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Colorimetric analyzer based on mobile phone camera for determination of available phosphorus in soil

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ABSTRACT

A field deployable colorimetric analyzer based on an "Android mobile phone" was developed for the determination of available phosphorus content in soil. An inexpensive mobile phone embedded with digital camera was used for taking photograph of the chemical solution under test. The method involved a reaction of the phosphorus (orthophosphate form), ammonium molybdate and potassium antimonyl tartrate to form phosphomolybdic acid which was reduced by ascorbic acid to produce the intense colored molybdenum blue. The software program was developed to use with the phone for recording and analyzing RGB color of the picture. A light tight box with LED light to control illumination was fabricated to improve precision and accuracy of the measurement. Under the optimum conditions, the calibration graph was created by measuring blue color intensity of a series of standard phosphorus solution (0.0–1.0 mg P L^{-1}), then, the calibration equation obtained was retained by the program for the analysis of sample solution. The results obtained from the proposed method agreed well with the spectrophotometric method, with a detection limit of 0.01 mg P L^{-1} and a sample throughput about 40 h^{-1} was achieved. The developed system provided good accuracy (RE < 5%) and precision (RSD < 2%, intra- and inter-day), fast and cheap analysis, and especially convenient to use in crop field for soil analysis of phosphorus nutrient.

1. Introduction

In agriculture, the determination of major plant nutrients (*e.g.* nitrogen, phosphorus and potassium) in soil and fertilizers are crucial for management of nutrient suitable for plant growth and avoiding the over dump of fertilizer to soil that will be lead to environmental pollution [1,2]. The phosphorus is the key essential nutrient which is widely distributed in nature. Although total amount of phosphorus in the soil may be high, it is often present in unavailable forms or in the forms that are only available outside of the rhizosphere. In the soil, more than 80% of the phosphorus can become immobile and unavailable for plant uptake because of adsorption, precipitation, or conversion to the organic form. Phosphorus is never found in a free or uncombined state because of its great affinity for oxygen. Orthophosphates are the most abundant form of phosphorus in an environment, and are readily available for assimilation that was used as fertilizers [3–5].

The available phosphorus is an essential plant nutrient, thus knowing the amounts of phosphorus in soils is useful for agricultural management. Many techniques were reported for determination of available phosphorus [6]. In a batchwise chemical analysis, the colorimetric method, such as vanadomolybdophosphoric acid method and molybdenum blue method based on stannous chloride or ascorbic acid as a reductant [7], is widely used for determination of available phosphorus. However, batch method consumes long time, large amount of chemicals and the big instrument is difficult to be employed in the field.

Nowadays, several commercial test kits are available for field test of phosphorus in soil. Five commercially available soil test kit were compared with standard laboratory analysis for phosphorus content as reported by Faber et al. [8]. All commercial test kits showed similar features such as fast analysis, inexpensive and useful for soil management, but the results of those products presented different accuracies. Nevertheless, the analytical laboratory required precise analytical values, but all of products could provide only the approximate value of nutrient content (*i.e.*, low, medium and high), thus, they are suitable for using as initial screening purposes only.

Recently, the colorimetric analysis could be done by determining RGB values of the digital photo image, which provide more precise and accurate results. The RGB color system represented by

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Table 1
Summary of various analyses based on smartphone camera since 2011-present.

Year	Operating system	Analyte	Linear range	LOD	Precision	Ref.
2011	Symbian 60*	Potassium	3.1×10^{-5}–0.1 M	3.1×10^{-5} M	< 2%RSD	[9]
2012	Android*	O_2	2–100%	0.7%O_2	N/A	[10]
2013	iOS	Trinitrotduene (TNT)	1–500 mg L^{-1}	3.67–4.0 mg L^{-1}	2.09–7.43%RSD	[11]
2013	Android*	Soil color	N/A	N/A	SD < 3	[12]
2013	Android and iOS	Age of blood stains	1 h–42 day	N/A	N/A	[13]
2013	Android*	Citrus yield	N/A	N/A	N/A	[14]
2014	iOS	Methamphetamine	0.1–2.5 mg L^{-1}	0.01–0.04 mg L^{-1}	2–6%RSD	[15]
2014	Android*	Cocaine	0–1 mg L^{-1}	0.25 mg L^{-1}	N/A	[16]
2014	iOS	Maltose	0–70 μmol mL^{-1} maltose	3.5 μmol mL^{-1}	4–6%RSD	[17]
2014	Android and iOS*	pH	5.0–9.0	< 1.66	N/A	[18]
		Protein	0–100 mg L^{-1}	< 41 mg dL^{-1}		
		Glucose	0–500 mg L^{-1}	< 92 mg dL^{-1}		
2014	Android*	Salivary cortisol	0.01–10 ng mL^{-1}	0.01 ng mL^{-1}	N/A	[19]
2015	Android	Salivary cortisol	0.3–60 ng mL^{-1}	0.3 ng mL^{-1}	10–16%RSD	[20]
2015	Android*	pH	1–14	N/A	N/A	[21]
In press	Android	CRP	0.3–81 ng mL^{-1}	0.4 ng mL^{-1}	1.7–3.7%CV	[22]
		HRP	0.114–7.3 ng mL^{-1}	0.114 ng mL^{-1}	2.6–7.1%CV	
		BCA protein	20–2000 μg mL^{-1}	N/A	N/A	
Present	Android*	Available phosphorus	0.0–1.0 mg L^{-1}	0.01 mg L^{-1}	< 2%RSD	This work

BCA: bicinchoninic acid; CRP: human C-reactive protein; CV: coefficient of variance; HRP: horse radish peroxidase; LOD: limit of detection; N/A: not available; SD: standard deviation; RSD: relative standard deviation.

* With application software development.

three component intensities (red, green and blue) is an important index for color expression. The digital cameras integrated in mobile phone and similar devices were interested and have been developed as image detectors. These mobile/smart phones are now available at low cost. Table 1 summarized smartphone camera systems that applied to various analyses. Different application softwares on various operating systems have been developed to be used with smartphone for specific purposes. Among different operating systems, the Android is more popular to develop application because of its low cost smartphone, having open code, free license program and a large community of developers.

In this work, we develop a new colorimetric analyzer based on an android mobile phone for application in agricultural field. The aims of the research were: (i) to design and create the more convenient device for the determination of available phosphorus in soil (ii) to develop software program in the android operating system for recording and analyzing RGB color of the picture (iii) to improve the accuracy and precision of the results by controlling the illumination on sample during taking a photograph. The developed method was successfully applied to convenient determination of phosphorus content in soil from fruit orchards for soil management of phosphorus nutrient.

2. Experimental

2.1. Chemicals and solutions preparation

All chemicals are analytical reagent grade and obtained from Ajax Finechem Co. Ltd. (Australia), except HCl was from B.D.H Co., Ltd (England). All solutions were prepared by using deionized water produced by water purification system (Millipore, Sweden). Acidic molybdate reagent was prepared from 25 mL of 3%(w/v) molybdate solution (dissolving 1.5 g of ammonium molybdate in 50 mL water) in 62.5 mL of 4 mol L^{-1} sulfuric acid (22 mL of 98%(w/v) sulfuric acid in 100.00 mL water) with 12.5 mL of 0.1%(w/v) antimony tartrate (dissolving 0.1 g of antimony tartrate in 100 mL water). Finally, the 100 mL of reagent solution was obtained. A 1%(w/v) of ascorbic acid solution was freshly prepared by dissolving 1 g of ascorbic acid in 100 mL of water. A stock solution of phosphorus (1000 mg P L^{-1}) was prepared by dissolving 0.4394 g potassium dihydrogen phosphate in 100.00 mL of water. Working standard solution of phosphorus was freshly prepared by diluting the stock solution with water. A Bray II solution for extraction of phosphorus from soil was prepared by mixing 100 mL of 0.5 mol L^{-1} HCl and 15 mL of 1.0 mol L^{-1} NH_4F and adjusted to volume of 500 mL with water. The final concentration of NH_4F and HCl are 0.03 and 0.1 mol L^{-1}, respectively.

2.2. Sample collection and extraction procedure

The soil samples were collected from mangosteen, durian and rambutan orchards in Chanthaburi, Thailand. All samples were air-dried and ground to less than 2 mm particles diameter. The extraction procedure [23,24] was carried out by shaking a portion of 1 g of each soil sample with 7 mL of bray II solution for 1 min. The extracted solution was filtered through a filter paper and the final volume of the filtrate was adjusted to 25.00 mL with water. Filtering of sample may take time about 10–15 min, however, several samples could be done at the same time.

2.3. Apparatus and instrument setup

The colorimetric analyzer box (Fig. 1) was made from a polystyrene foam box, covered outside of the box with black plastic board for protecting the environmental light. Dimension of the box (width × length × height) is as follow: outside: 8 × 10 × 18 cm and inside: 5 × 6 × 11.5 cm). A battery operated flashlight with 7 LED was used to control illumination inside the box by attaching it with the lid of the box at a position over the sample test tube. At this position there is no reflected light on side of the test tube being observed when a rough surface back screen was used, as described in Section 3.5. A mobile phone was stuck on the front of the box at about the center position of the box that providing good focus to the tube. A sample holder was fixed far from mobile phone camera about 3–4 cm, that giving the best focus. In this work, the Nokia X Dual SIM RM-980 was used. However, any Andriod smartphone may be used, with the following setup/characteristics, *i.e.*, LCD touchscreen, back camera at least 3 megapixel (2048 × 1536 pixel), fixed-focus camera without flash, automatic ISO control, automatic white balance, and single image mode.

Spectrophotometer (T60 visible spectrophotometer, PG instruments Ltd., England) was used to perform a standard method for