitoring, smartphone-enabled gadgets have been developed. Examples include examining water samples for heavy metals, phenols, pesticides, and microbiological pollutants [11]. Among various approaches, colorimetric techniques are highly preferred for water quality monitoring due to their simplicity and ease of visualization.

Colorimetric analysis involves detection of analytes in samples by measuring changes in the color intensity caused by the presence of the analytes. The underlying principle of colorimetric assay is the formation of colored complexes or compounds by reaction of analytes with specific reagents. Quantification of colorimetric assay can be achieved by various tools. While naked-eye visual observation is the simplest approach, it is usually qualitative and does not allow determination of concentration of analyte in the sample. Typical instruments for quantitative colorimetric analysis include UV-Vis spectrophotometer and colorimeter [12]. However, these equipment are generally located in research laboratories and are not easily accessible to common populace. Recent efforts have been focused on use of simpler and relatively common alternative tools for quantitative colorimetric analysis such as digital camera, scanner and smartphones [13]. The use of a digital camera as an image acquisition device for paper-based assay was demonstrated for detection of Hg(II) [14]. The images captured were processed to determine color intensity for Hg(II) concentration range 0.1–100 ppm. In another study, a document scanner was used for recording digital images of solutions placed in a 96-well microplate for glucose assay [12]. The concentration measurements determined by digital colorimetric analysis were in close agreement with those obtained by spectrophotometric method.

Although effective, digital cameras and scanners come with inherent limitations that warrant consideration.

These limitations encompass factors such as bulkiness and cost. They often require additional equipment and power sources, making them less convenient for fieldwork or situations where mobility is essential. In contrast, smartphones have emerged as a superior alternative, offering portability, cost-effectiveness, standardized imaging capabilities, extensive connectivity, and remarkable energy efficiency. Modern smartphones include high-resolution cameras that can capture and analyse images of exceptional quality. Notably, smartphones also offer the possibility of using integrated apps for analysis, further enhancing their utility in quantification. With the use of specific applications or attachments, they may be repurposed as useful colorimetric analytical instruments. These merits collectively position smartphone as an optimal tool for quantification in colorimetric assays, particularly for on-site detection where portability and ease of use are paramount.

Perchlorate ( $\text{ClO}_4^-$ ) salts, recognized as potent thyroid disruptors, have polluted ecosystems across the world and emerged as a new subject of study in several disciplines [15–19], particularly in ecology and health. The imperative to detect perchlorate arises from its potential adverse impacts on both human health and the environment. Present methods for detecting perchlorate use cumbersome or pricey analytical equipment [20–23]. These include ion chromatography, liquid chromatography-mass spectrometry, surface-enhanced Raman spectroscopy, infrared spectroscopy, and capillary electrophoresis. These methods come with their own set of limitations, including inadequate selectivity, labor-intensive pre-treatment procedures, and high sample throughput requirements. More extensive research has been conducted on chemosensors compared to biosensors, such as those utilizing  $\text{ClO}_4^-$  reductase-based biosensor [24] and *Caenorhabditis elegans* biosensor [25]. Spectrophotometric techniques have been employed for detecting perchlorate in aqueous solutions, using methylene blue and brilliant green [26–28]. Additionally, fluorescent chemosensors have shown promise in perchlorate detection. For instance, the presence of perchlorate [29] can transform a previously fluorescence-inactive imidazole bithiocarbohydrazone-Cu complex into an active one. When a 1-(4-biphenyl)benzimidazolium-based dipodal system is exposed to  $\text{ClO}_4^-$  in water, it significantly increases the intensity of fluorescent emission of the system. Furthermore, colorimetric detection of aqueous perchlorate has been achieved using platinum complex salt through solid-state anion exchange [30]. In another study, researchers developed a device for detecting improvised explosive compounds, including perchlorate [31]. Recent work has also involved the treatment of gold nanoparticles with methylene blue, resulting in increased sensitivity to perchlorate [32]. Although many of these methods offer notable sensitivity, they are not instrument-free and may involve some complexity that limits their suitability for portable detection. Hence, there is a pressing need to develop a straightforward, rapid, cost-effective yet efficacious technique for on-site quantitative estimation of perchlorate in water.

The objective of this study is to develop a facile low-cost microfluidic paper-based device for the detection of perchlorate in contaminated water. A simple perchlorate-methylene blue assay on paper is integrated with smartphone-assisted digital colorimetric analysis for quantification. The research

outcome should pave way for the development of a practical, eco-friendly and affordable point-of-use technique for on-site monitoring of water pollutants. This novel work is poised to make a substantial impact in addressing environmental concerns related to perchlorate contamination.

## 2. Materials and methods

### 2.1. Chemicals and equipment

The design of the microfluidic paper-based analytical device ( $\mu$ -PAD) essentially incorporates hydrophilic circular elements surrounded by hydrophobic barriers. The substrate used was filter paper (Whatman Qualitative No. 1). White candle wax was obtained from a nearby commercial store in Chennai, India, due to its excellent thermal and hydrophobic properties, making it an ideal choice for creating hydrophobic barriers on a paper substrate. When exposed to high temperatures, wax quickly melts due to heat and penetrates the porous matrix of the paper substrate. Subsequently, it solidifies and fills the pores as it cools. As a result, hydrophobic barriers can be formed. All chemicals used in the experiments were of analytical grade. Sodium perchlorate, sodium fluoride, potassium bromide, potassium sodium tartrate, sodium nitrate, methylene blue, Coomassie brilliant blue, and Whatman filter paper were purchased from Sigma-Aldrich (St. Louis, USA). Distilled water was used for the preparation of solutions. A smartphone (Oppo F19 Pro+, model number CPH2213) was used for image capture.

### 2.2. Collection of samples and study area

The water samples were taken from the southern coast of India, encompassing various districts in Tamil Nadu, India, including Sivakasi, Thoothukudi, Kalpakkam, Coimbatore, Salem, Erode, Madurai, Chengalpattu, Vellore, and Kanchipuram. These samples were collected in 100 mL sample bottles and stored at 4°C for subsequent analysis. Fig. 1 illustrates the geographical locations where the water samples were collected for the study. A total of thirty samples were gathered from various districts and categorized as surface water, groundwater, and drinking water. Sampling took place during the period May–June 2022. Drinking water samples were obtained from public areas such as bus stops, railway stations, temples, and churches, while surface water was sourced from rivers, lakes, and ponds. Groundwater sampling was done at bore wells, public wells, and tube wells.

### 2.3. Design and fabrication of $\mu$ -PAD

The development of a microfluidic paper-based analytical lab-on-a-chip platform was achieved through the wax permeation principle. Fig. 2 depicts the steps involved in the fabrication process. A custom-designed rubber stamp and its plastic holder, weighing a total of 52 g, were crafted by a local shop in Chennai, Tamil Nadu. The rubber pad absorbed the molten wax, which was then imprinted onto the paper substrate in the form of a circular pattern with a 14 mm diameter for the detection region [33]. Subsequently,

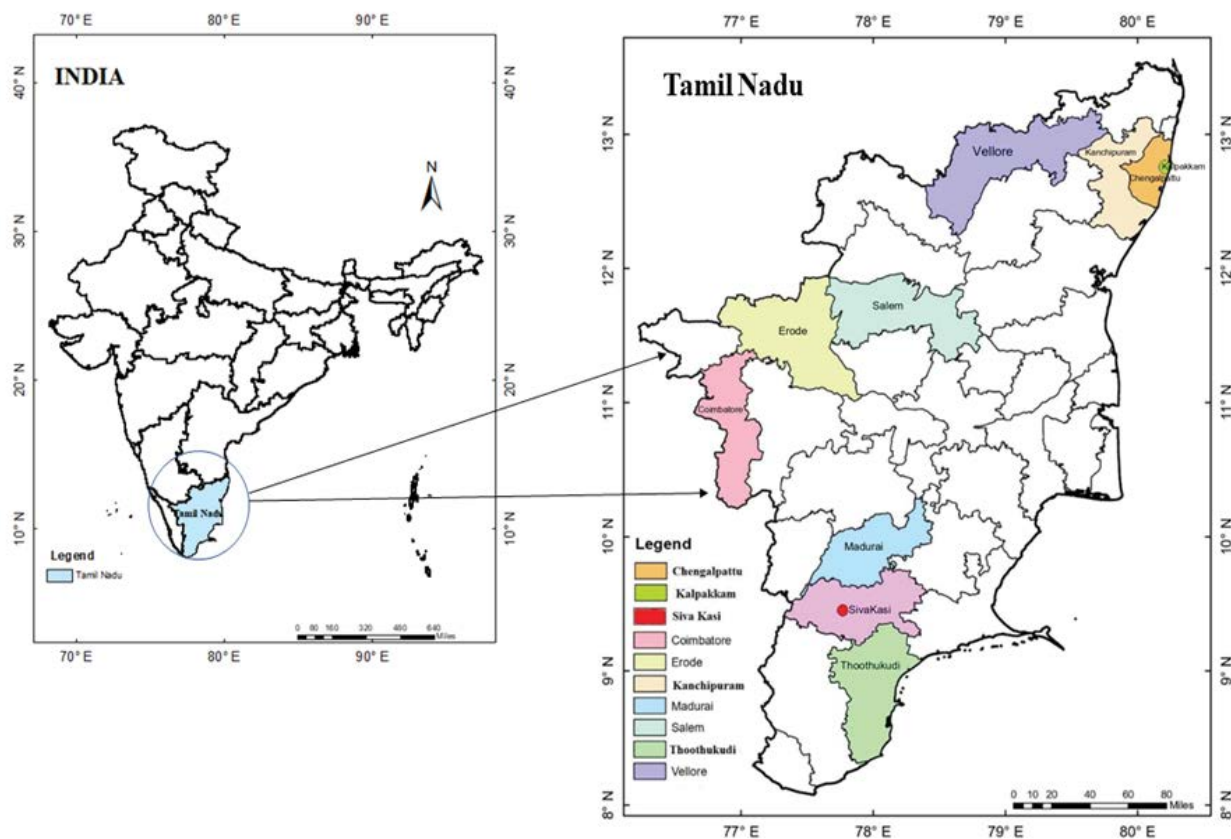


Fig. 1. Location map showing different sample collection sites in various districts of Tamil Nadu.

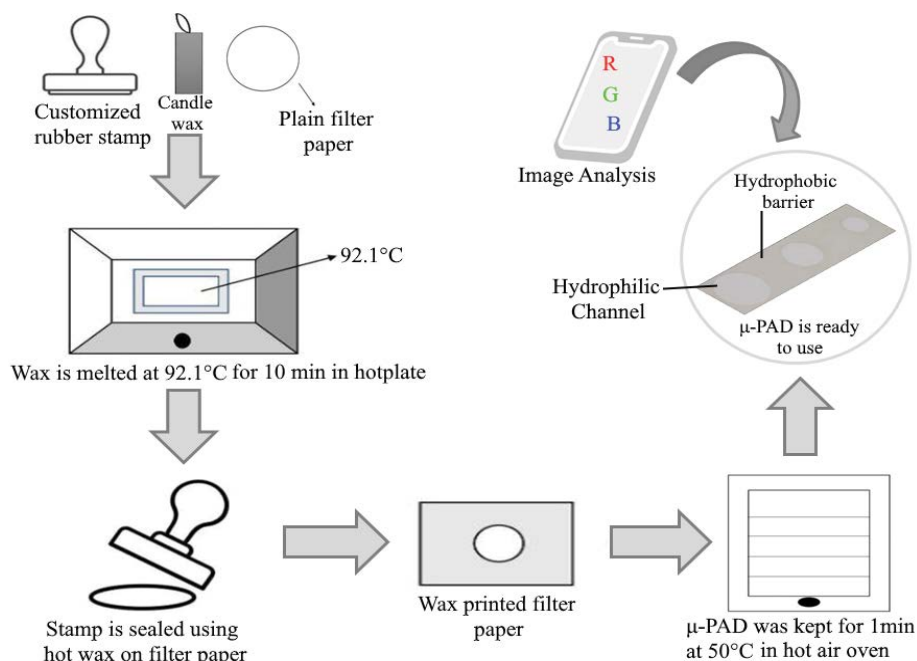


Fig. 2. Schematic illustration of procedure for fabrication of paper-based analytical device. Integration of device with digital colorimetric analysis enables perchlorate detection.

the substrate with the wax-coated pattern was placed on a hot plate and heated to 90°C for 10 min. The heat treatment caused the wax to melt and permeate the thickness of the paper [34]. Thereafter, the substrate was allowed to cool for 10 min before being stored in a clean and dry area for conducting experiments.

#### 2.4. Perchlorate determination using $\mu$ -PAD

The detection zone was coated with 7.5  $\mu$ L of methylene blue and allowed to settle for about 2 min. Water samples were spiked with a known concentration of perchlorate. 0.9  $\mu$ L of the spiked sample was pipetted onto the detection zone and the color change was recorded. In comparison to macroscale analytical equipment, a paper-based device requires smaller quantities of chemicals and samples. Images were captured using a smartphone [35].

#### 2.5. Image analysis and processing

All images were taken using a smartphone camera in default mode without flash. Image acquisition was done by placing devices inside an imaging box with dimensions of (10 cm  $\times$  10 cm  $\times$  10 cm), equipped with light emitting diodes (LEDs) providing a light intensity of 4,000 lx, ensuring no interference from surrounding illumination conditions [36]. The captured images were transferred to a computer system for analysis. ImageJ was employed for image analysis and to gather detailed information for each RGB channel.

#### 2.6. Analytical performance evaluation

It is essential to assess the specificity, interference, and stability of the microfluidic assay. Specificity and interference

were evaluated using perchlorate and other anions such as fluoride, tartrate, bromide, and nitrate. The specificity of the reagent was also checked by following the standard experimental protocol. In this case, Coomassie brilliant blue was used as a reagent instead of methylene blue. The color intensities were recorded and compared with those of the methylene blue-coated paper-based device [37,13]. Furthermore, the stability of the developed microfluidic device was evaluated by observing the color stability of the product resulting from the chemical interaction between methylene blue and perchlorate for a duration of 24 h after conducting the experiment.

#### 2.7. Parametric measurements of real samples

A digital pH meter was used to determine the pH, salinity, total dissolved solids (TDS), and conductivity of real water samples (Oakton PCS Tester 35). Instruments and sensors were calibrated and washed with deionized water before testing the solutions at room temperature.

### 3. Results and discussion

#### 3.1. Digital colorimetric analysis for perchlorate detection

The colorimetric assay used for perchlorate detection on paper-based analytical devices is based on formation of a purple color complex due to the interaction between perchlorate and methylene blue. The presence or absence of a target analyte can be derived from an observed color shift in qualitative colorimetric tests; no extra procedures are frequently required to interpret the data. However, further examination of the colorimetric signal generated by the reaction is necessary for quantitative colorimetric tests [38].

To validate the formation of hydrophilic detection zones, an easily accessible methylene blue solution was used. Methylene blue solution diffuses radially outward from the center of the zone. When the fluid comes into contact with the wax border the diffusion stops indicating confinement on all sides of the detecting zone. A color change in the detection zone in the presence of perchlorate served as an indicator of perchlorate contamination in the water sample.

Methylene blue, a cationic dye, interacts with the perchlorate ion and forms a complex. This interaction results in the formation of an ion pair between the perchlorate anion and the adsorbed methylene blue cation.  $MB^+ ClO_4^-$  has a weaker hydration sphere suitable for hydrophobic expulsion than  $MB^+ Cl^-$  produced with the smaller and thus more hydrophilic chloride anion. Consequently, the addition of perchlorate leads to the formation of a purple color in the detection zone. Fig. 3a illustrates the colorimetric response when water samples with varying perchlorate concentrations

are added to the paper-based analysis devices. Observing a change in the color signal is a qualitative method of perchlorate detection. It is also quantifiable, as discussed in the subsequent subsection. The integration of paper-based sensors with digital smartphone imaging facilitates quick data gathering, processing, analysis, and transmission to stakeholders, making the approach a likely contender for commercial acceptance.

### 3.2. Quantitative analysis and performance testing

The digital images are processed to obtain quantitative information for correlating perchlorate concentration with color intensity. The grayscale color intensity value is calculated for each detection zone using Eq. (1) from primary color intensity values.

$$GSI = 0.2126R + 0.7152G + 0.0722B \quad (1)$$

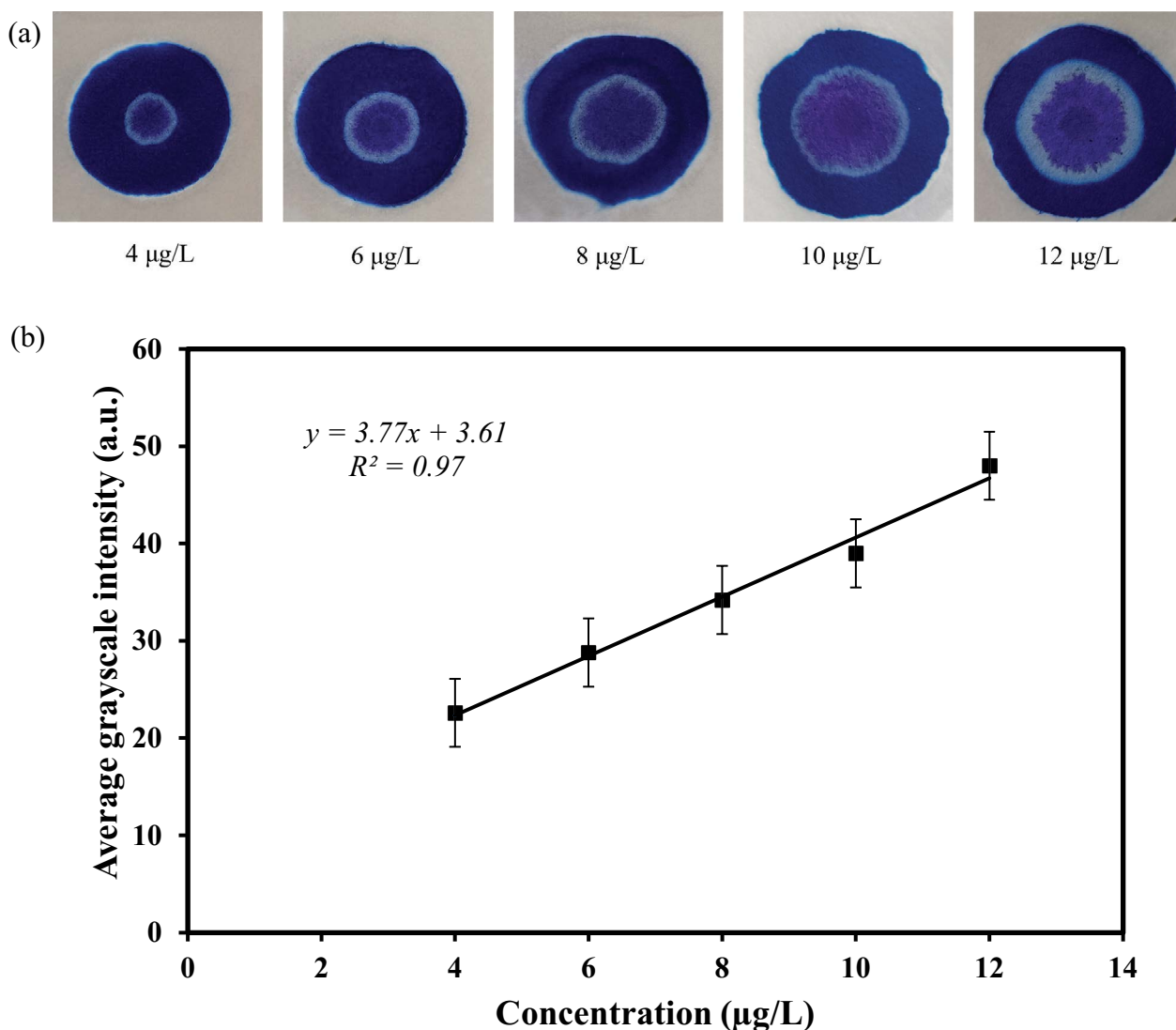


Fig. 3. Paper-based colorimetric assay for perchlorate detection. (a) Digital images of paper-based analytical devices for various concentrations of perchlorate in range between 4 and 12 µg/L. (b) Calibration plot for average grayscale color intensity as a function of perchlorate concentration.



where GSI indicates grayscale intensity and *R*, *G*, and *B* represent the relative intensities of the red, green, and blue color channels, respectively. The scale was inverted to achieve the final average grayscale intensity (*I*) by subtracting GSI from 255, using Eq. (2):

$$I = 255 - \text{GSI} \tag{2}$$

Fig. 3b illustrates the calibration plot with average grayscale intensity values on *y*-axis for and perchlorate concentration on *x*-axis. The grayscale is advised to avoid the subjectivities of diverse observers. The data points were fitted and the linear equation ( $y = 3.77x + 3.61$ ) provided the best fit with coefficient of determination ( $R^2 = 0.97$ ). The calibration graph plot can readily determine the perchlorate concentration in unknown water samples using the platform when imaged under standard conditions.

The intensities of the red, green, and blue color channels were recorded for different real water samples followed by the calculation of grayscale intensity values. The samples indicated the maximum gray scale intensities in the drinking water sample of Chengalpattu (CP-1) and groundwater samples of Kanchipuram District (KP-3). The surface water samples of Erode (ED-2) indicated the second-highest intensities, trailed by the drinking water samples of Coimbatore (CT-1) and Erode Districts (ED-1). Thus, only slight differences in the grayscale intensities were observed in different types of water samples from other districts, as evident in Fig. 4. However, all the samples indicated the presence of perchlorate in them. Thus, paper-based device could be used for rapid screening of perchlorate in samples. The positive results in water samples suggest continuing cautiously in case of further testing or using water samples. Adverse test outcomes do not approve a lack of perchlorate unless it is known that the sampling techniques were satisfactory.

Further, the analytical figures of merit of the paper-based assay were examined. The limit of detection (LOD) is calculated using the expression given in Eq. (3):

$$\text{LOD} = \frac{3.3\sigma}{s} \tag{3}$$

where  $\sigma$  is the standard deviation of the blank response and *s* is the slope of the calibration.

The limit of quantification (LOQ), defined as the minimum concentration of analyte in a sample that can be quantitatively determined with sufficient precision and accuracy, is calculated using Eq. (4). [39].

$$\text{LOD} = \frac{10\sigma}{s} \tag{4}$$

LOD and LOQ of the paper-based perchlorate assay were estimated to be 3.41 and 10.34  $\mu\text{g/L}$ , respectively. These findings demonstrate the viability of the approach for measuring low levels of perchlorate in water.

### 3.3. Stability and specificity analysis

The stability of the signal readout in the assay was assessed by monitoring the colorimetric signal intensity over time after the reaction [37]. Images were captured at various time intervals within a 24-h period following the completion of the reaction. Fig. 5 displays the average grayscale intensity results for experiments conducted with a perchlorate concentration of 10  $\mu\text{g/L}$ . The intensity levels stayed within 9% of the initial signal intensity for the first 6 h. After 12 h the signal decreased by approximately 12% compared to the initial value, and after 24 h, it decreased by about 28%. These findings suggest that capturing images within 6 h of

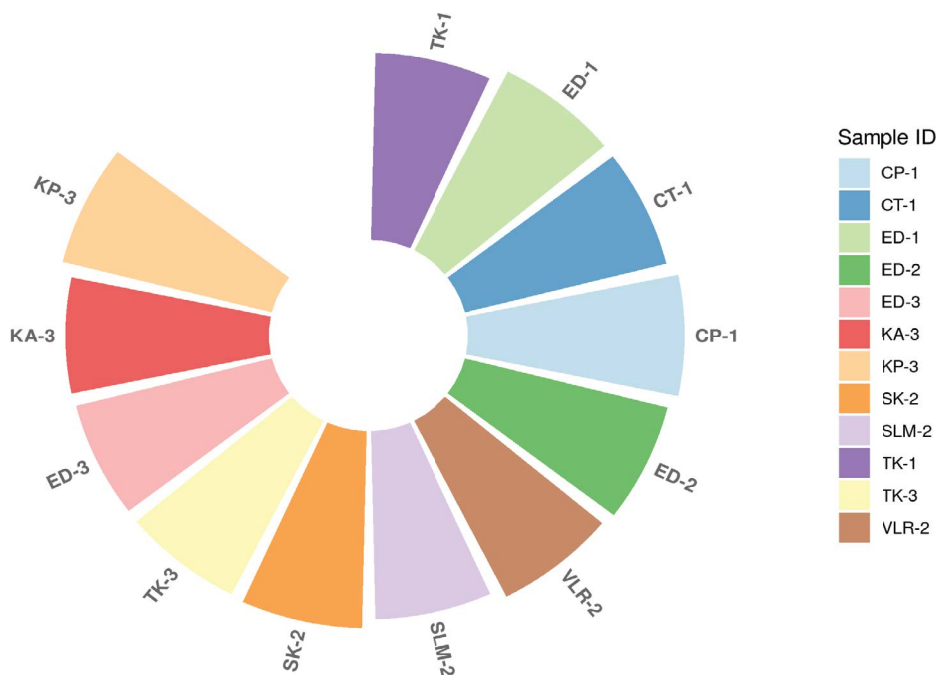


Fig. 4. Real water sample analysis using paper-based analytical devices.

the reaction yields the most reliable results, as the intensities remained relatively steady within this duration. This time frame is reasonable for the user, given the quick nature of the imaging step. The experiments were limited to a short duration within the scope of this work due to the simplicity of the procedure involved. Long-term studies can be conducted in a similar manner to explore specific aspects and diverse applications [13].

The specificity of the reagent was evaluated using the standard experimental procedure employed for paper-based perchlorate assays. Coomassie brilliant blue was used as the reagent coated on the paper, replacing methylene blue. Color intensities were recorded and compared with the methylene blue-coated paper-based device. The results showed a reduction in color intensity of approximately 23% when compared to methylene blue, as seen in Fig. 6. Also, no purple color complex formation occurred, indicating that Coomassie brilliant blue-coated paper-based devices are unsuitable for perchlorate detection. Therefore, it is evident that methylene blue-coated paper-based devices are specifically apt for determination of perchlorate in water.

The efficacy of perchlorate detection requires consideration of potential interfering components that can affect the accuracy and reliability of results. Co-ions present in the water may exhibit similar colorimetric responses or spectral properties, potentially leading to cross-reactivity and false positives. Fig. 7 provides a visualized representation of the

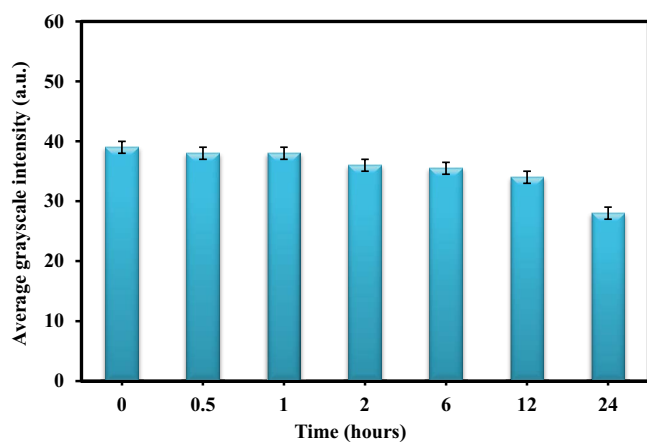


Fig. 5. Stability of the paper-based colorimetric assay. The colorimetric signal output was examined over time for a 24-h period after the completion of the reaction.

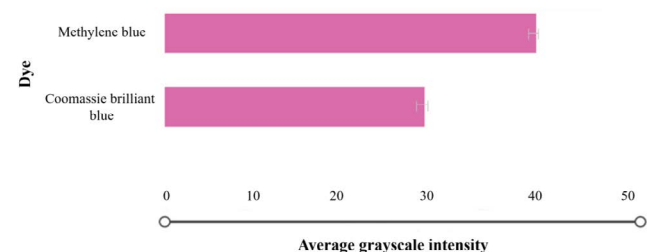


Fig. 6. Specificity of the reagent for paper-based colorimetric assay. The color intensities were compared for devices coated with methylene blue and Coomassie brilliant blue.

results of specificity and interference tests using different anions in water samples. Specificity tests involved examining the interaction of methylene blue with individual ions, specifically nitrate, fluoride, tartrate, and bromide, and comparing their responses with that of perchlorate. The colorimetric signal output was found to be specific to the presence of perchlorate. This specificity could be attributed to the absence of complex formation between methylene blue and other ions or only minimal interaction, resulting in a very weak color intensity. Interference was assessed by comparing the colorimetric signal of a mixture of anions ( $\text{Br}^-$ ,  $\text{F}^-$ ,  $\text{NO}_3^-$ ,  $\text{C}_4\text{H}_4\text{O}_6^{2-}$ ) added to a perchlorate-containing solution with that of perchlorate alone. The colorimetric signals showed almost identical intensities, indicating an absence of interference from the investigated anions. Moreover, oxidative compounds within the samples could plausibly interfere with the detection if they interact with methylene blue in an analogous manner to perchlorate. These compounds might induce reactions that modify the colorimetric response, leading to erroneous outcomes. For instance, the presence of any interfering oxidants could cause color changes unrelated to perchlorate concentrations, resulting in misleading interpretations. While such reports are not readily available in the open literature, this aspect warrants systematic exploration. In such scenarios, it would be advisable to incorporate appropriate pre-treatment steps to eliminate undesirable interactions. Thus, understanding the influence of oxidative compounds and potential interferences is crucial to ensure the specificity and reliability of perchlorate detection methods, contributing to accurate assessments of water quality.

#### 3.4. Parametric measurements

The pH level, salinity, TDS concentration, and conductivity of the water samples from each district were analyzed as part of the characterization process. The outcomes of these parametric measurements are summarised here. In field circumstances, pH is a significant factor in perchlorate biodegradation. It is often observed to influence the biodegradation process. Perchlorate contamination tends to alter the pH levels of water bodies. Upon its introduction, it can lead to a decrease in pH, rendering the water more acidic. This pH shift can disrupt the delicate balance of aquatic ecosystems, affecting the health and survival of organisms that are sensitive to alterations in acidity. Significant shifts in pH are frequently associated with the release of harmful effluents from industrial processes or the disposal of chemical waste products. The pH range conducive to perchlorate decomposition typically falls between 5 and 9, with the most effective reduction occurring within the alkaline range of 7.5–8.5 [40]. The pH range measured in this study was 7.02–8.3, as shown in Fig. 8. For both surface water and groundwater, it is recommended to maintain a pH level within the range of 6.5 to 8.5 [41]. Consequently, it was observed that the pH of numerous samples approached neutrality at approximately 7.2, and all the samples adhered to the recommended pH range.

Furthermore, the presence of perchlorate can influence salinity levels in water. Perchlorate contamination often occurs alongside other ions, potentially leading to an increase in overall salinity. Elevated salinity levels can

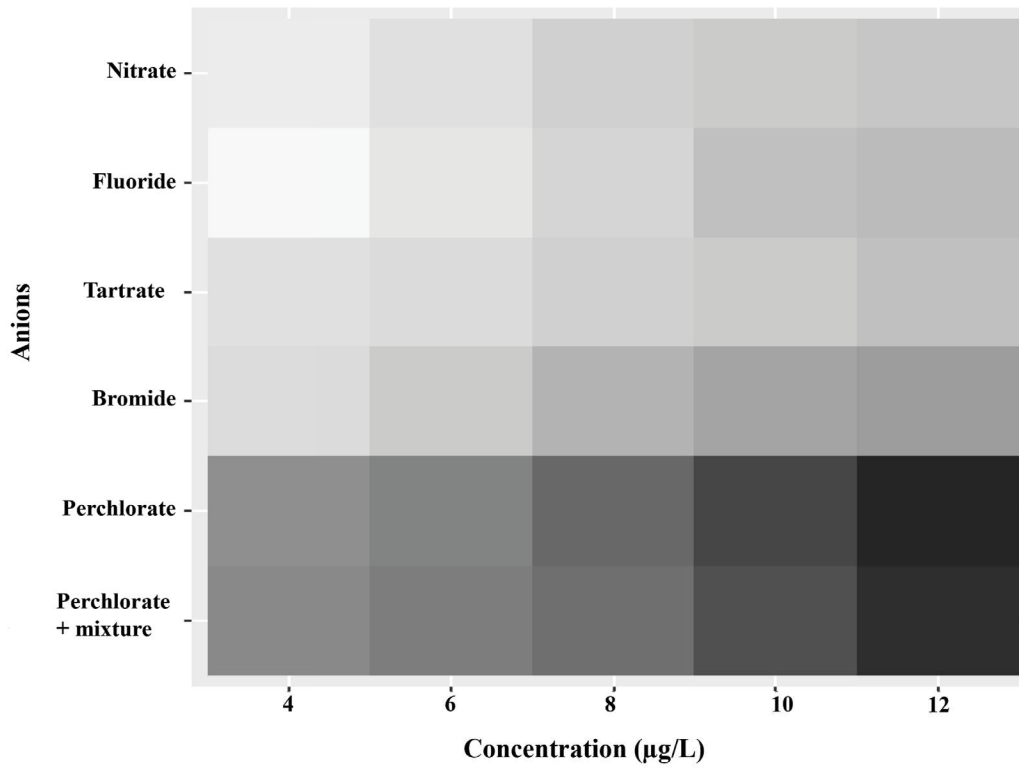


Fig. 7. Specificity and interference tests on the paper-based analytical devices.

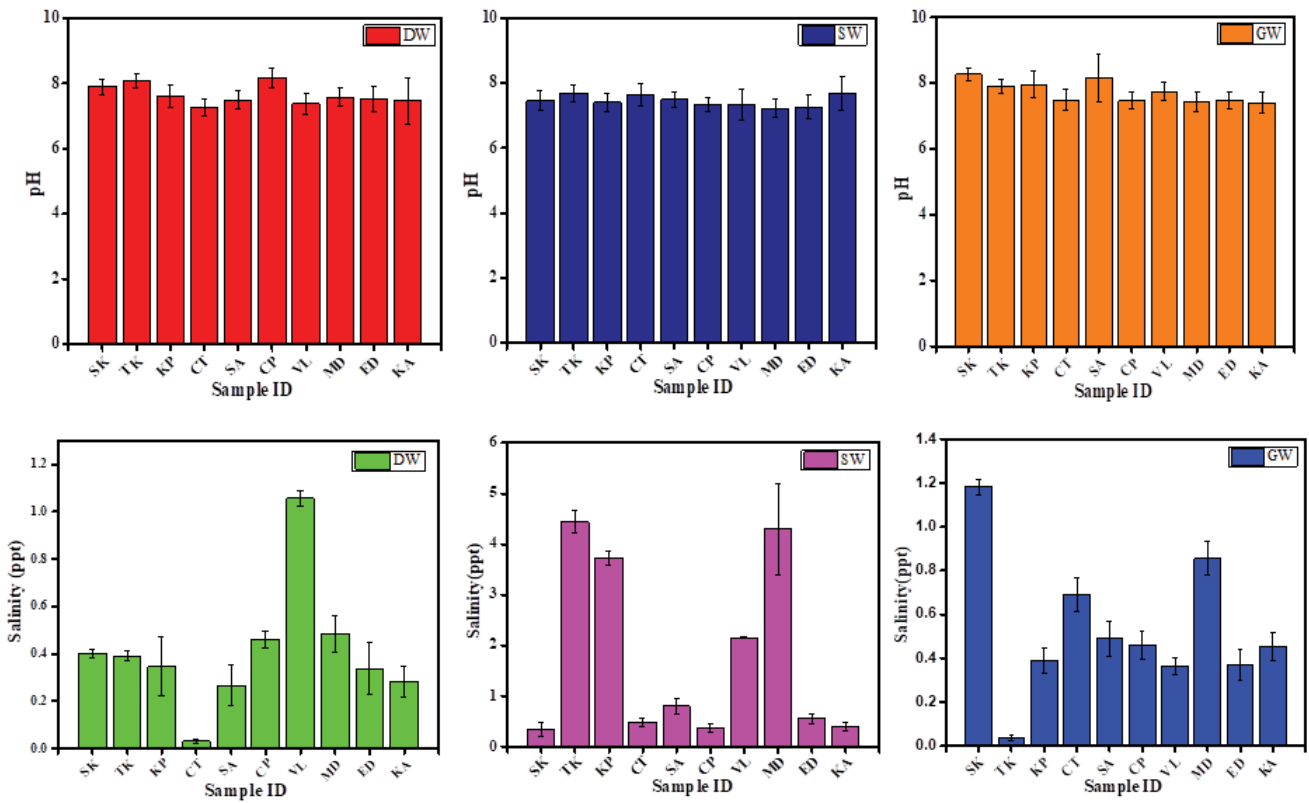


Fig. 8. pH and salinity of drinking water, surface water, and groundwater samples from various locations.



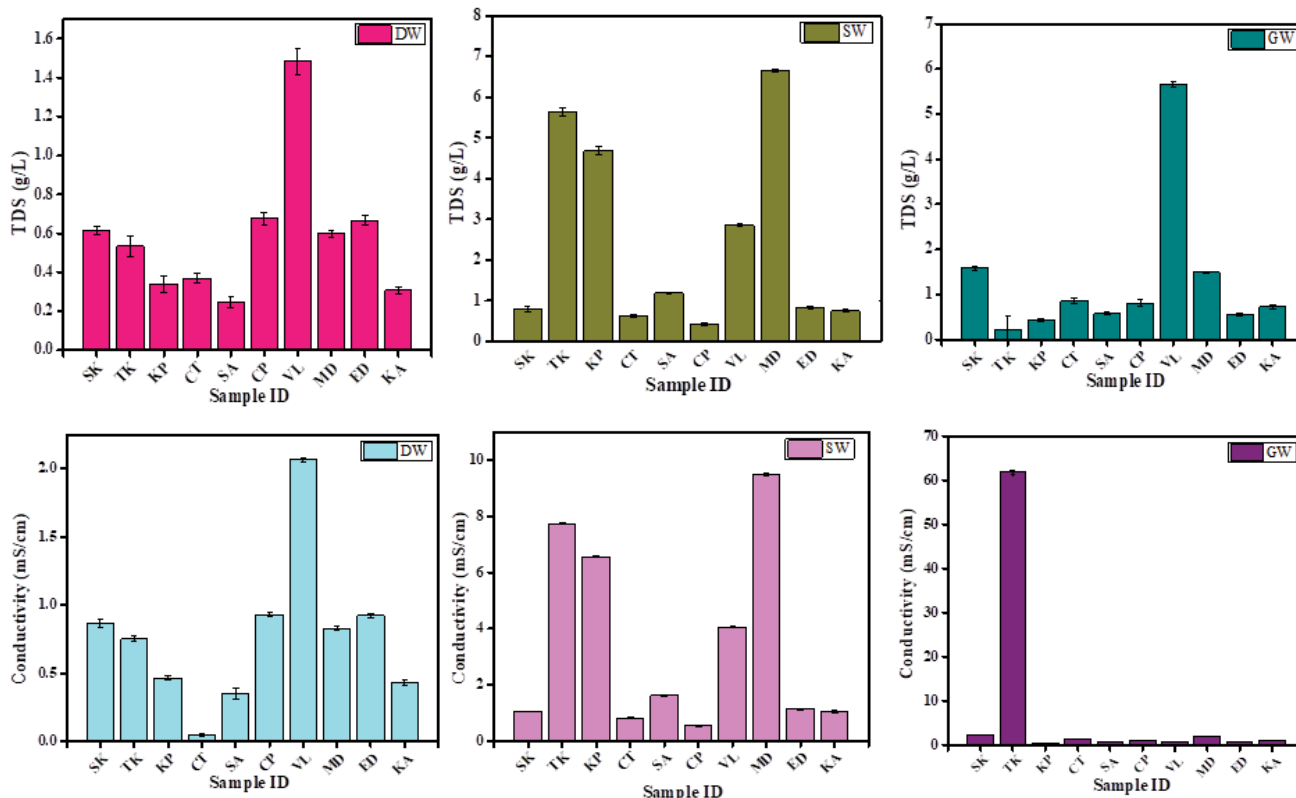


Fig. 9. Total dissolved solids and conductivity of drinking water, surface water, and groundwater samples from various locations.

have detrimental effects on aquatic organisms adapted to specific salt concentrations, potentially disrupting their physiological processes. The water samples were subjected to quantification of their salinity and the results ranged from 0.03 to 5.2 ppt, as displayed in Fig. 8. It is reported that the microorganisms involved in perchlorate biodegradation tend to thrive under high saline conditions [42]. Fig. 8 illustrates the differences in salinity among drinking water, groundwater, and surface water samples. The hardness of ground or surface water often rises as pH levels decrease. Higher salinity can affect microorganisms because the solubility of oxygen decreases. An increase in salinity levels can be attributed to industrial effluents released into water sources, potentially impacting crop yields. Therefore, it is recommended to treat industrial effluents before they are released into the environment.

Conductivity is also influenced by the presence of ions like perchlorate. Higher concentrations of perchlorate lead to an increase in conductivity due to the greater number of charged particles in the water. This increased conductivity can be indicative of pollution and may affect the behavior of aquatic organisms, particularly those sensitive to changes in their electrochemical environment. The water samples were also subjected to quantification of TDS and conductivity. Conductivity is affected by factors like mobility, temperature, and total ionic concentration. TDS refer to the concentration of inorganic and organic substances dissolved in water. Perchlorate, being a soluble ion, contributes to total dissolved solids levels in water bodies. Elevated TDS can impact water quality, affecting the taste, odor, and overall

suitability for consumption. Additionally, increased TDS levels can lead to problems in water treatment processes, posing challenges for municipal water supply systems. It should be noted that total dissolved solids primarily indicate physico-chemical characteristics, and may not necessarily indicate pollution or environmental hazards. Regulatory bodies typically recommend that the TDS level in drinking water should not exceed 0.5 g/L. Fig. 9 displays the variation in total dissolved solids, ranging from 0.28 to 6.68 g/L, suggesting that TDS exceeded the standard limit in some cases. The conductivity values of all water samples fell within the range of 0.05 to 9.52 mS/cm. According to the results, drinking water exhibited the lowest conductivity (0.05 mS/cm), while surface water showed the highest values. In most circumstances, a range of 0.05 to 1.5 mS/cm is considered acceptable for drinking water. Therefore, samples from various regions that fall outside this range should not be regarded as suitable for human consumption.

#### 4. Conclusion

This study has successfully demonstrated the development of a cost-effective paper-based microfluidic platform for the rapid and precise detection of perchlorates in water samples. The approach utilizes readily available materials and harnesses smartphone image acquisition and digital analysis for quantitative data interpretation, making it a highly practical and suitable solution for analysis of environmental contaminants. The microfluidic device designed and fabricated in this study holds great promise as a rapid

screening tool for detecting perchlorates in water samples. Furthermore, the study rigorously assessed the stability and specificity of the microfluidic assay. By combining visual colorimetric detection with smartphone technology, this technique has the potential to advance the development of portable devices for on-site detection of contaminants, promoting sustainability by reducing the need for extensive laboratory resources and infrastructure. Overall, this study contributes to the development of an innovative and sustainable method for the quantitative detection of perchlorate, offering promise for a wide range of applications in the field of water quality monitoring.

### Acknowledgments

The authors would like to acknowledge the Department of Biotechnology, School of Bioengineering, and SRM Institute of Science and Technology for providing the necessary facilities for the research.

### Author contribution

MuthuKumar Raghunathan: was involved in the supervision, resources, investigation, and conceptualization.

Praveen Kumar: contributed to the conceptualization of the study, visualization, experimentation, validation, and data analysis, writing-original draft preparation, review and editing. Ashish Kapoor: contributed to data analysis, review and editing.

### Funding

The execution of the project was not supported by any funding agencies.

### Data and material availability

All data generated or analyzed during this study are included in this article. We would like to declare that the work described was original research that has not been published previously and is not under consideration for publication elsewhere, in whole or in part.

### Declarations

#### Ethics approval and consent to participate

Not applicable.

#### Competing interests

The authors declare no competing interests.

### References

- [1] I. Narin, M. Soylak, L. Elçi, M. Doğan, Determination of trace metal ions by AAS in natural water samples after preconcentration of pyrocatechol violet complexes on an activated carbon column, *Talanta*, 52 (2000) 1041–1046.
- [2] T. Minami, W. Konagaya, L. Zheng, S. Takano, M. Sasaki, R. Murata, Y. Sohrin, An off-line automated preconcentration system with ethylenediaminetriacetate chelating resin for the determination of trace metals in seawater by high-resolution inductively coupled plasma mass spectrometry, *Anal. Chim. Acta*, 854 (2015) 183–190.
- [3] B. Bansod, T. Kumar, R. Thakur, S. Rana, I. Singh, A review on various electrochemical techniques for heavy metal ions detection with different sensing platforms, *Biosens. Bioelectron.*, 94 (2017) 443–455.
- [4] N.J. Vickers, Animal communication: when I'm calling you, will you answer too?, *Curr. Biol.*, 27 (2017) R713–R715.
- [5] M.B. Gumpu, S. Sethuraman, U.M. Krishnan, J.B.B. Rayappan, A review on detection of heavy metal ions in water-an electrochemical approach, *Sens. Actuators, B*, 213 (2015) 515–533.
- [6] D. Santra, S. Mandal, A. Santra, U.K. Ghorai, Cost-effective, wireless, portable device for estimation of hexavalent chromium, fluoride, and iron in drinking water, *Anal. Chem.*, 90 (2018) 12815–12823.
- [7] R. Pol, F. Cespedes, D. Gabriel, M. Baeza, Microfluidic lab-on-a-chip platforms for environmental monitoring, *TrAC, Trends Anal. Chem.*, 95 (2017) 62–68.
- [8] S. Solanki, C.M. Pandey, R.K. Gupta, B.D. Malhotra, Emerging trends in microfluidics based devices, *Biotechnol. J.*, 15 (2020) 1900279, doi: 10.1002/biot.201900279.
- [9] H. Lim, A.T. Jafry, J. Lee, Fabrication, flow control, and applications of microfluidic paper-based analytical devices, *Molecules*, 24 (2019) 2869, doi: 10.3390/molecules24162869.
- [10] M.M. Gong, D. Sinton, Turning the page: advancing paper-based microfluidics for broad diagnostic application, *Chem. Rev.*, 117 (2017) 8447–8480.
- [11] M. Rezazadeh, S. Seidi, M. Lid, S. Pedersen-Bjergaard, Y. Yamini, The modern role of smartphones in analytical chemistry, *TrAC, Trends Anal. Chem.*, 118 (2019) 548–555.
- [12] A. Fatoni, Supiani, D.W. Dwiasi, M.D. Anggraeni, Introducing colorimetric analysis with document scanner for high school students, *J. Phys. Conf. Ser.*, 1494 (2020) 012026, doi: 10.1088/1742-6596/1494/1/012026.
- [13] S. Balasubramanian, A. Udayabhanu, P.S. Kumar, P. Muthamilselvi, C. Eswari, A. Vasantavada, S. Kanetkar, A. Kapoor, Digital colorimetric analysis for estimation of iron in water with smartphone-assisted microfluidic paper-based analytical devices, *Int. J. Environ. Anal. Chem.*, 103 (2023) 2480–2497.
- [14] A. Apilux, W. Siangproh, N. Praphairaksit, O. Chailapakul, Simple and rapid colorimetric detection of Hg(II) by a paper-based device using silver nanoplates, *Talanta*, 97 (2012) 388–394.
- [15] A.M. Leung, L.E. Braverman, E.N. Pearce, History of U.S. iodine fortification and supplementation, *Nutrients*, 4 (2012) 1740–1746.
- [16] F. Cao, J. Jaunat, N. Sturchio, B. Cances, X. Morvan, A. Devos, P. Ollivier, Worldwide occurrence and origin of perchlorate ion in waters: a review, *Sci. Total Environ.*, 661 (2019) 737–749.
- [17] P. Kumarathilaka, C. Oze, S.P. Indraratne, M. Vithanage, Perchlorate as an emerging contaminant in soil, water and food, *Chemosphere*, 150 (2016) 667–677.
- [18] R. Calderon, F. Godoy, M. Escudey, P. Palma, A review of perchlorate (ClO<sub>4</sub><sup>-</sup>) occurrence in fruits and vegetables, *Environ. Monit. Assess.*, 189 (2017) 82, doi: 10.1007/s10661-017-5793-x.
- [19] T.W. Collette, T.L. Williams, E.T. Urbansky, M.L. Magnuson, G.N. Hebert, S.H. Strauss, Analysis of hydroponic fertilizer matrices for perchlorate: comparison of analytical techniques, *Analyst*, 128 (2003) 88–97.
- [20] F. Zapata, C. Garcia-Ruiz, The discrimination of 72 nitrate, chlorate and perchlorate salts using IR and Raman spectroscopy, *Spectrochim. Acta, Part A*, 189 (2018) 535–542.
- [21] P. Kuban, I.K. Kiplagat, P. Bocek, Electrokinetic injection across supported liquid membranes: new sample pretreatment technique for online coupling to capillary electrophoresis. Direct analysis of perchlorate in biological samples, *Electrophoresis*, 33 (2012) 2695–2702.
- [22] C. Wang, H. Chen, L. Zhu, X. Liu, C. Lu, Accurate, sensitive and rapid determination of perchlorate in tea by hydrophilic interaction chromatography-tandem mass spectrometry, *Anal. Methods*, 12 (2020) 3592–3599.

- [23] S. Zhu, X. Zhang, J. Cui, Y.E. Shi, X. Jiang, Z. Liu, J. Zhan, Silver nanoplate-decorated copper wire for the on-site microextraction and detection of perchlorate using a portable Raman spectrometer, *Analyst*, 140 (2015) 2815–2822.
- [24] B.C. Okeke, G. Ma, Q. Cheng, M.E. Losi, W.T. Frankenberger Jr., Development of a perchlorate reductase-based biosensor for real time analysis of perchlorate in water, *J. Microbiol. Methods*, 68 (2007) 69–75.
- [25] S.A. Alsaleh, L. Barron, S. Sturzenbaum, Perchlorate detection via an invertebrate biosensor, *Anal. Methods*, 13 (2021) 327–336.
- [26] I. Iwasaki, S. Utsumi, C. Kang, The spectrophotometric determination of micro amounts of perchlorate by the solvent-extraction method, *Bull. Chem. Soc. Jpn.*, 36 (1963) 325–331.
- [27] H. Sun, Y. Liang, L. Zhou, X. Zhang, F. Luo, Z. Chen, Visual detection of perchlorate in aqueous solution using alkali methylene blue, *Anal. Sci.*, 38 (2022) 525–531.
- [28] A.G. Fogg, C. Burgess, D.T. Burns, A critical study of Brilliant green as a spectrophotometric reagent: the determination of perchlorate particularly in potassium chlorate, *Analyst*, 96 (1971) 854–857.
- [29] S.M. Basheer, M. Muralisankar, T.V. Anjana, K.N. Aneesrahman, A. Sreekanth, Multi-ion detection and molecular switching behaviour of reversible dual fluorescent sensor, *Spectrochim. Acta, Part A*, 182 (2017) 95–104.
- [30] S.D. Taylor, W. Howard, N. Kaval, R. Hart, J.A. Krause, W.B. Connick, Solid-state materials for anion sensing in aqueous solution: highly selective colorimetric and luminescence-based detection of perchlorate using a platinum(II) salt, *ChemComm*, 46 (2010) 1070–1072.
- [31] K.L. Peters, I. Corbin, L.M. Kaufman, K. Zreibe, L. Blanes, B.R. McCord, Simultaneous colorimetric detection of improvised explosive compounds using microfluidic paper-based analytical devices ( $\mu$ PADs), *Anal. Methods*, 7 (2015) 63–70.
- [32] B. Keskin, A. Uzer, R. Apak, Colorimetric sensing of ammonium perchlorate using methylene blue-modified gold nanoparticles, *Talanta*, 206 (2020) 120240, doi: 10.1016/j.talanta.2019.120240.
- [33] X. Wang, F. Li, Z. Cai, L. Liu, J. Li, B. Zhang, J. He, Sensitive colorimetric assay for uric acid and glucose detection based on multilayer-modified paper with smartphone as signal readout, *Anal. Bioanal. Chem.*, 410 (2018) 2647–2655.
- [34] A.K. Yetisen, M.S. Akram, C.R. Lowe, Paper-based microfluidic point-of-care diagnostic devices, *Lab Chip*, 13 (2013) 2210–2251.
- [35] T. Akyazi, J. Saez, J. Elizalde, F. Benito-Lopez, Fluidic flow delay by ionogel passive pumps in microfluidic paper-based analytical devices, *Sens. Actuators, B*, 233 (2016) 402–408.
- [36] R. Ghosh, S. Gopalakrishnan, R. Savitha, T. Renganathan, S. Pushpavanam, Fabrication of laser printed microfluidic paper-based analytical devices (LP- $\mu$ PADs) for point-of-care applications, *Sci. Rep.*, 9 (2019) 7896, doi: 10.1038/s41598-019-44455-1.
- [37] I. Ortiz-Gomez, M. Ortega-Muñoz, A. Salinas-Castillo, J.A. Álvarez-Bermejo, M. Ariza-Avidad, I. de Orbe-Payá, F. Santoyo-Gonzalez, L.F. Capitan-Vallvey, Tetrazine-based chemistry for nitrite determination in a paper microfluidic device, *Talanta*, 160 (2016) 721–728.
- [38] E. Azuaje-Hualde, S. Arroyo-Jimenez, G. Garai-Ibabe, M.M. de Pancorbo, F. Benito-Lopez, L. Basabe-Desmots, Naked eye Y amelogenin gene fragment detection using DNAzymes on a paper-based device, *Anal. Chim. Acta*, 1123 (2020) 1–8.
- [39] A. Shrivastava, V.B. Gupta, Methods for the determination of limit of detection and limit of quantitation of the analytical methods, *Chron. Young Sci.*, 2 (2011) 21–25.
- [40] C. Wang, L. Li, S. Chi, Z. Zhu, Z. Ren, Y. Li, G. Cao, Thorium-doping-induced superconductivity up to 56 K in  $Gd_{1-x}Th_xFeAsO$ , *Europhys. Lett.*, 83 (2008) 67006, doi: 10.1209/0295-5075/83/67006.
- [41] J.R. Anoop, L. Muruganandam, Occurrence of perchlorate in drinking, surface, ground and effluent water from various parts of South India, *Int. J. Innovation Eng. Technol.*, 4 (2014) 1–7.
- [42] J.D. Coates, U. Michaelidou, S.M. O'Connor, R.A. Bruce, L.A. Achenbach, The Diverse Microbiology of (Per)Chlorate Reduction, In: *Perchlorate in the Environment*, Springer, Boston, MA, 2000, pp. 257–270.