



On-site detection of heavy metals in wastewater using a single paper strip integrated with a smartphone

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Abstract

A field paper-based heavy metal strip was designed and implemented for simultaneous detection of the heavy metals Zn, Cr, Cu, Pb and Mn in wastewater samples. The colorimetric paper strip was fabricated by drop-casting of chromogenic reagents onto detection zones. When the fabricated paper strip was exposed to Zn, Cr, Cu, Pb and Mn, multiple colors appeared that were then recorded with a smartphone followed by processing in the Color Picker application. After optimizing the analytical parameters, such as the chromogenic concentration, pH and reaction time, the paper strip achieved detection limits of 0.63, 0.07, 0.17, 0.03 and 0.11 mg/L for Zn, Cr, Cu, Pb and Mn, respectively. Five heavy metals analyses were able to be performed within 1 min on one paper strip. This paper strip is accurate with recoveries from 87 to 107%. The results of the proposed paper strip correlated well with those determined by inductively coupled plasma-optical emission spectrometry of wastewater samples. The use of a single paper strip integrated with a smartphone for the detection of five heavy metals in wastewater represents an all-in-one device with on-site detection, leading to cost-effective and rapid assays that show a great application potential for on-site environmental monitoring.

Keywords Heavy metal · Paper strip · Smartphone · Wastewater

Introduction

The rapid growth of industrial factories has caused increased environmental concerns [1]. Factories are major contributors to water pollution through contaminated radioactive or toxic organic compounds or heavy metals that are released into waterways [2–4]. Heavy metal contamination has become an environmental problem because the metals are difficult to degrade and easily accumulate in ecological systems [5, 6]. Among the various heavy metals, zinc (Zn), hexavalent chromium (Cr), copper (Cu), lead (Pb) and manganese (Mn) are highly toxic to humans, causing various adverse health effects such as cancer, neurodegenerative cardiovascular disease and

kidney disease [7, 8]. The Ministry of Natural Resources and Environment (MNRE) in Thailand established maximum concentrations of Zn, Cr, Cu, Pb and Mn for wastewater released from factories as 5, 0.25, 2, 0.2 and 5 mg/L, respectively [9].

As a result, a convenient detection method was needed to monitor heavy metal concentrations. Several detection approaches, such as spectrophotometry [10–12], electrochemical method [13, 14], inductively coupled plasma-optical emission spectrometry (ICP-OES) [15, 16] and atomic absorption spectroscopy (AAS) [17–20], have been developed for heavy metal detection. Although these techniques are highly sensitive and precise, they all require expensive instruments, skilled technicians and laborious operations, which are not suitable for on-site analysis. One of the key objectives for method development is to rapidly and easily analyze heavy metals in wastewater to monitor water quality.

Over the past decade, paper-based analytical devices (PADs) have garnered increasing attention due to their attractive features including the ability for concurrent on-site detection, cost-effectiveness and multiple analysis detection. A paper sheet is used as a device substrate, which is a cheap and easy-to-handle support [21–23]. PADs such as microfluidic PADs (μ PADs) [24], lateral flow assays [25] or paper strip

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test assays [26] have increased the development of analytical tools for applications in food safety [27], health care [28] and environmental science [29]. Many reports have demonstrated PADs that can detect heavy metals. For example, Marzo et al. [25] presented a lateral flow assay to detect cadmium (Cd) by gold nanoparticles on the basis of color change. Rattanarat et al. [30] developed three-dimensional μ PADs for multiple heavy metals—nickel (Ni), ferric (Fe), Cu and Cr—using colorimetric detection and electrochemical detection of Pb and Cd. In addition, Li et al. [31] fabricated a colorimetric PAD to detect Fe^{2+} in tap water. Phenanthroline was added on a paper strip to react with Fe^{2+} , generating an orange-red complex. The intensity of the color was measured based on the light reflectance principle. However, previous work has not been suited for on-site applications to monitor environmental pollutants, due to the desktop equipment, complicated procedures and time-consuming processes required. Various types of detectors for PAD smartphones, including fluorescence-, electrochemistry- and chemiluminescence-based applications, have shown potential for use in portable detection, thus representing approaches for on-site detection.

With the above considerations, we aimed to develop a paper strip for simple and on-site heavy metals detection using a smartphone as a detector. Five heavy metals—Zn, Cr, Cu, Pb and Mn—are formed in complexes with selective chromogenic reagents to form colored products on a single paper strip. For quantitative analysis, the photographs of the reaction color were acquired by a smartphone. All parameters affecting the colorimetric response were optimized. We evaluated the linear range, limit of detection, quantitation, accuracy, precision and selectivity of the assay. To determine the applicability of the paper strip for field detection, on-site detections of Zn, Cr, Cu, Pb and Mn in wastewater were performed. The proposed and analytical results were compared with the ICP-OES technique. Compared with other colorimetric PADs, the proposed paper strip has several advantages, including cost-effectiveness, speed and simplicity of fabrication (see Electronic Supplementary Material [ESM] Table S1). This smartphone-based paper strip detection system will be a potential device applied in field pollutant analysis. The proposed paper strip exhibits good accuracy with a low detection limit and a potential device applied in field pollutant analysis.

Materials and methods

Reagents and apparatus

Zinc (II) sulfate (ZnSO_4), potassium chromate (K_2CrO_4), copper (II) chloride (CuCl_2), lead (II) nitrate ($\text{Pb}(\text{NO}_3)_2$), nickel (II) nitrate ($\text{Ni}(\text{NO}_3)_2$), manganese (II) sulfate monohydrate ($\text{MnSO}_4 \cdot \text{H}_2\text{O}$), sodium hydroxide (NaOH), sodium dihydrogen phosphate dehydrate ($\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$), disodium

hydrogen orthophosphate (Na_2HPO_4), ammonium chloride (NH_4Cl) and sodium cyanide (NaCN) were purchased from Ajax Finechem (Victoria, Australia). Zincon monosodium salt (ZI; $\text{C}_{20}\text{H}_{15}\text{N}_4\text{NaO}_6\text{S}$), 1,5-diphenylcarbazide (DPC; $\text{C}_{13}\text{H}_{14}\text{N}_4\text{O}$), cuprizone (CPZ; $\text{C}_{14}\text{H}_{22}\text{N}_4\text{O}_2$), alizarin red S (ALS; $\text{C}_{14}\text{H}_7\text{NaO}_7\text{S}$) and 1-(2-pyridylazo)-2-naphthol (PAN; $\text{C}_{15}\text{H}_{11}\text{N}_3\text{O}$) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Ammonia was purchased from Applichem Panreac (Ottoweg, Darmstadt, Germany). Cyclohexanone was purchased from Alfa Aesar (Heysham, Lancashire, UK). Hydrochloric acid, sulfuric acid, methanol, ethanol and chloroform were purchased from RCI Labscan (Pathumwan, Bangkok, Thailand). Standard solutions of Zn, Cr, Cu, Pb and Mn were prepared by buffer solutions. Warning: Sodium cyanide may be hazardous to health and safety.

An Alcatel Shine Lite 5080X smartphone (5.0" HD display and camera at 13.0 megapixel) was used to take an image of the paper strip, and the image was then converted to an intensity value by the Color Picker application. A 10×10 -cm homemade box was constructed using gray paper cardboard sheets. A white light-emitting diode (LED, 36 W) was used as control illumination in the box. An optical window with a diameter of 0.5 cm was drilled on the top of the box to contact the smartphone camera.

The quantitative determination of Zn, Cr, Cu, Pb and Mn in real samples was performed by the ICP-OES technique under the following conditions: 1100–1500 watts of forward power, 14–18-mm viewing height, 15–19 L/min of argon-coolant flow, 0.6–1.5 L/min of argon-nebulizer flow, 1.0–1.8-mL/min sample-pumping rate with a 1-min pre-flush time and measurement time near 1 s/wavelength peak for sequential instruments and 10 s/sample for simultaneous instruments.

Fabrication of a paper strip for the detection of Zn, Cr, Cu, Pb and Mn

The paper strip was fabricated by a simple and cost-effective cutting technique. As shown in Fig. 1a, the paper strip consisted of five detection zones, where the selective chromogenic reagent was immobilized for analysis of Zn, Cr, Cu, Pb and Mn. To prepare each detection zone, chromatography paper was cut in dimensions of 0.5×0.5 cm. Each piece of paper was then placed on drawing paper (0.5×6 cm) using double-sided adhesive tape as a connector.

Detection of Zn, Cr, Cu, Pb and Mn using a paper strip integrated with a smartphone

First, 3 μL of selective chromogenic reagents were added to each detection zone. The detection zones on the paper strip were numbered 1–5 as shown in Fig. 1a. Position number 1 was modified with 0.4 mM ZI, cyanide and cyclohexanone; number 2 was modified with 4 mM DPC; 2 mM CPZ was

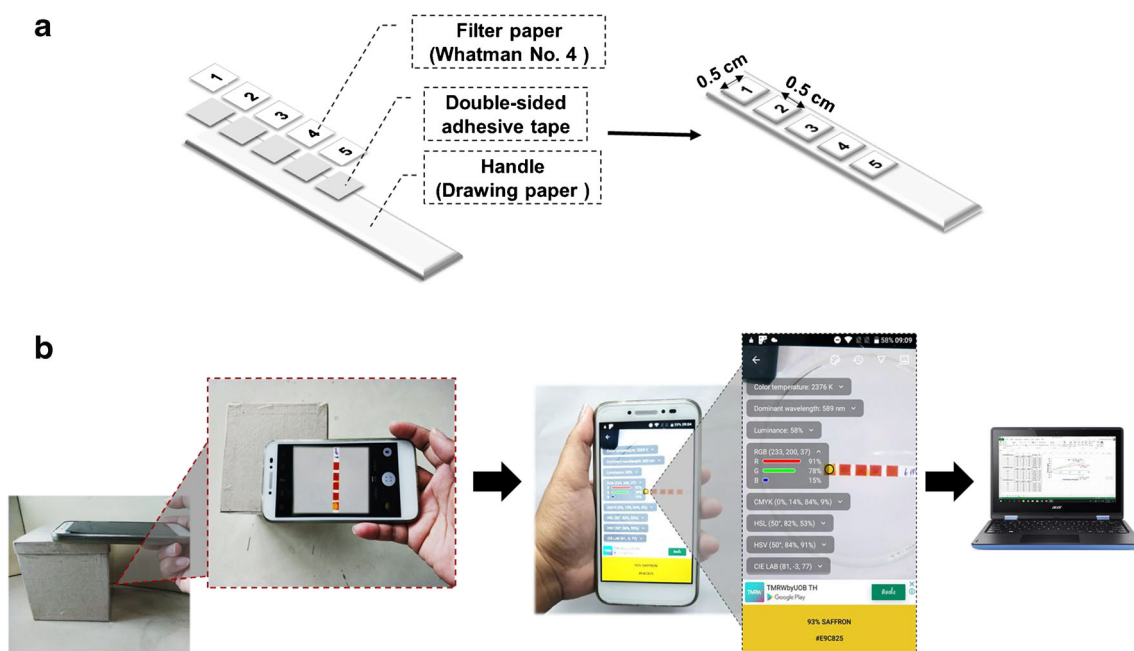


Fig. 1 Schematic illustration of **(a)** paper strip fabrication and **(b)** image processing

added to number 3; number 4 was filled with 2 mM ALS; and number 5 was modified with 5 mM PAN and cyanide. Then, a standard solution (Zn, Cr, Cu, Pb and Mn) or real sample solution was dropped onto each detection zone. In the case of a wastewater sample, the solution was adjusted to a pH 9.00 for Zn, Cu and Mn; pH 1.00 for Cr; and pH 6.00 for Pb detection. The paper strip was kept at room temperature for 1 min. After completely reacting, the paper strip was inserted into a homemade box to protect against external radiation by keeping the same lighting conditions (Fig. 1b). Then, a smartphone was placed on top of the box to take a photograph of the colorimetric paper strip. Finally, the concentrations of five metals were evaluated using the red-green-blue (RGB) color analysis-based Color Picker application in the smartphone.

Image processing using the Color Picker application

The Color Picker application was applied to capture the image from the smartphone. Images obtained from the CMOS sensor consisted of 1280×720 pixels, which were located in the circular pointer and were matched to the detection zone on a paper strip. Image analysis was performed through RGB profiling, and the RGB values of the image were measured. The RGB values were used for analysis of five metals, utilizing the red channel for Zn, Cu and Pb and the green channel for Cr and Mn. The intensity signal was calculated by subtraction of the red and green values acquired from the blank area and from the metals' detection zones (numbered 1–5). The concentrations of Zn, Cr, Cu, Pb and Mn in the real sample were obtained from the linear regression equation of

the calibration curve between the intensity and concentrations of the metal ions.

Results and discussion

Colorimetric paper strip for analysis of Zn, Cr, Cu, Pb and Mn

In this work, we demonstrate the simultaneous analysis of five metals using a single paper strip. To detect Zn, Cr, Cu, Pb and Mn, specific colorimetric reagents were dropped on the detection zone of the paper strip. We then added solution containing known concentrations of 2–6 mg/L Zn, 0.1–0.4 mg/L Cr, 1–8 mg/L Cu, 0.08–0.6 mg/L Pb and 1–7 mg/L Mn onto the detection zones numbered 1–5, respectively. Figure 2a shows the five detection zones with their full color responses of the paper strip. Detection zone number 1 generated a blue color, resulting from the reaction between Zn and ZI. Detection zone number 2, used for the detection of Cr, generated a pink color obtained by the Cr complex with DPC. In detection zone number 3, the Cu reacted with CPZ to create a complex with a blue color. In detection zone number 4, the Pb created a complex with ALS, resulting in a purple color. Finally, Mn reacted with PAN to form a complex and generated a red color on detection zone number 5. The color changes corresponded to the concentration of metals. Figure 2b shows the RGB values of images for the detection of Zn, Cr, Cu, Pb and Mn with the different concentrations. Based on the regression method, the results of the red channel provided the best linearity and the highest detection of Zn, Cu and Pb, whereas the

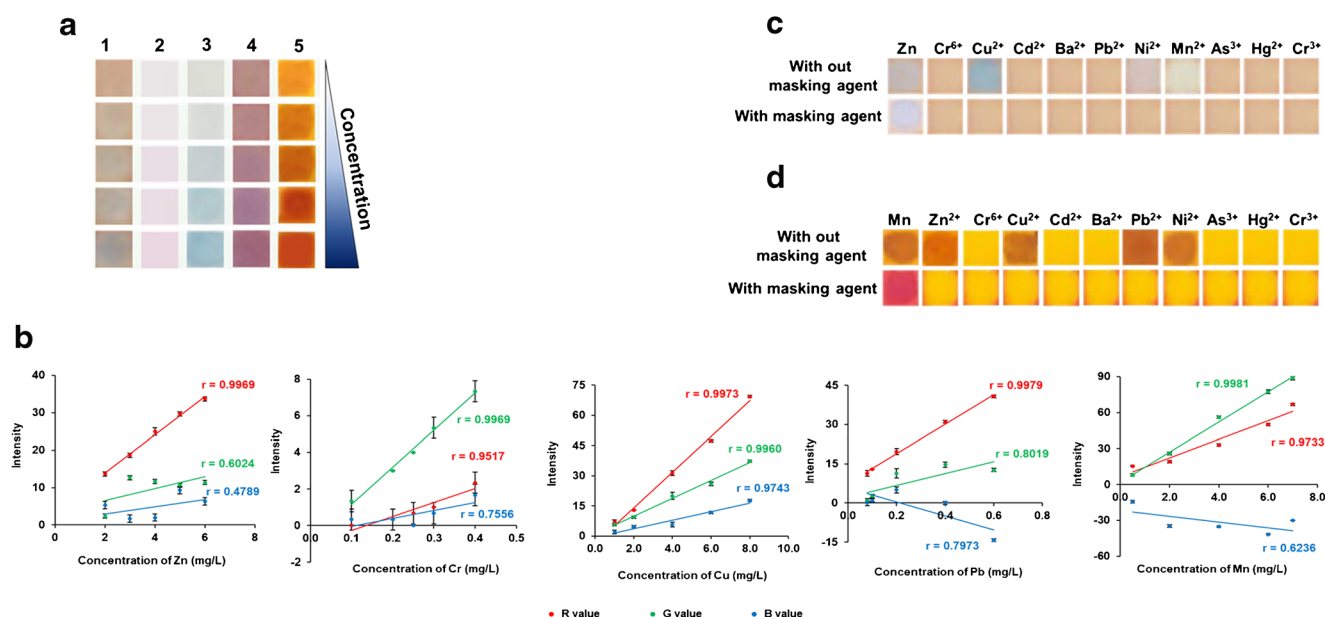


Fig. 2 (a) The color changes on the paper strip corresponding to different concentrations of Zn, Cr, Cu, Pb and Mn, (b) RGB values of the complex between metals and a chromogenic agent, (c) the color change of

detection zones modified with ZI in the presence of Cu, Ni and Mn, and (d) color change of detection zones modified with PAN in the presence of Zn, Cu, Pb and Ni with and without masking agents

green channel was chosen for Cr and Mn detection. Therefore, we used the red channel for quantitative analysis of Zn, Cu and Pb, and the green channel was used for Cr and Mn analyses.

In the preliminary study, we found that some metal ions interfered with the Zn-ZI complex and Mn-PAN complex for the detection of Zn and Mn, respectively. Eleven metal ions, including Zn²⁺, Cr⁶⁺, Cu²⁺, Cd²⁺, Ba²⁺, Pb²⁺, Ni²⁺, Mn²⁺, As³⁺, Hg²⁺ and Cr³⁺, were tested. As shown in Fig. 2c, the presence of 5 ppm of Cu and Mn, and 10 ppm Ni resulted in a color change of the detection area to blue, which was the same as Zn. To suppress the effect of Cu, Ni and Mn on Zn detection, 3 μ L of 100 mM cyanide and 3 μ L of cyclohexanone were used as masking agent. Cyanide was added to complex with Zn and other metals. Adding cyclohexanone causes a selective release of Zn from its cyanide complex so that it can be complexed with ZI to form a blue color. Similarly, the effect of Zn, Cu, Pb and Ni masking by cyanide was tested for Mn detection by comparing the colorimetric response obtained for the paper strip with and without the masking agent (Fig. 2d). Cyanide forms stable complexes with Zn, Cu, Pb and Ni, but does not form a complex with Mn. Therefore, in weakly alkaline solutions, only Mn reacts with PAN to form a red complex. Based on the above results, masking agents were applied for the detection of Zn and Mn.

Optimization of reagent volume

To study the optimum reagent volume on detection zones, we used methylene blue because it has a deep blue color, which

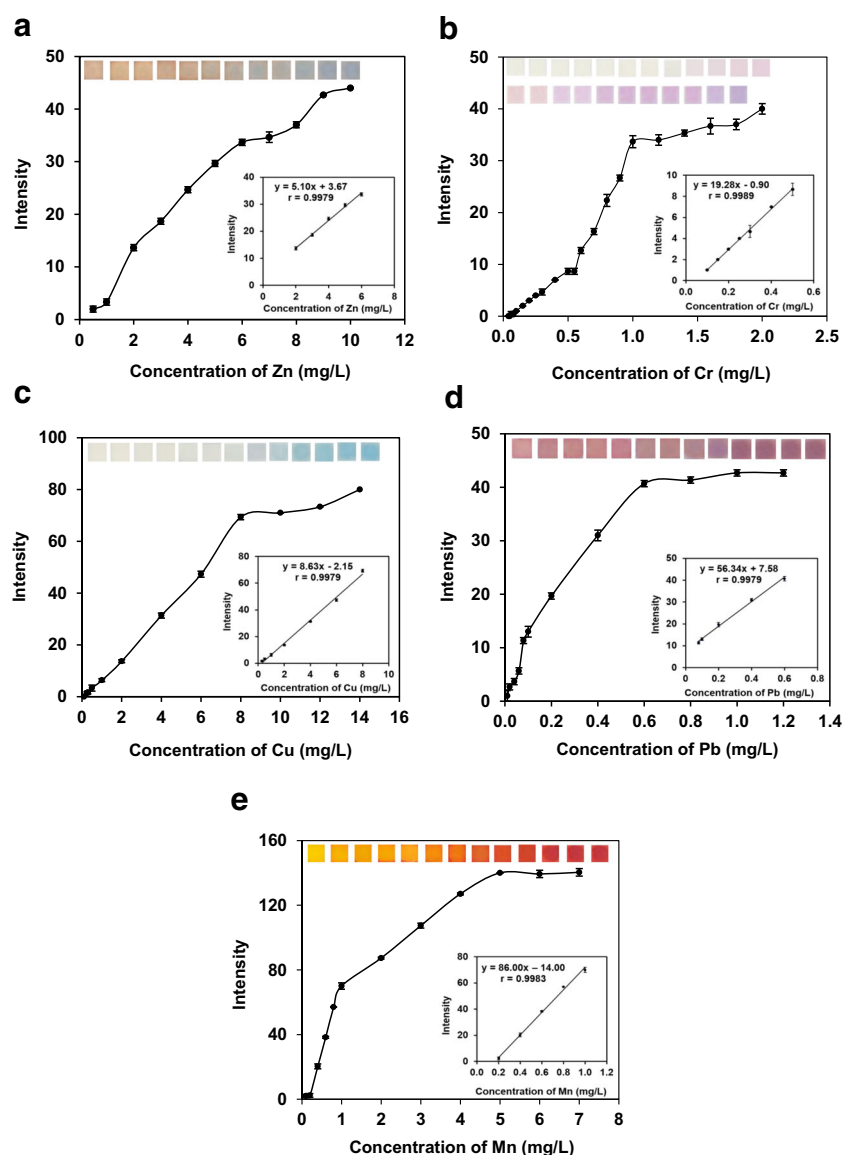
allows for easy identification of the volume of reagent on the detection zone. We added methylene blue at different volumes ranging from 1 to 10 μ L. For a reagent volume lower than 6 μ L, the solution was not large enough to cover the detection zone. In contrast, with a volume higher than 6 μ L, the reagent was found outside the detection area. Thus, 6 μ L of reagent volume was selected as an optimal volume for reagents (see ESM Fig. S1).

Optimization of detection conditions

Zn detection

The assay in this work is based on the reaction between the metal and chromogenic reagent. Therefore, the colorimetric response was dependent on the ZI concentration, pH of the solution and reaction time. The effect of the ZI concentration on the colorimetric reaction was optimized in the range of 0.1–1.0 mM. The results showed that when the ZI concentration was low, the ZI did not effectively react with Zn. As shown in ESM Fig. S2a, the intensity increased with increased ZI concentration. When the concentration of ZI was greater than 0.4 mM, the intensity decreased due to an increase of the blank signal. As a results, 0.4 mM was chosen as the appropriate ZI concentration. The color of the formed Zn complex highly depends on the pH of the media. Thus, the pH of the solution was optimized in the range 6.00–11.00. The obtained results are shown in ESM Fig. S2b. Low-intensity signals were observed under acidic conditions, and the signal increased in the alkaline solution and reached a maximum

Fig. 3 The calibration curves of (a) Zn, (b) Cr, (c) Cu, (d) Pb, and (e) Mn



intensity at pH 9.00. The Zn-ZI complex is stable in the pH range of 8.50–9.50 because ZI has acidic functional groups that become ionized at an alkaline pH. Therefore, an alkaline condition assists in Zn dissolution and facilitates the reaction of Zn with positively charged metal ions [32]. Finally, the reaction time was optimized by maintaining the concentration of ZI and pH for different

reaction times at room temperature. The longer the reaction time, the more Zn-ZI complexes are formed (ESM Fig. S2c). The intensity was amplified by increasing the reaction time from 0.5 to 1 min, where 1 min provided the highest response. Thus, 1 min was selected as the optimal reaction time. In summary, 0.4 mM ZI, pH of 9.00 and 1 min of reaction time were used for the detection of Zn.

Table 1 Summary of the sensitive detection of metals using a paper strip

Metal	Linear range (mg/L)	Regression equation	r	LOD (mg/L)	LOQ (mg/L)
Zn	2.00–6.00	$y = 5.10x + 3.67$	0.9979	0.63	2.10
Cr	0.10–0.50	$y = 19.28x - 0.90$	0.9989	0.07	0.24
Cu	0.30–8.00	$y = 8.63x - 2.15$	0.9979	0.17	0.57
Pb	0.08–0.60	$y = 56.34x + 7.58$	0.9979	0.03	0.09
Mn	0.20–1.00	$y = 86.00x - 14.00$	0.9983	0.11	0.30

Table 2 Summary of the precision and accuracy of the paper strip ($n = 3$)

Metal	Precision (%RSD)		Concentration (mg/L)			Recovery (%)
	Intra-assay	Inter-assay	Initially	Added	Found	
Zn	3.54	3.80	ND	2.10	1.83 ± 0.00	87 ± 0.00
			ND	4.00	4.13 ± 0.00	103 ± 0.00
			ND	6.00	6.10 ± 0.11	102 ± 1.73
Cr	0.00	0.00	ND	0.24	0.25 ± 0.00	106 ± 0.00
			ND	0.30	0.32 ± 0.00	106 ± 0.00
			ND	0.40	0.38 ± 0.00	95 ± 0.00
Cu	3.67	3.98	ND	0.57	0.59 ± 0.00	104 ± 0.00
			ND	2.00	2.14 ± 0.07	107 ± 3.46
			ND	4.00	3.70 ± 0.07	93 ± 1.64
Pb	2.48	4.25	ND	0.09	0.08 ± 0.01	89 ± 0.00
			ND	0.20	0.19 ± 0.00	93 ± 0.00
			ND	0.40	0.35 ± 0.00	87 ± 0.00
Mn	1.08	2.49	ND	0.30	0.32 ± 0.01	106 ± 2.24
			ND	0.60	0.62 ± 0.01	104 ± 1.12
			ND	0.80	0.81 ± 0.01	101 ± 0.84

ND: not detected

Cr detection

The detection zone with Cr and DPC had a pink color, and the DPC concentration influenced the color intensity. Therefore, the effect of DPC concentration on color intensity was studied. The results in ESM Fig. S3a show that the intensity increased up to 4.0 mM, and then a plateau was observed. The concentration of DPC at 4.0 mM was optimal. The complex reaction between Cr and DPC depends on pH, as shown in ESM Fig. S3b. The results were given at the optimal pH of 1.00 due to the formation of a Cr-DPC complex in strongly acidic media [33]. In addition, the reaction time was also optimized. When the reaction time reached 1 min, DPC reacted almost completely with Cr, and the intensity was no longer increased (ESM Fig. S3c). Therefore, we chose 1 min as the optimal reaction time of the DPC and Cr complex. The optimal conditions for the detection of Cr were 4.0 mM DPC, pH of 1.00 and 1 min of reaction time.

Cu detection

An increase in analytical response was found with increasing concentrations of CPZ from 0.25 mM to 2.0 mM due to the increased complex binding of CPZ molecules to Cu (ESM Fig. S4a). The plateau after 2.0 mM is due to the limited amount of Cu to form a complex with CPZ. Hence, CPZ at 2.0 mM was selected. The effect of the pH on blue intensity was examined by an ammonia-ammonium chloride buffer solution within the pH range of 6.00–12.00. As shown in ESM Fig. S4b, the color signal increased with the gradual increase of pH from 6.00 to 9.00 and then continuously decreased at higher pH values. CPZ reacts with Cu in a slightly alkaline medium (optimum pH 8.00–9.50) to form a blue complex. This complex is not formed below pH 6.50, and the color fades above pH 12.00 [34]. Some assays based on a CPZ reagent have also reported pH 9.00 as the

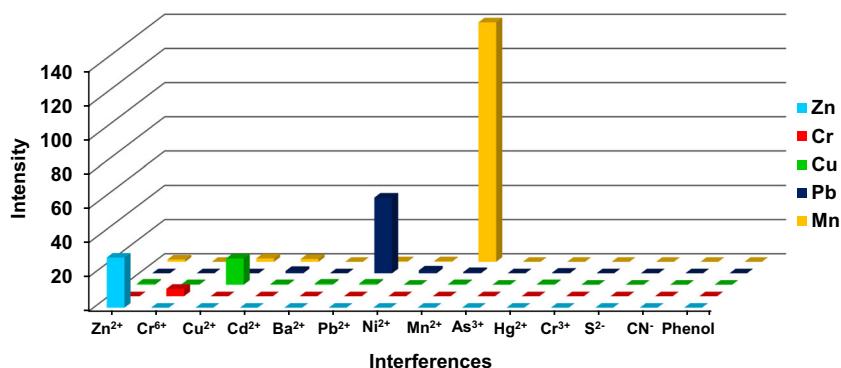
Fig. 4 Selectivity test of the paper strip to other interferences

Table 3 Selectivity of the paper strip for the detection of Zn, Cr, Cu, Pb and Mn

Interferences	10% Tolerance ratio				
	Zn	Cr	Cu	Pb	Mn
Zn ²⁺	—	>10	>10	>10	4
Cr ⁶⁺	>10	—	>10	>10	>10
Cu ²⁺	>10	>10	—	>10	3
Cd ²⁺	>10	>10	>10	>10	10
Ba ²⁺	>10	>10	>10	>10	>10
Pb ²⁺	>10	>10	>10	—	>10
Ni ²⁺	>10	>10	>10	>10	>10
Mn ²⁺	>10	>10	>10	>10	—
As ³⁺	>10	>10	>10	>10	>10
Hg ²⁺	>10	>10	>10	>10	4
Cr ³⁺	>10	>10	>10	>10	>10
S ²⁻	>10	>10	>10	>10	>10
CN ⁻	>10	>10	>10	>10	>10
Phenol	>10	>10	>10	>10	>10

optimum pH for solution [35]. Therefore, pH 9.00 was selected as the pH of the solution in this experiment. Moreover, the effect of the reaction time on the colorimetric reaction was evaluated at different times from 0.5 to 5 min. As shown in ESM Fig. S4c, the intensity increased proportionally to the reaction time until 1 min and then became constant. Thus, the optimal CPZ concentration, solution pH and reaction time were achieved at 2.0 mM, pH of 9.00 and 1 min, respectively.

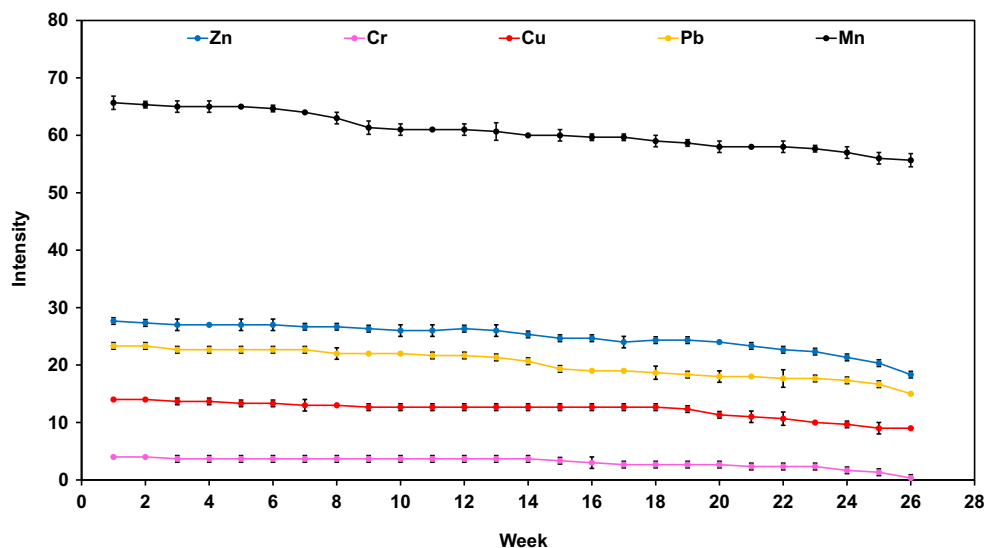
Pb detection

First, we optimized the concentration of ALS for the detection of 0.2 mg/L Pb. Too low of concentrations of ALS

could not successfully bind to metal enough, resulting in a weak-intensity signal and poor detection limit; too high of concentrations might cause a high color background and waste reagents (ESM Fig. S5a). Therefore, the best concentration of ALS was 2.0 mM. Then, the effect of the pH solution was studied, ranging from 1.00–10.00. As shown in ESM Fig. S5b, the mean amplified with increasing pH, peaking at a pH of 6.00 and dropping at 7.00. pH > 6.00 is likely a result of hydroxide in the solution, which causes precipitation of Pb, while pH < 6.00 may be due to the competition of hydronium ions toward complexes with ALS, which results in a decrease in the complex product [36]. Thus, pH of 6.00 was chosen for the formation of the Pb-ALS complex on the paper strip for subsequent experiments. Finally, the effect of the reaction time was optimized. As shown in ESM Fig. S5c, the intensity gradually increased with increasing reaction time up to 1 min; after that, the change in intensity was negligible, suggesting that the Pb-ALS complexes were completely bonded. Based on these results, 2.0 mM of ALS, 6.00 of pH solution and 1 min of reaction time were used for Pb detection.

Mn detection

The influence of PAN concentration, pH value and reaction time was investigated for the detection of 5 mg/L Mn. As shown in ESM Fig. S6a, increasing the concentration of PAN from 1.0 to 5.0 mM led to the colorimetric response, first an increase and then a decline, which might be due to an excess concentration of PAN, yielding high background. Thus, 5.0 mM was selected as the optimum concentration of PAN. The pH solution affected the Mn-PAN complex on the paper strip. Different pH values from 6.00 to 12.00 were tested. As shown in ESM Fig.

Fig. 5 Stability of the paper strip

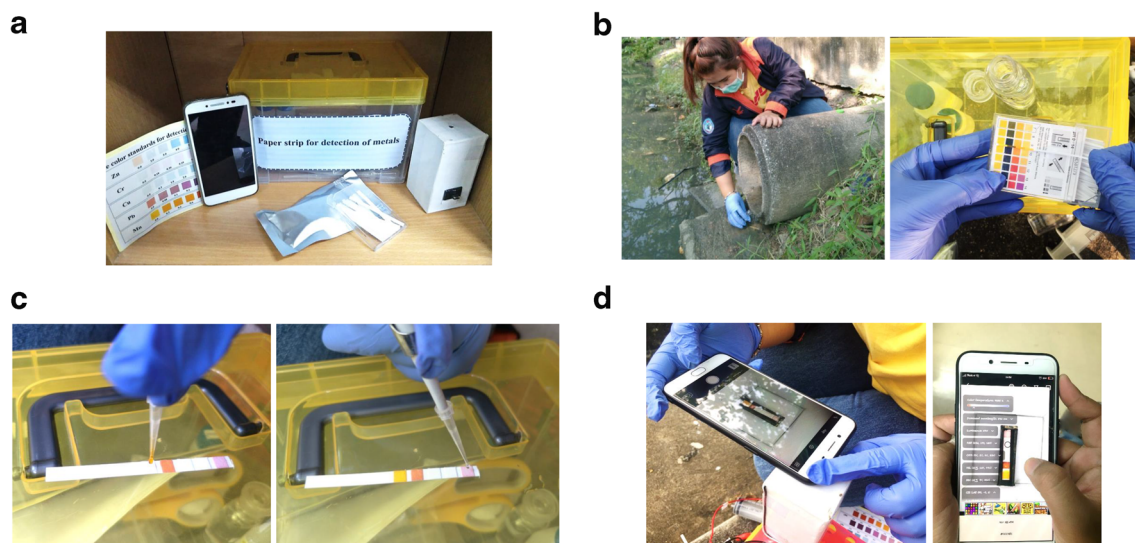


Fig. 6 The paper strip was applied for on-site detection of Zn, Cr, Cu, Pb and Mn in a wastewater sample. **(a)** Portable paper strip kit, **(b)** collection of sample and adjustment the pH of sample, **(c)** addition of chromogenic

reagents and sample solution on paper strip and **(d)** reading signal on-site using a smartphone application

S6b, the intensity response amplified with increased pH values from 6.00 to 9.00 and reached a maximum at pH 9.00. This finding might explain why manganese and PAN can generate a stable complex in an alkaline condition [37, 38]. Therefore, pH of 9.00 was chosen for further experimentation, which is similar to previous works [39]. To evaluate the effect of the reaction time, different times ranging from 0.5 to 5.0 min were investigated. The time for the completed reaction between Mn and PAN was within 1 min (ESM Fig. S6c). Finally, the best values for the experimental conditions were found as follows: 5.0 mM PAN, pH at 9.00 and 1 min of reaction time.

According to the reaction time results, the complete reaction for all heavy metals was almost 1 min.

Therefore, the optimal time for taking photographs was only 1 min.

Sensitive detection of Zn, Cr, Cu, Pb and Mn using a paper strip

Under the optimum conditions, the color intensity of the paper strip for the simultaneous detection of Zn, Cr, Cu, Pb and Mn amplified with increasing concentrations of the heavy metals. Figure 3 displays the calibration plots of the color intensity obtained from varying concentrations of Zn, Cr, Cu, Pb and Mn. The linearity of calibration curves was good for five heavy metals, with correlation coefficients (r) greater than 0.995 (Table 1). The limit of detection (LOD) and limit of quantification (LOQ) were then evaluated by 3 SD/m and 10

Table 4 Summary of the analytical results of Zn, Cr, Cu, Pb and Mn in wastewater ($n = 3$)

Sample	Quantitative analysis (mg/L)									
	Zn		Cr		Cu		Pb		Mn	
	Paper strip	ICP-OES	Paper strip	ICP-OES	Paper strip	ICP-OES	Paper strip	ICP-OES	Paper strip	ICP-OES
1	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
2	ND	ND	ND	ND	ND	ND	ND	ND	0.82 ± 0.02	0.83 ± 0.05
3	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
4	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
5	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
6	ND	ND	ND	ND	ND	ND	ND	ND	0.82 ± 0.01	0.82 ± 0.01
7	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
8	2.28 ± 0.20	2.19 ± 0.18	ND	ND	ND	ND	ND	ND	ND	ND
9	ND	ND	ND	ND	0.61 ± 0.07	0.55 ± 0.01	ND	ND	ND	ND

ND: not detected

SD/m, where SD is the standard deviation of the blank, and m is the slope of the linear calibration curve. The LOD and LOQ results for the analysis of Zn, Cr, Cu, Pb and Mn are presented in Table 1. The LODs were calculated as 0.63 mg/L for Zn, 0.07 mg/L for Cr, 0.17 mg/L for Cu, 0.03 mg/L for Pb and 0.11 mg/L for Mn. The LOQs for Zn, Cr, Cu, Pb and Mn were 2.10, 0.24, 0.57, 0.09 and 0.30 mg/L, respectively, with acceptable precision and accuracy. It should be noted that LOQs of all heavy metals were lower than the maximum residue limits established by Thailand regulations. Therefore, the proposed assay meets the performance requirements, which is suitable for the analysis of Zn, Cr, Cu, Pb and Mn in pollutant water samples.

Many methods for the quantitative detection of Zn, Cr, Cu, Pb and Mn have been developed based on various assays. A comparison between the proposed paper strip and previous methods is presented in ESM Table S2. As shown, our paper strip had advantages over the mentioned methods based on the ability of concurrent on-site testing, cost-effectiveness and multiple analysis detection at the same time. The paper sheet, which is cheap and easy to use, required less time for the analysis of five metals.

Precision and accuracy

The intra-assay and inter-assay precision for all heavy metal detection using the developed paper strip were studied. To evaluate intra-assay precision, seven measurements of Zn, Cr, Cu, Pb and Mn were performed on the same day. For inter-assay testing, paper strip reproducibility was evaluated by detecting five heavy metals over 7 days with different batches of paper strip fabrication. Both of them were estimated from the detection of 5 mg/L Zn, 0.25 mg/L Cr, 2 mg/L Cu, 0.2 mg/L Pb and 5 mg/L Mn ($n = 7$). The relative standard deviations (RSD) of intra-assay and inter-assay precision are shown in Table 2. Intra-assay and inter-assay values varied from 0.00–3.67% and 0.00–4.25%, respectively, demonstrating that the precision of the paper strip was acceptable.

The accuracy of the analytical method was evaluated through recovery studies at three different concentrations levels of each metal by spiking a wastewater sample. The recovery values were equal to 87–107%. These results indicate acceptable precision and accuracy of the proposed paper strip.

Selectivity and stability of the paper strip

The selectivity of the paper strip was investigated. The possible interferences can be caused by different metals and other compounds that can be found in a wastewater sample, including Zn^{2+} , Cr^{6+} , Cu^{2+} , Cd^{2+} , Ba^{2+} , Pb^{2+} , Ni^{2+} , Mn^{2+} , As^{3+} , Hg^{2+} , Cr^{3+} , S^{2-} , CN^- and phenol. As described in Fig. 4, the color intensity was significantly amplified in the presence of

Zn, Cr, Cu, Pb and Mn, whereas no significant differences were observed from other interferences. Besides, the tolerable concentration ratios of studied interferences to the target ions is presented in Table 3. In most cases, tolerance levels higher than 10-fold were achieved.

Another key requirement of the paper strip is long-term stability. Thus, the stability of the developed paper strip was determined by keeping the reagent and paper strip at 4 °C and measuring the response every week. As shown in Fig. 5, the intensity response of Zn, Cr, Cu, Pb and Mn showed no significant changes over time, after 8 weeks. As a result, the developed paper strip showed good long-term stability.

On-site detection of Zn, Cr, Cu, Pb and Mn in wastewater

The paper strip was applied to analyze Zn, Cr, Cu, Pb and Mn in industrial wastewater samples. As shown in Fig. 6, a sample was collected from wastewater of an electronics factory (sample 1). To use the paper strip (Fig. 6a), 10 mL of the sample was collected (Fig. 6b). Then, the pH of the samples was adjusted to pH 9.00 for Zn, Cu, Mn, pH 1.00 for Cr and pH 6.00 for Pb analysis. Next, chromogenic reagents and sample solution were drop-cast onto the paper strip (Fig. 6c). After 1 min, the metal concentration was determined using a smartphone (Fig. 6d). Table 4 shows the analytical results. All heavy metal concentrations detected by the paper strip were compared to the ICP-OES method. The concentration of metal ions in the four wastewater samples ranged from 0.61 mg/L to 2.28 mg/L. The analytical results of both methods agreed at the 95% confidence interval which shows that our paper strip has good accuracy for the determination of practical samples.

Conclusions

This work presents a portable sensor for the on-site quantitative detection of trace heavy metals in wastewater samples by coupling a paper strip and a smartphone. The concentrations of chromogenic substrate, pH of solution and reaction time were optimized to achieve a sensitive paper strip for the detection of Zn, Cr, Cu, Pb and Mn in wastewater. The LOQs of all metals were lower than the regulations for Thailand. The analytical results of real samples tested using the proposed paper strip were statistically similar to the results from ICP-OES. In summary, there are four benefits of our paper strip: (1) the paper strip is made of paper by a cutting method, which can be easily fabricated with low cost; (2) the paper strip coupled with a smartphone is a powerful portable device for on-site detection; (3) the analysis time of five metals is only 1 min; and (4) the total cost of the assay is approximately \$0.01 per five metals. These benefits would significantly expand the use of the proposed paper strip for environmental monitoring in developing countries.

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Compliance with ethical standards

The authors declare that they have no competing interests.

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