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Determination of available phosphorus in soils by using a new extraction procedure and a flow injection amperometric system

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ABSTRACT

A new extraction procedure based on an off-line extraction column was proposed for extracting of available phosphorus from soils. The column was fabricated from a plastic syringe fitted at the bottom with a cotton wool and a piece of filter paper to support a soil sample. An aliquot (50 mL) of extracting solution (0.05 M HCl + 0.0125 M H_2SO_4) was used to extract the sample under gravity flow and the eluate was collected in a polyethylene bottle. The extract was then analyzed for phosphorus contents by a simple flow injection amperometric system, employing a set of three-way solenoid valves as an injection valve. The method is based on the electrochemical reduction of 12-molybdophosphate which is produced on-line by the reaction of orthophosphate with acidic molybdate and the electrical current produced was directly proportional to the concentration of phosphate in range of 0.1–10.0 mg L^{-1} PO_4-P, with a detection limit of 0.02 mg L^{-1}. Relative standard for 11 replicate injections of 5 mg L^{-1} PO_4-P was 0.5%. A sample through put of 35 h^{-1} was achieved, with consumption of 14 mg KCl, 10 mg ammonium molybdate and 0.05 mL H_2SO_4 per analysis. The detection system does not suffer from the interferences that are encountered in the photometric method such as colored substances, colloids, metal ions, silicate and refractive index effect (Schlieren effect). The results obtained by the column extraction procedure were well correlated with those obtained by the steady-state extraction procedure, but showed slightly higher extraction efficiency.

1. Introduction

Phosphorus is a major nutrient for plants and is typically present in soils for agricultural purposes in the concentration range of 0.03–0.22%. However, the major fractions of P in soil are in the insoluble forms, which plants cannot utilize. Therefore, it is the plant-available P, the amount of P extracted by the chemical extractant, which is correlated well with the amount of P taken up by the plant that is more important than the total amount of P in the soil [1]. Orthophosphate is the dominant form of available P useful for plant grown. Release of available P from soil depends on several factors such as pH, humidity and type and amount of soil minerals. In agricultural practices, P is usually obtained from the applied fertilizer. However, in soils which contain high levels of iron and aluminum, P in fertilizer may be absorbed by soils, thus only small fractions (3–25%) would be available for plants. On the other hand, application of excessive amounts of fertilizer may cause impact to the environment due to leaching of the nutrient to the water body.

The determination of available P in soil is necessary for agricultural practices and research in this field. The test values obtained are useful for the producers to best manage for the application of fertilizer and make decisions concerning the profitability of their operations while managing for impacts such as erosion, nutrient runoff and water quality [2]. Several chemicals have been used as extractants for available P. The suitable extractant would give the best correlation between the amounts of P extracted and P taken up by the particular plant, which depends on several factors, such as type and amount of soil minerals, and pH of soil. There are some commonly used extraction protocol for available P such as Bray No. 1 (0.025 M HCl + 0.03 M NH_4F), Bray No. 2 (0.10 M HCl + 0.03 M NH_4F), North Carolina (0.05 M HCl + 0.0125 M H_2SO_4), Troug (0.001 M H_2SO_4 + $(NH_4)_2SO_4$ (pH 3.0)), Citric acid (1.0% citric acid), Egner (0.01 M calcium lactate + 0.02 M HCl), Morgan (0.54 M HOAc + 0.7 M NaOAc (pH 4.8)) and Olsen (0.50 M $NaHCO_3$ (pH 8.5)) [3,4]. The procedure for soil extraction is based on shaking known amounts of soil with an accurate volume of the extractant for extended periods of time to reach a steady-state condition between the solid and liquid phases. The extract is then filtered and analyzed for P content by spectrophotometric methods, employing either molybdenum blue or vanadomolybdate reactions. A stirred flow extraction chamber [5] and an extraction micro-column [6] have been proposed for on-line fractionation of P from soil and sediment under non-steady-state condition. In these procedures, a fresh extracting solution was continuously propelled though the

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sample bed, resulting in better extraction efficiency to be obtained. However, on-line extraction procedure requires a pump and a micro-column for each soil sample and concerning a long extraction time, so it is not suitable for extraction of available P involving a large number of soil samples. Such a protocol is usually applied for generating the extraction profiles for kinetic study of the leaching process [6]. Thus, we proposed here a batchwise gravity feed column extraction procedure, which on the other hand utilized a simple syringe as extraction vessel for each sample and despite it takes long extraction time, extraction of several samples at the same time could be carried out in order to increase sample throughput.

The spectrophotometric detection based on molybdenum blue method either in batch or flow systems is commonly used for determination of P. The flow system provided higher degrees of automation and reduced consumption of the chemicals. A multi-syringe flow injection analysis was proposed for determination of available P in soils [7]. All injection analysis was developed for determination of P in soil and sediment extracts [8]. However, the spectrophotometric detection method suffers potential interference from colored and colloidal substances and silicate. In flow analysis, the effect of refractive index or the Schlieren effect can also be a problem [6,8]. These interferences may present in the soil extract at high extents. We have developed the FI amperometric systems, which have higher tolerant to these interferences [9], thus they should be appropriate for soil analysis.

In this work, a new extraction procedure based on off-line column extraction was developed for extracting available P from soil. The collected extract was analyzed for P concentration by using a FI amperometric method which was based on the electrochemical reduction of the on-line formed 12-molybdophosphate, producing an electrical current, which was directly proportional to P concentration. The column extraction was simpler, more convenient to use and provided higher extraction efficiency than the standard extraction procedure based on batchwise shaking. The amperometric detection provided higher selectivity than the spectrophotometric one, as it did not suffer from colored substances, particulates and refractive index effect (Schlieren effect), and could tolerate to silicate at high concentration (up to 1000 mg L^{-1}). The system could be applied to soil extract without the requirement of masking agent and with low consumption of chemicals (2 mL each of KCl and reagent solutions per analysis).

2. Experimental

2.1. Chemicals and materials

All chemicals used were of analytical reagent grade. Deionized water (obtained by a system of Milli-Q, Millipore, Sweden) was used throughout. Acidic molybdate solution (0.5%, w/v in 2.5%, v/v sulfuric acid) was prepared by dissolving 1.25 g of ammonium molybdate (Ajax Finechem, Australia) in water and 6.50 mL of concentrated sulfuric acid (Merck, Germany) was added before adjusting the final volume to 250 mL. A 0.1 M potassium chloride was prepared by dissolving 1.86 g KCl (Merck, Germany) with water and adjusting volume to 250 mL. Orthophosphate stock solution (1000 mg L^{-1} PO_4-P) was prepared by dissolving 0.4394 g KH_2PO_4 (Merck, Germany) with water and adjusting volume to the mark of a 100-mL volumetric flask. Working standard solutions in the concentration range of 0.1–10 mg L^{-1} PO_4-P were daily prepared from an intermediate solution (10 mg L^{-1} PO_4-P), diluted from the stock solution. A solution for extraction of phosphate from soil (0.05 M HCl + 0.0125 M H_2SO_4) was prepared by diluting 2.10 mL conc. HCl and 0.35 mL conc. H_2SO_4 in water and adjusting the volume to 500 mL.

Electrode polishing kit (model MF-1000, BAS, USA) was used for cleaning of the working electrode.

2.2. FI manifold

The FI system used is schematically depicted in Fig. 1(a). It consisted of a peristaltic pump (Ismatec, Switzerland) with pump tubing, an injection device assembled from a set of three-way solenoid valves (Biochemvalve, USA) [10], a flow through electrochemical cell (cross-flow cell, Model MF-1093, BAS, USA) assembling with a 3-mm diameter glassy carbon working electrode (GCE), a stainless steel auxiliary electrode and a Ag/AgCl reference electrodes (3 M KCl), and a compact potentiostat (Palmsens Vs 3.6, Netherlands) with connecting to a personal computer. The three-way solenoid valves were assembled to be used as an injection valve, as shown in Fig. 1. Actuation of the valves for loading and injecting positions was performed by manually switching the electrical power supplied to the valves. PTFE tube of inner diameter of 0.5 mm was used for assembling the system.

2.3. Sample collection and extraction procedures

Soil samples were collected from longan orchards and vegetable growing field in Chiang Mai, northern of Thailand. A soil sample was taken from 15 points at a depth of 7 cm and combined together. The sample was dried in air and ground to a particle sizes of less than 100 mesh.

The standard extraction procedure was carried out by accurately weighing 1.00 g of soil, putting in a 100-mL Erlenmeyer flask, adding 15.00 mL of the extracting solution and shaking for 30 min. The extract was filtered through a Whatman No. 5 filter paper into a polyethylene bottle.

Column extraction was carried out batchwise employing a plastic syringe fitted at the bottom with cotton wool and a

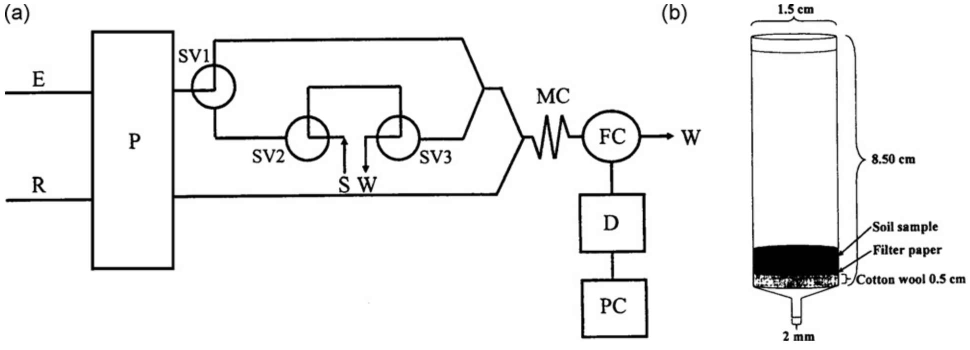

Fig. 1. (a) FI manifold of flow injection amperometric system for determination of phosphate; (*E* = 0.1 M KCl, *R* = 0.5%, w/v molybdate in 2.5%, v/v sulfuric acid, P = peristaltic pump, S = standard/sample, SV1–SV3 = three-way solenoid valves, MC = mixing coil, FC = electrochemical flow through cell, W = waste, D = potentiostat/amperometric detector, PC = personal computer) and (b) an extraction column for off-line leaching of soil samples (adapted from a 10-mL plastic syringe).

piece of filter paper to support the sample (Fig. 1(b)). A portion (1.0000 g) of ground soil was accurately weighed and put in the column. An extracting solution (50.00 mL) was carefully poured into the column and the leachate was collected in a polyethylene bottle.

Extraction was carried out at room temperature of about 25 ± 2 °C.

2.4. FI amperometric determination procedure

A KCl electrolyte solution and an acidic molybdate solution were pumped at $1.0\ \mathrm{mL\,min^{-1}}$ each. A standard/sample solution (75 μL) was injected into the stream of 0.1 M KCl and then merged with the stream of the acidic molydate solution which then flowed through a mixing coil (50 cm in length) to the amperometric flow cell. The 12-molybdophosphate complex produced on-line was then electrochemically reduced at the glassy carbon working electrode producing an electrical current, that was directly proportional to the concentration of phosphate (PO_4-P). The current was converted to voltage and continuously recorded as a FI peak. A calibration graph was a plot of peak height obtained vs. P concentration. Concentration of P in sample was then evaluated from the calibration graph.

3. Results and discussion

3.1. FI amperometric determination of orthophosphate

The FI amperometric method for orthophosphate determination is based on the electrochemically reduction of the 12-molybdophosphate which formed on-line by the reaction of orthophosphate and molybdate in an acidic medium [9]. The reduction current is linearly proportional to orthophosphate concentration. The FI amperometric system for the determination of orthophosphate in the soil extract was modified from the previously reported system [9] by replacing the six port injection valve with the injection valve assembled from three solenoid valves (SV1–SV3) and a three-way connector, as shown in Fig. 1(a). At the loading position (as shown in Fig. 1(a)), a standard/sample was loaded into a sample loop and then by manually switching current, the solenoid valves were actuated to another position to allow the carrier solution (0.1 M KCl) to inject sample into the reagent solution (0.5%, w/v ammonium molybdate in 2.5%, v/v H_2SO_4). The reaction product, 12-molybdophosphate was reduced at the GCE, at a constant applied potential of 0.20 V vs. Ag/AgCl, producing a FI peak that was recorded by a portable amperometer. Fig. 2 illustrates the FI profiles of phosphate standard solution (1.0–10.0 $\mathrm{mg\,L^{-1}}$ PO_4-P) and some samples. Linear calibration graphs for phosphate in the concentration ranges of 0.1–1.0 ($y = 0.2455x + 0.0014$, $r^2 = 0.9996$) and 1.0–10.0 ($y = 0.2111x + 0.1389$, $r^2 = 0.9966$) $\mathrm{mg\,L^{-1}}$ PO_4-P were obtained. The detection limit calculated from three times standard deviation of blank/slope of the calibration graph was found to be 0.02 $\mathrm{mg\,L^{-1}}$ PO_4-P.

Relative standard deviations for 11 replicate injections of 5 $\mathrm{mg\,L^{-1}}$ PO_4-P were 0.5%. A sample throughput of 35 $\mathrm{h^{-1}}$ was achieved, with the low consumption of reagents (about 2 mL each of electrolyte and reagent solutions, corresponding to 14 mg KCl, 10 mg ammonium molybdate and 0.05 mL sulfuric acid per analysis).

As reported previously for the determination of P in water samples [9,10], the FI amperometric method has higher selectivity than those based on spectrophotometric detection. The method can tolerate silicate up to 1000 $\mathrm{mg\,L^{-1}}$, so no masking agent was needed for soil analysis. The interferences from some metals such as Fe and Al at high concentration can be avoided by passing the sample solution through a cation exchange resin column. However, no interference was observed for the studied soil samples, so such a treatment was not necessary. The developed system did not suffer from colored substances, colloids and Schlieren effect.

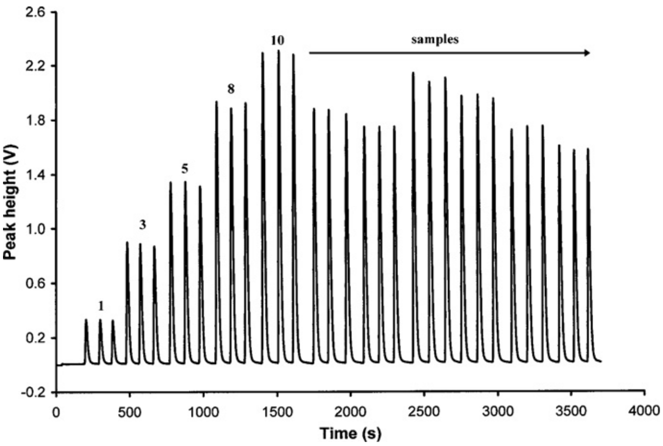

Fig. 2. FI profiles for standard phosphate solutions and samples, determined by FI amperometric system in Fig. 1(a); condition: applied potential 0.20 V, $E = 0.1$ M KCl, $R = 0.5\%$, w/v molybdate in 2.5%, v/v sulfuric facid, and flow rate of each line $1.0\ \mathrm{mL\,min^{-1}}$. Values indicate concentration of phosphate in $\mathrm{mg\,L^{-1}}$ PO_4-P.

3.2. Leaching of available P from soil by extraction column

The standard procedure for extraction of available phosphorus from soil is based on shaking of the suspension of soil in the extractant for a period of time to reach equilibrium (45 min) and filtering of the extract. Different extracting solutions can be utilized, which may give slightly different available P contents dependent on the type of soils. The proposed extraction procedure was based on column extraction and the North Carolina solution (0.05 M HCl + 0.0125 M H_2SO_4) was selected as the extracting solution. A simple 10 mL plastic syringe was adopted to use as a column, by taking out the plunger and placing a cotton wool and a piece of a Whatman No. 40 filter paper fitted to the syringe for supporting a soil sample as shown in Fig. 1(b). An accurate amount of soil sample (about 1.00 g) was put in the syringe and an accurate volume of extracting solution was added carefully to fill the remainder volume of the syringe. The extraction gravity feed profiles were investigated by collecting the eluate fractions (15.00 mL each) to be determined for phosphate concentration. It was found that the similar profiles were obtained for different soil samples and more than 99% of the phosphate could be extracted within fraction number 3, corresponding to extraction volume of 45.00 mL, as shown in Fig. 3. The volume of extracting solution of 50.00 mL was selected for further extraction of soil samples. Although the extraction time of about 50 min was needed, several extractions could be performed in parallel at the same time, leading to high sample throughput. The relative standard deviation was 6.0%, for six replicate extractions of the same soil sample, which was comparable to that of the standard extraction procedure (6.8%). Although the mini-column could be incorporated to the FI amperometric system in order to perform on-line extraction/determination, the low sample throughput was resulted due to the long extraction time involved. Thus the on-line extraction was not suitable for determination of available P in soil, but it might be appropriate for study on sequential extraction of P associated with different phases of soil employing various extracting solutions [6].

It should be noted that some extracted solutions obtained had intense color due to the organic matter present in the soils, which

Table 1
Available P contents in soil samples, extracted by the proposed and the standard procedures, and determined by the FI amperometric and standard spectrophotometric [11] methods.

Sample	Available phosphorus content (mg kg^{-1} as PO_4-P)*			
	Column extraction		Standard extraction	
	FI amperometric	Spectrophotometric	FI amperometric	Spectrophotometric
1	1173 ± 4	1152 ± 5	1083 ± 1	1095 ± 7
2	1349 ± 14	1368 ± 3	1235 ± 10	1244 ± 2
3	1085 ± 1	1119 ± 1	960 ± 2	995 ± 2
4	1718 ± 8	1725 ± 9	1535 ± 7	1505 ± 6
5	1810 ± 1	1729 ± 1	1567 ± 4	1533 ± 1
6	473 ± 6	467 ± 1	421 ± 4	422 ± 2
7	1387 ± 8	1352 ± 2	1217 ± 5	1182 ± 2
8	73.9 ± 1.3	60.4 ± 0.1	58.6 ± 0.9	51.5 ± 0.1
9	2160 ± 5	2040 ± 1	1859 ± 4	1749 ± 1
10	1951 ± 1	2060 ± 7	1527 ± 6	1697 ± 1
11	3.7 ± 0.2	4.3 ± 0.1	2.3 ± 0.1	3.2 ± 0.2
12	35.4 ± 0.3	30.8 ± 0.3	28.2 ± 0.1	24.4 ± 0.3
13	45.2 ± 0.2	44.6 ± 0.4	31.0 ± 0.1	31.4 ± 0.2
14	12.9 ± 0.1	13.6 ± 0.1	7.1 ± 0.1	7.6 ± 0.1
15	38.5 ± 1.0	34.9 ± 0.2	24.0 ± 0.2	23.1 ± 0.2
16	65.8 ± 1.0	59.2 ± 0.5	56.6 ± 2.1	52.2 ± 0.3
17	22.4 ± 0.6	26.0 ± 0.2	29.7 ± 0.4	31.9 ± 0.1

* Mean of triplicate results.

may interfere in the spectrophotometric determination, but not in the FI amperometric system.

3.3. Determination of available P in soil samples

The developed method was applied to the determination of available P in different soil samples, collected from Longan orchards and vegetable field in Chiang Mai province of Thailand. Soil extraction was performed using the developed procedure and a standard extraction procedure for comparison. The extracts were determined for available P contents by the proposed FI amperometric method and standard spectrophotometric method [11] based on molybdenum blue reaction using ascorbic acid as a reducing agent and potassium antimonyl tartrate acting simultaneously as a catalyst and masking agent. According to *t*-test at 95% confidence level, the results obtained from both methods were in good agreement ($t_{critical} = 2.021$, $t_{calculated} = 0.025$, $n = 17$) [12], as presented in Table 1. It should be noted that the FI amperometric method consumed much smaller amounts of chemicals and was simpler, faster, more convenient to use and provided better tolerance to the interferences presented in soil (e.g., colored substances, colloids and silicate) than the standard spectrophotometric method.

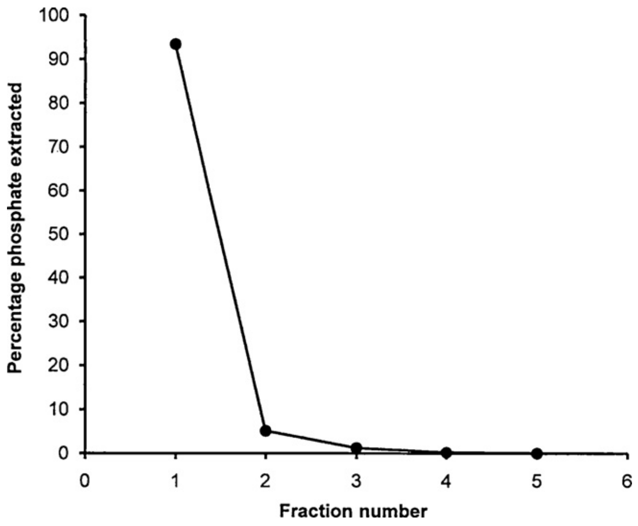

Fig. 3. Percentage extraction of phosphate from soil in different fractions of the extracting solution.

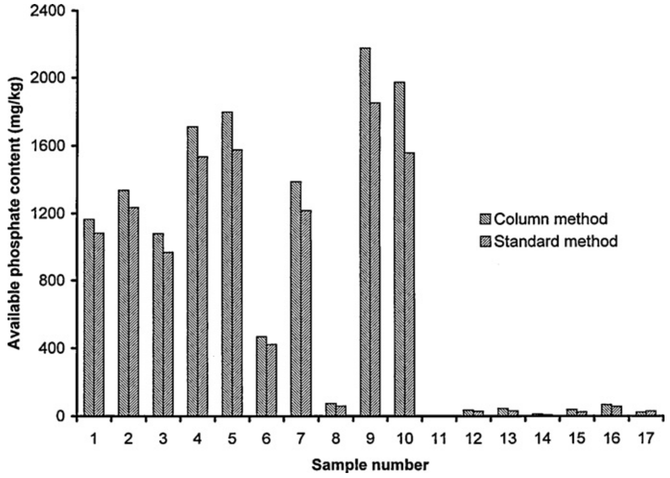

Fig. 4. Available P contents extracted by using the column extraction and standard extraction procedures.

It was found that the phosphate contents found in the extracts from both the extraction procedures were in good correlation. However, the proposed extraction procedure provided better extraction efficiency than the standard method, as shown in Fig. 4. This may due to the advantage of dynamic extraction that the sample was in contact with the fresh extracting solution, which flowed through the sample bed in the extraction column, so no re-adsorption of the extracted P was occurred. Moreover, the proposed extraction procedure was simpler, and more convenient to use than the standard method. The extraction of several samples could be performed at the same time.

4. Conclusion

A flow injection amperometric method and a new extraction procedure were proposed for the determination of available phosphorus in soil samples. Although the extraction was done by using a simple column made from a plastic syringe, a better extraction efficiency than the standard extraction procedure could be accomplished, which was more convenient to perform. The proposed extraction procedure may be a new alternative way to perform extraction of available P in routine analysis of soil. The FI amperometric method provided the precise and accurate results, with using of simple and cost-effective components to assemble the system, and consumed of smaller amount of chemicals. The amperometric detection also provided significant advantages over the spectrophotometric detection of the standard method, e.g., it can tolerate to silicate up to 1000 mg L^{-1} and does not suffer interference from turbidity and colored substances present in soil samples. The system could be developed to higher degrees of automation by control of the injection valve via a computer.

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