# Paper-Based Sensor for Preliminary Screening of Lead in Industrial Wastewater

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Water is life 2016, 5-11 June 2016, Maurick College, The Netherlands

# Paper-Based Sensor for Preliminary Screening of Lead in Industrial Wastewater

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## Abstract

Nowadays, several heavy metals have been continuously used in industry, where lead (II) ion is the most harmful metal in Thailand. People nearby industrial areas have been inflected with serious human disorders due to lead contamination in consumed water. The objective of this research is to develop an inexpensive, portable testing device, paper-based sensor, for rapid determination of lead (II) level in wastewater. In this paper-based sensor, dithizone ligand was used as a colorimetric agent for lead (II) detection. The principle of this paper-based sensor is reaction between lead (II) ion and dithizone to produce a distinctively red-colored complex on our proposed sensor, which can be clearly observed by naked eyes. After this reaction, lead (II) concentration was quantified from color intensity of product images through a scanner coupled withImageJ, the image processing software. Moreover, various colorimetric conditions in the lead (II) quantitation were investigated including dithizone concentration, pH, reaction time, and other metals interference. We found that paper-based sensor can detect lead (II) concentration in the range of 10-500 ppm with NaOH 0.1 M (at pH 13), dithizone 2.5 mM, and 5 minutes of reaction time as the optimal conditions. Finally, we have successfully tested the sensor for lead (II) determination in wastewater samples with acceptable correlation with the standard method using inductively coupled plasma optical emission spectrometry (ICP-OES) with 5% error acceptance.

## Keywords

lead (II) ion**,** dithizone, paper-based sensor, color intensity

## Introduction

Nowadays, a number of chemical substances, especially the heavy metals i.e. lead, cadmium, mercury, are prevalently used in industry. If environment or food is contaminated by these substances, it will be dangerous. Heavy metals are deadly substances to organisms and ecosystem. Even their little amount can cause many health problems and diseases. More importantly, industrial parts have become bigger and bigger consequently resulting in severe damages to us. In Thailand, the most harmful heavy metal is **lead**. Lead (II) ions dissolved in water are responsible for danger to water organisms and people taking them through daily activities.

The graph illustrated below shows the trend of lead (II) poisoning in Thailand (Source: BUREAU OF EPIDEMIOLOGY National Trustworthy and Competent Authority in Epidemiological Surveillance and Investigation, Thailand). As can be seen, in 2012, there has been an increasing number of people inflected by lead (II) ions throughout all regions, except for the southern part. This trend was very remarkable in the northeast region, where the local people suffered from water contaminated by lead (II) ions.

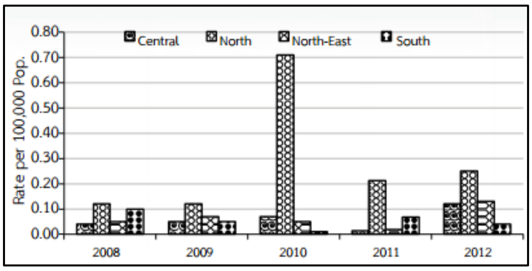



Fig. 1 Reported cases of lead (II) poisoning per 100,000 populations, by region, Thailand, 2008-2012

Nowadays, lead quantifying can be determined by three conventional methods— Atomic Absorption Spectrometry (AAS), Inductively Coupled Plasma- Optical Emission Spectrophotometry (ICP-OES), and electrochemistry. However, these methods are far from practical, such asexpensive equipment and high operation cost, time-consuming procedures, and high requirement for experts. Therefore, we intended to eliminate these problems by trying to invent a practical lead (II) determination test-kit.

To invent the test kit, we studied the behavior of **dithizone** ($C_{13}H_{12}N_4S$), the substance that can react with lead (II) ions. Dithizone acts as a ligand forming the complexation with lead (II) ions, which then changes its molecular structure, as shown in Fig. 2. This new structure leads to color changing in reacted substance. Varying lead (II) concentrations provide different rates of reaction which result in various color. Then, we can determine the quantity of lead (II) ions from the color intensity based on colorimetric analysis.

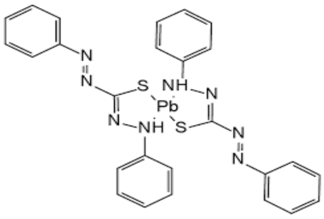



Fig. 2 Structure of complex compound from the reaction between lead (II) ion and dithizone

## The purpose of the investigation

1. To determine the conditions optimal for the colorimetric technique used in investigating the reaction between lead (II) ion and dithizone.

2. To develop a novel lead paper-based analytical device (PAD).

## Method of the investigation

In our study, there are many samples representing various colors. Observe with the naked eyes cannot provide precise results, so we measure the color intensity by using software ImageJ determining color intensity as quantitative information


Fig. 3 The process of measurement color intensity

This study contains five main experimental phases.

**1. Design and Fabrication of Paper-Based Analytical Device (PAD)**

In this phase, there are three steps to design and fabricate paper-based analytical device (PAD)

1) Through Microsoft Power Point, the feature of the test kit was designed as shown Fig. 4. The 0.7-cm diameter circle area functioned as the detection zone. The rest area, shaded in blue, was positioned as the waxing zone.


Fig. 4 Design of paper-based analytical device (PAD)

2) Our paper-based sensor was made of No.1 Whatman paper, as illustrated in the design shown in Fig. 4. Then, the waxing zone was printed by the wax printer. Next, the printed paper was heated on the hot plate at 175˚C for 40 seconds to dissolve wax on the paper, where this wax functioned as the hydrophobicity. Finally, the process was completed with the clear adhesive tape being putted on one side of the printed paper. These steps are shown in Fig. 5.

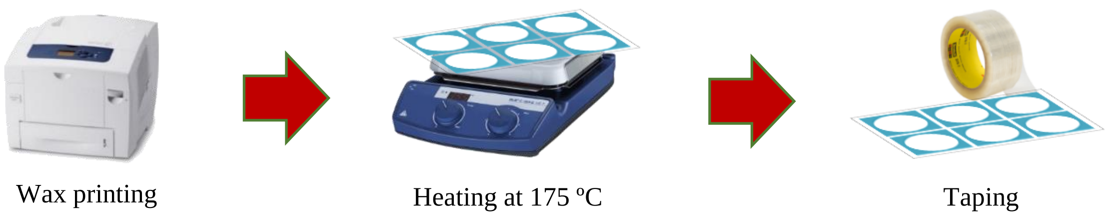



Fig. 5 Preparation of paper by wax-printing, heating, and taping

**2. Determination of the Optimal Conditions**

Subtracted color intensity can be identified by the difference of color intensity between lead samples and distilled water (control).

This phase contains three major steps to determine the optimal conditions of reaction between lead (II) ion and dithizone

2.1 Dithizone Concentration Optimization

2.1.1 Varying of dithizone concentration (1.25, 2.5, 5, 10, 25 mM respectively) was performed on two groups of the samples –500-ppm lead (II) solution and distilled water.

2.1.2 Color intensity was investigated through ImageJ.

2.1.3 Subtracted color intensity was calculated to find the best dithizone concentration that can detect the most remarkable lead (II) concentration.

2.2 pH Optimization

According to the literature review,dithizone can formulate a complex well in basicity pH because in acidity, protonation of sulfer and nitrogen atom reduces the donor-acceptor interactions between dithizone and lead (II) ion (Zargoosh& Babadi, 2011). Therefore, varying of NaOH concentration was performed throughout this step.

2.2.1 Varying of NaOH concentration (0.05, 0.1, 0.25, 0.5, 1 M respectively) was performed on two groups of the samples –500-ppm lead (II) solution and distilled water.

2.2.2 Color intensity was investigated through ImageJ.

2.2.3 Subtracted color intensity was calculated to find the best NaOH concentration that can detect the most remarkable lead (II) concentration.

2.3 Time Optimization

2.3.1 Varying of reaction time was performed every minute on two groups of the samples –500-ppm lead (II) solution and distilled water.

2.3.2 Color intensity was investigated through ImageJ.

2.3.3 Subtracted color intensity was calculated for the best reaction time that can detect the most remarkable lead (II) concentration.

**3. Analysis of Multi-Concentration Lead Samples**

After deriving the best condition that can detect lead (II) ion concentration clearly, we used reagents with the optimal condition to test the reaction of lead (II) solution with varying concentration (0, 10, 20, 50, 100, and 250 ppm) to investigate the relation between subtracted color intensity and lead (II) concentration.

**4. Investigation of Interference of Other Heavy Metal Ions**

This phase consists of two major steps to investigate color interference taking place as a result of other heavy metal ions contaminated in the water samples.

1) Mentioned in previous research, various types of metal ions ($Cu^{2+}$, $Co^{2+}$, $Ni^{2+}$, $Ag^{+}$ etc.) that can react with dithizone were investigated under the same optimal condition.

2) Types of metal ions that can disturb our optimal condition were found, and then varying concentrations of the metal ions were performed to determine the lowest/highest concentration that cannot disturb our optimal condition.

**5. Investigation of PAD Effectiveness**

The effectiveness of our PAD was compared to the standard method, ICP-OES, used in wastewater samples determination. If the difference of value is lower than 5% RSD, it indicates no significant difference.

Our device is distinguished from the color intensity with its easy-using and on-site testing capability difference from standard method, ICP-OES. This method has limitation like only well-trained can use this method, and the equipment have expensive cost.

## Results of the experiment

### 1. Determination of the Optimal Conditions

1.1 Dithizone concentration optimization

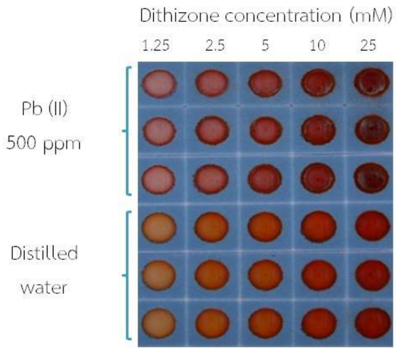
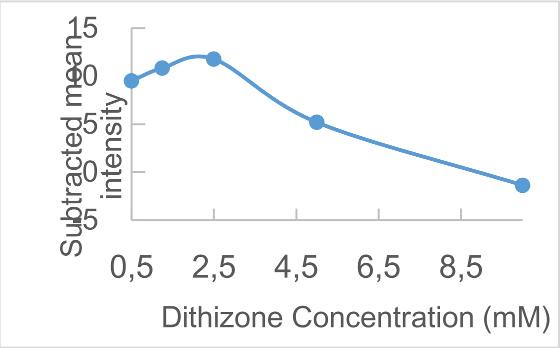



Fig. 6 The experimental result varying dithizone concentration

Fig. 6 shows the results of our experiment. The first three rows represent the mixture between 500 ppm lead (II) ion solution and dithizone at each dithizone concentration while the last three rows represent the mixture between lead (II) ion and dithizone at each dithizone concentration. Next, we determined it as a quantitative data using software ImageJ.




Graph 1 Relationship between subtracted mean intensity and dithizone concentration from ImageJ analysis on Fig. 6

We determined the color intensity in Fig. 6, then calculated the subtracted mean intensity of each dithizone concentration, and plotted graph shown in Graph 1. Considering from Graph 1, the dithizone concentration which provided the maximum value of subtracted mean intensity is 2.5 mM.

1.2 pH optimization

We found that dithizone can form a complex well in pH base because in acidity protonation of sulfer and nitrogen atom reduces the donor-acceptor interactions between dithizone and Pb (II) ions (Zargoosh K. & Babadi F. F., 2015). Therefore, we used NaOH as a reagent to vary pH in our optimization.

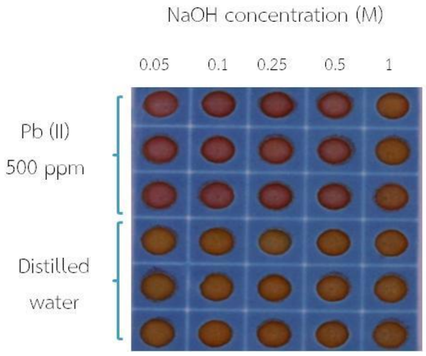
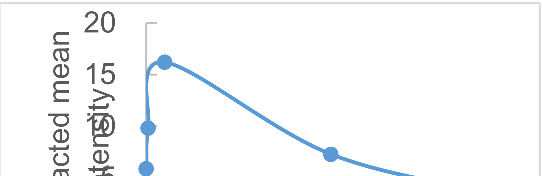



Fig. 7 The experimental result varying NaOH concentration

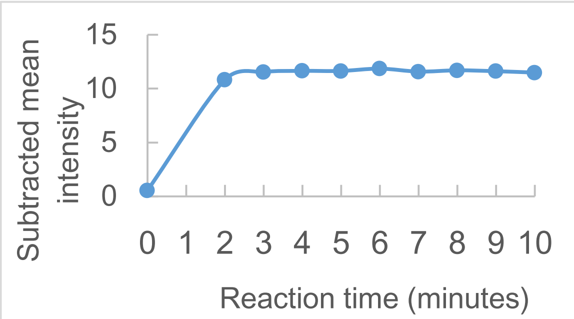
Fig. 7 shows the results of our experiment which is similar to varying NaOH concentration. The first three rows represent the mixture between 500 ppm lead (II) ion solution and dithizone at each NaOH concentration while the last three rows represent the mixture between lead (II) ion and NaOH at each dithizone concentration.



Graph 2 Relationship between subtracted mean intensity and NaOH concentration from ImageJ analysis on Fig. 7

Similar to 1.1 from Graph 2, the NaOH concentration which provided themaximum value of subtracted mean intensity is 0.1 M (pH=13).

1.3 Reaction time optimization




Graph 3 Relationship between subtracted mean intensity and reaction time from ImageJ analysis

Considering from Graph 3, the reaction time which provided themaximum value of subtracted mean intensity is 5 minutes. After optimization of 3 crucial factors, we got the value of optimal condition shown on Table 1.

| Parameters | Examined values | Optimal value |
|---|---|---|
| Dithizone concentration | 1.25-25 mM | 2.5 mM |
| NaOH concentration | 0.05-2 M | 0.1 M |
| Reaction time | 1-10 minutes | 5 minutes |

Table 1 Concluded optimal condition consisting of dithizone 2.5 mM, NaOH 0.1 M (pH=13) and reaction time 5 minutes

## 2. Analysis of Multi-Concentration Lead Samples

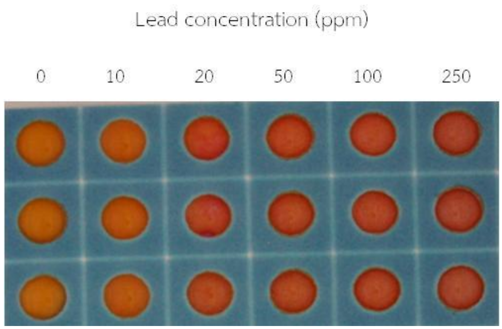
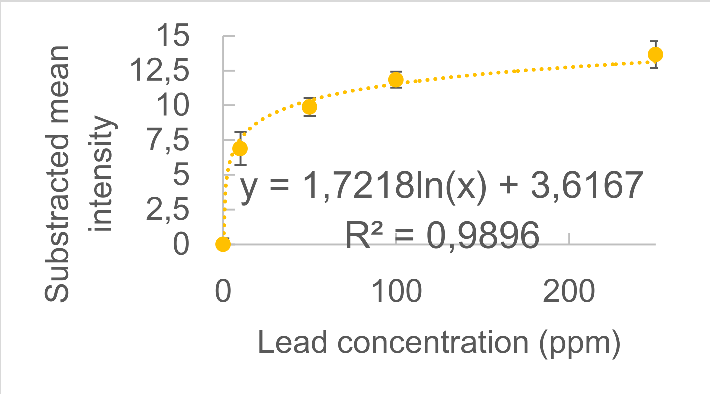


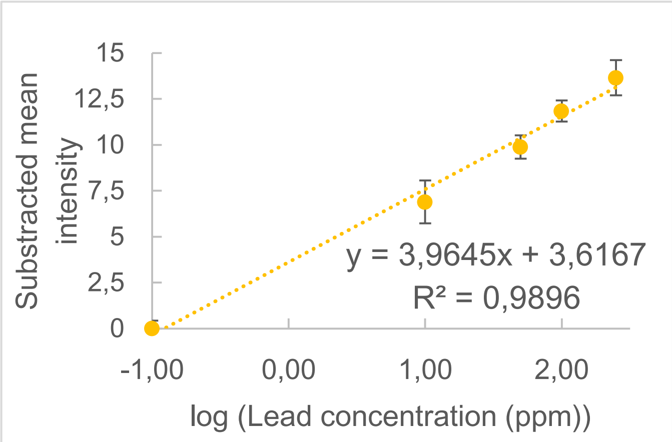
Fig. 8 The experimental result varying lead (II) ion concentration under the optimal condition

We reacted multi-concentration of lead (II) ions consisting of 0, 10, 20, 50, 100, 250 ppm with dithizone under then optimal condition shown in Table 1. The results with 3 repeats are represented in Fig. 8 Then, we determined the numerical data using ImageJ, calculated subtracted mean intensity, and plotted graph shown in Fig. 8.




Graph 4 Relationship between subtracted mean intensity and lead (II) ion concentration from ImageJ analysis

Considering from Graph 4, this curve looked like exponential curve. Therefore, we took logarithm base 10 on lead concentration (x-axis).




Graph 5 Relationship between subtracted mean intensity and lead (II) ion concentration from ImageJ analysis.

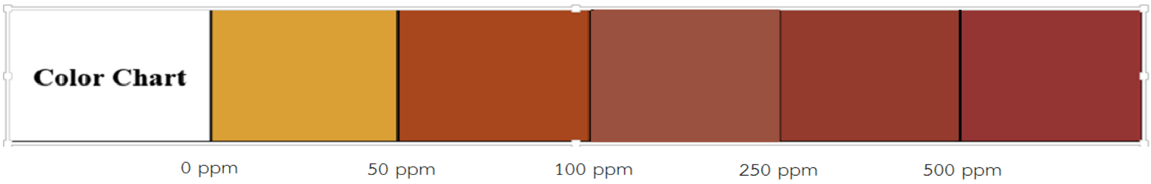



Fig. 9 Color chart for lead determination using Paper-based analytical device
(got color from Fig. 8)

Considering from Graph 5, this curve is almost linear with R2=0.989.The high linearity of Graph 5 represents that the optimal condition worked and can be used to detect lead (II) ions efficiently based on color chart in Fig. 9.

### 3. Investigation of Interference of Other Heavy Metal Ions

We considered 11 metal ions which can react with dithizone ligand.

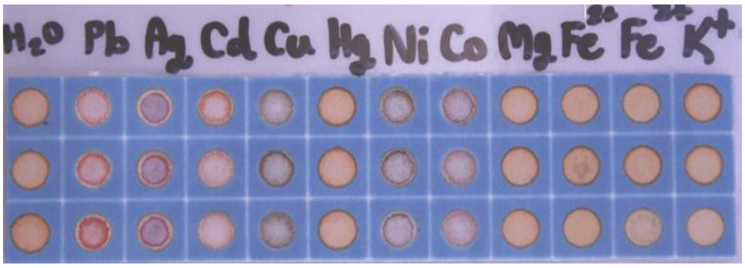



Fig. 10 11 kinds of other metals ions reacted with dithizone under the optimal condition

From Fig. 10 , 4 of 11 kinds of heavy metal ions including Co(II), Cu(II), Ni(II), Ag(I)formed a new color with dithizone whereas other 6 heavy metal ions including Cd(II), Hg(II), Mg, Fe(III), Fe(II), K didn't.

3.1 Co(II), Cu(II), Ni(II), Ag(I)

These 4 metals could form new color so we studied analytical performance of each metal ion similar to section 2 that studied lead (II) ion but studied the others instead. We varied the concentration of each metal, observed color intensity trend separately using subtracted mean intensity.

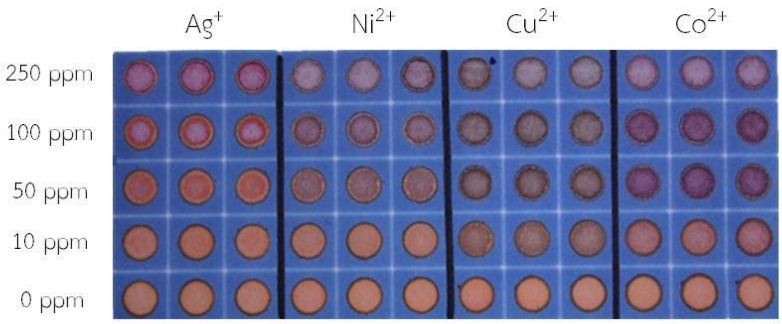
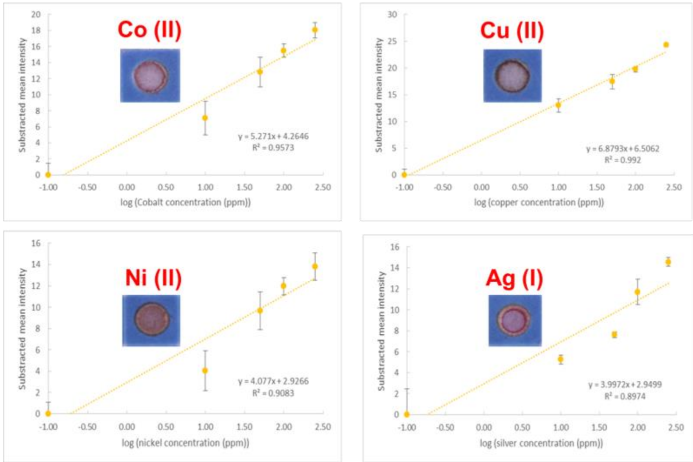



Fig. 11 The experimental result varying Co(II), Cu(II), Ni(II), Ag(I) concentration under the optimal condition




Graph 6 Relationship between subtracted mean intensity and Co(II), Cu(II), Ni(II), Ag(I) concentration from ImageJ analysis

According to Fig. 11 and Graph 6, in range of concentration 10-250 ppm Co(II), Cu(II), Ni(II), Ag(I) have their own linearity trends independently which causes the disturbance on detection of lead when their concentrations are more than 10 ppm, so their tolerance concentration must be lower than 10 ppm.

3.2 Cd(II), Hg(II), Mg, Fe(III), Fe(II), K

These 6 metals couldn't form new color, so we studied their behavior combining it with lead (II) ion 250 ppm and reacted with dithizone in various ratios under the optimal condition. The result is shown in Fig. 12.

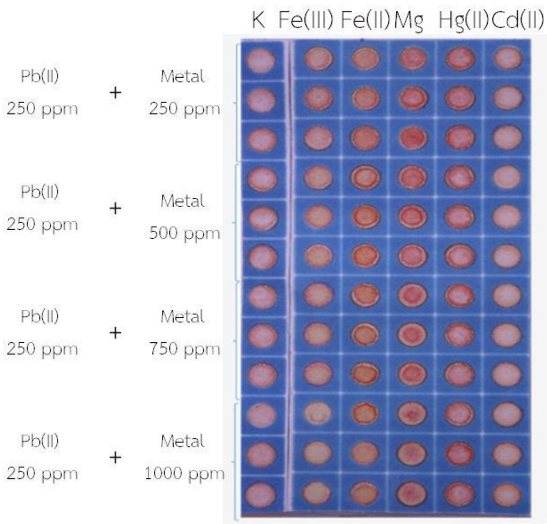



Fig. 12 11 kinds of other heavy metal ions react with dithizone under the optimal condition

From Fig. 12, from ImageJ all zones' color intensities are approximately near to color intensity of lead (II) ion 250 ppm (lower than 5% error). If these ions are lower than 1000 ppm, no inference will occur. Therefore, their tolerance concentration must be more than 1000 ppm.

From 3.1 and 3.2, we summarized the tolerance concentration shown in Table 2.

| Heavy metal ions | 5% Tolerance concentration |
|---|---|
| Co(II), Cu(II), Ni(II), Ag(I) | Lower than 10 ppm |
| Cd(II), Hg(II), Mg, Fe(III), Fe(II), K | Higher than 1000 ppm |

Table 2 Concluded tolerance concentration of each heavy metal ions using testing solution Pb(II) 250 ppm

**5. Investigation of PAD Effectiveness**

To determine whether Paper-based analytical device is practical or not, we collected 4 water samples spiked them with lead (II) ion and measured lead concentration in both ways, conventional method ICP-OES and our proposed method. The result is shown in Table 3.

| Type of samples | Spiked lead concentration (ppm) | ICP-OES results | Proposed method | |
|---|---|---|---|---|
| | | Concentration (ppm) | Concentration (ppm) | %RSD |
| Water Sample#1 | no spike | 0.01 ± 0.00 | ND[1] | - |
| | 50 | 49.21 ± 0.41 | 51.91 ± 2.11 | 4.06 |
| | 100 | 99.11 ± 0.09 | 104.11 ± 2.11 | 2.03 |
| Water Sample#2 | no spike | 0.02 ± 0.00 | ND[1] | - |
| | 50 | 50.01 ± 0.13 | 52.03 ± 1.89 | 3.63 |
| | 100 | 100.99 ± 0.15 | 103.25 ± 2.45 | 2.37 |
| Water Sample#3 | no spike | 0.02 ± 0.02 | ND[1] | - |
| | 50 | 49.87 ± 0.04 | 50.87 ± 2.71 | 5.33 |
| | 100 | 102.14 ± 0.31 | 105.51 ± 3.39 | 3.21 |
| Water Sample#4 | no spike | 0.01 ± 0.02 | ND[1] | - |
| | 50 | 50.19 ± 0.21 | 50.31 ± 1.92 | 3.82 |
| | 100 | 98.11 ± 1.21 | 104.97 ± 3.29 | 3.13 |

Table 3 Comparing conventional method ICP-OES to our proposed method Paper-based analytical device with lead spike 0, 50, 100 ppm using water sample from laboratory of Mahidol Wittayanusorn and Chulalongkorn University

According to Table 3, the values of conventional method and our proposed method are no significantly different (almost all specimens got lower than 5% RSD).

## Conclusion

1. The optimal conditions for reaction are using NaOH 0.1 M, dithizone 2.5 mM, and reaction time 5 minutes.

2. 10 ppm is the minimum lead concentration which PAD can detect.

3. The interference heavy metals considering 5% tolerance, Co (II), Cu (II), Ni (II), Ag (I) concentration is lower than 10 ppm. Cd(II), Hg(II), Mg2+, Fe(II), Fe(III), K+ concentration is more than 1000 ppm.

4. The value of conventional method and PAD are no significantly different
(lower than 5% error).

## Acknowledgements

This research can be success by many people especially Mrs. Orawon Chailapakul, Mr.Poomrat Rattanarat, from Chulalongkorn University and Mrs. Usa Jeenjenkit from Mahidol Wittayanusorn School. Sincerely thanks to these people who provided insight and expertise that greatly assisted the research

## Reference

[1] Shammas, N.K., Wang, L.K., Hung, Y.T. (2012). Handbook of Environment and Waste Management: Air and Water Pollution Control.Tambekar, Singapore.

[2] Tambekar, P., Batra, R. J., &Weginwar, R. G.(2014).Physico-chemical and microbial analysis of certain water sources and industrial wastewater samples in Chandrapur District, Maharastra, India. Journal of Chemical & Pharmaceutical Research, 6 (5), 710.

[3] Wu, S., Lv, T., Li, C., Kuschk, P., Wiessner, A., Pang, C., & Dong, R. (2012). Advances in Environmental Science and Engineering. Selected, peer reviewed paper from the 2012 International Conference on Energy and Environmental Protection (ICEEP 2012), June 23-24, 2012, Hohhot, China.