Article

A Multi-Pumping Flow System with Pulsed Fluidization to Evaluate Soil Capacity for Phosphate Adsorption

Jeane M. C. Machado,[a,b] Wanessa R. Melchert,[a] Elias A. G. Zagatto[b] and Marcos Y. Kamogawa*,[a]

[a]*Escola Superior de Agricultura Luiz de Queiroz, Universidade de São Paulo, CP 9, 13418-900 Piracicaba-SP, Brazil*

[b]*Centro de Energia Nuclear na Agricultura, Universidade de São Paulo, CP 96, 13400-970 Piracicaba-SP, Brazil*

Soils with high levels of iron and aluminum, thus negatively the phosphorous availability, affecting crop yields. A prior determination of the soil capacity to adsorb phosphate is relevant to attaining good agricultural performance. To this end, a multi-pumping flow system with pulsed fluidization is proposed. The procedure takes into account the amount of remaining phosphorus (P-rem) in the solution after the adsorption step to indirectly estimate the soil retention capacity. A 1.0 mg L^{-1} P solution is passed through a fluidized bed column with 50 mg of soil (particles < 0.15 mm), where the analyte is adsorbed. P-rem was spectrophotometrically quantified by the molybdenum blue method. The coefficient of variation (n = 20), limits of detection and quantification (99.7% confidence level), and sampling rate were estimated as 1.54%, 17 and 51 µg L^{-1} P, and 87 h^{-1}, respectively. Only 0.27 mg $(NH_4)_6Mo_7O_{24}$ and 0.10 mg $SnCl_2$ were consumed *per* determination, generating 2.4 mL of waste. The results followed a linear relationship [y = 0.9702x + 1.483 (r = 0.9635; n = 20)] with the reference procedure. In comparison to the reference procedure, 100, 40 and 180-fold reductions in the required soil amount, waste generation and analytical time were attained.

Keywords: solenoid micro-pumps, adsorption, kinetic, solid-liquid interaction

Introduction

Productivity in tropical soils is closely related to soluble or available phosphorus, usually *o*-phosphate species. These species are adsorbed on oxides and aluminosilicates through bonds exchange with surface OH$^-$ groups, or precipitated as insoluble compounds with e.g. Ca, Fe and Al (Metal-PO$_4$ ⇋ PO$_4^{3-}$). Soils with high clay, iron and aluminum contents are generally characterized by a high phosphate adsorbing capacity, resulting in low availability of this species to plants, causing nutritional deficiency and thereby affecting plant growth and production.[1]

Knowledge of the soil capacity to adsorb phosphate is thus required to estimate the concentration of soluble phosphorus in the soil solution (P-sol) in order to support the recommendation for a suitable fertilization. In this context, the determination of remaining phosphorus (P-rem), i.e., the amount of P in the solution after adsorption in soil, has been often used to estimate the adsorption capacity. The P-rem determination proposed in 1971[2] and adapted to Brazilian soils in 2000,[3] presents drawbacks such as the large required volumes of reagent and the cumbersome steps of dilutions, transfers and filtrations, which are time-consuming and susceptible to errors. Consequently, mechanization of the entire procedure and increased sampling rates are aimed at, especially in routine laboratories complying with the principles of Green Chemistry.[4,5]

Flow analysis fulfills these requirements, as flow systems allow for different sample preparation procedures and detection techniques, thus minimizing the possibility of contamination and human errors.[6] The main advantages of flow analyzers in relation to monitoring dynamic extraction processes are reduced analysis time and reagent consumption, the possibility to implement a continuous or segmented extraction process, as well as the instrumental versatility and robustness. These advantages have been emphasized in relation to phosphorus fractionation in mini-columns for *o*-phosphate monitoring in a multi-syringe flow injection analyzer,[7] and to the fractionation of organic and inorganic phosphorus in cereals in a multi-pumping flow analyzer.[8]

*e-mail: kamogawa@usp.br

Multi-pumping flow systems utilize solenoid micro-pumps to promote turbulent mixing, which improves radial mixing, and thus sample/reagent interactions. This aspect becomes more relevant in relation to analytical procedures involving relatively slow reactions where sensitivity is critical, as the mixing conditions may influence the analytical sensitivity.[9] Moreover, this aspect is a positive factor regarding the efficient implementation of fluidized beds for in-line analyte separation-concentration by solid phase extraction.[10] Sample movement and particle refluxing lead to an improvement in solid-liquid interactions, a condition efficiently attained with fluidized beds. The solution drag force through the particles is enough to support them, providing a pressure drop in the bed. Consequently, a chaotic yet reproducible geometry of the refluxing solid particles is established, characterizing fluidization.[11]

With fluidized beds, the limitations inherent to packed columns, such as overpressure, swelling effects, low availability of sites for interaction, and the establishment of preferential pathways are eliminated.[10] In this context, fluidization of solid waste samples has improved the efficiency of Cd, Cr, Cu and Zn extraction, resulting in better accuracy and repeatability in comparison to the batch-wise analogous procedure.[12]

The aim of this work was therefore to exploit the turbulent mixing inherent to pulsed flows and the establishment of a fluidized bed to evaluate the soil capacity to adsorb phosphorus. To this end, an advanced multi-pumping flow system was designed for the spectrophotometric determination of P-rem in soils.

Experimental

Reagents and solutions

All solutions were prepared with distilled-deionized water (resistivity > 18.2 MΩ cm) provided by a Milli-Q system. Chemicals were of analytical grade quality, except for calcium chloride, a technical-grade reagent.

The solution (R_1, Figure 1), a 10.0 mmol L^{-1} CaCl$_2$ plus 60.0 mg L^{-1} phosphorous (as KH$_2$PO$_4$) solution, was similar to that used in the batch-wise procedure,[3] but prepared with a lower (1.0 mg L^{-1}) phosphorus concentration.

For phosphate determination by the batch-wise procedure, a 3.9 mmol L^{-1} bismuth as (BiO)$_2$CO$_3$ plus 85 mmol L^{-1} ammonium heptamolybdate as (NH$_4$)$_6$Mo$_7$O$_{24}$ plus 2.7 mol L^{-1} sulfuric acid solution, and a 3.0% (m/v) ascorbic acid (C$_6$H$_8$O$_6$) solution were used. In the flow system, 2.0 mmol L^{-1} (NH$_4$)$_6$Mo$_7$O$_{24}$ in 1.0 mol L^{-1} HNO$_3$ and 4.0 mmol L^{-1} stannous chloride as SnCl$_2$ in 0.3 mol L^{-1} HCl solutions were used.[13]

Soil samples

Soil samples, classified as Typic Hapludox (TH), Aquent (A), Mollic Epiaquent (ME), Typic Argiudolls (TA) and Rhodic Hapludox (RH),[14] were collected from the topsoil (0-20 cm). Their main attributes were evaluated, and results are presented in Table 1. Sand was used as a reference in the procedure, in view of its low P adsorption (P-rem determined as 60 mg L^{-1}). To validate the proposed procedure, 16 samples with different adsorption capacities were analyzed by the batch-wise and proposed procedures.

Batch-wise procedure for P-rem estimation

The adsorption procedure was performed by adding 5.0 g of soil sample and 50 mL of a 10.0 mmol L^{-1} CaCl$_2$ plus 60.0 mg L^{-1} P solution into an Erlenmeyer flask and shaking it for 1.0 h at 250 rpm in a horizontal circular shaker. The extracts were filtered through quantitative filter paper. Thereafter, 5.0 mL of 3.9 mmol L^{-1} (BiO)$_2$CO$_3$ and 1.0 mL of 3.0% (m/v) ascorbic acid solutions were added to 5.0 mL of the extract. After 30 min, the P-rem was spectrophotometrically determined by the molybdenum blue method.[18]

Table 1. Soil attributes. Physical properties, oxide composition, soluble P and P-rem concentration by conventional procedure. Clay by hydrometer method;[15] oxides levels by sulfuric and alkaline attack;[16] phosphorus by colorimetry extracted with Mehlich 1[17]

| Soil | Total | | | Texture | SiO$_2$ / % | Al$_2$O$_3$ / % | Fe$_2$O$_3$ / % | MnO / % | TiO$_2$ / % | K$_i$ | K$_r$ | P Mehlich 1 / (mg L^{-1}) | P-rem / (mg L^{-1}) |
	Sand / (g kg^{-1})	Silt / (g kg^{-1})	Clay / (g kg^{-1})										
TH	831	43	126	loamy sand	2.30	4.42	1.68	0.014	0.75	0.88	0.71	11	46.36 ± 1.06
A	115	198	687	clay	10.10	10.01	3.42	0.012	1.45	1.71	1.41	6	32.29 ± 0.40
ME	179	309	512	clay	16.70	11.86	2.84	0.022	1.30	2.39	2.07	89	24.44 ± 0.45
TA	600	80	320	sandy clay loam	21.00	14.68	21.80	0.334	7.77	2.43	1.25	22	15.35 ± 0.53
RH	99	249	652	clay	12.70	24.43	31.92	0.177	10.46	0.88	0.48	6	6.48 ± 0.36

TH: Typic Hapludox; A: Aquent; ME: Mollic Epiaquent; TA: Typic Argiudolls; RH: Rhodic Hapludox.

System dimensioning

The proposed multi-pumping flow system (Figure 1) was comprised of two modules, related to in-line phosphorus adsorption (Figure 1a) and to flow-based phosphate determination (Figure 1b), which were dimensioned as follows.

Phosphorus adsorption module

For optimization of the adsorption module (Figure 1a), different mini-columns (10, 20, 30, 40 or 50 mm long, 3 mm i.d.) with ca. 25, 50, 75, 100 or 125 mg of soil (particles smaller than 0.149 mm) were evaluated. In these experiments, the Mollic Epiaquent soil (ME) was selected because of its intermediate P-rem value (Table 1). Cotton wool was placed at the bottom end of the mini-column to hold the soil particles, and a filter support (Millipore Swinnex, Darmstadt, Germany) with a 0.45 μm cellulose acetate membrane was placed at the top end to retain particulate material. In this way, particle losses during system operation were avoided. Each sample was added in its own column being discarded after use.

A $1.0\ mg\ L^{-1}$ phosphorus in $10.0\ mmol\ L^{-1}\ CaCl_2$ solution was directed to flow through the mini-column, and the extracts were collected after the model 120SP solenoid micro-pump (Biochem Valve Inc., Boonton, NJ, USA) had sent 100 pulses. As the stroke volume was 20 μL, a total volume of ca 2.0 mL was used *per* extraction. The phosphorous concentration in the extract was spectrophotometrically determined, as in the conventional procedure.

Phosphorus determination module

The multi-pumping flow system proposed for P-rem determination (Figure 1b) comprised four model 120SP and 140SP solenoid micro-pumps (Biochem Valve Inc., Boonton, NJ, USA) that dispensed 22.7 ± 0.1, 22.8 ± 0.1, 22.7 ± 0.1 and 43.7 ± 0.5 μL, as experimentally determined and a three-way solenoid valve used to clean the system or exchange the solutions (NResearch, West Caldwell, NJ, USA).

The model USB 2000 multi-channel CCD spectrophotometer (Ocean Optics, Dunedin, FL, USA) was used with a model LS-1 tungsten-halogen radiation source from the same manufacturer. Optical fibers (400 μm) were used to transmit the radiation towards a 1.0 cm optical path quartz flow cell (80 μL internal volume). This spectrophotometer allows the simultaneous acquisition of two wavelengths and was used to subtract the signals relating to Schlieren effect.

The manifold was built up with (0.8 mm i.d.) PTFE tubing. The flow set-up was controlled by using an Arduino board and a potency interface based on the ULN2803 integrated circuit, a 12 V DC power supply, and a push button to select the functions and to start or stop the program.[19] The program for operating the devices was developed in Arduino 1.5.5 software.
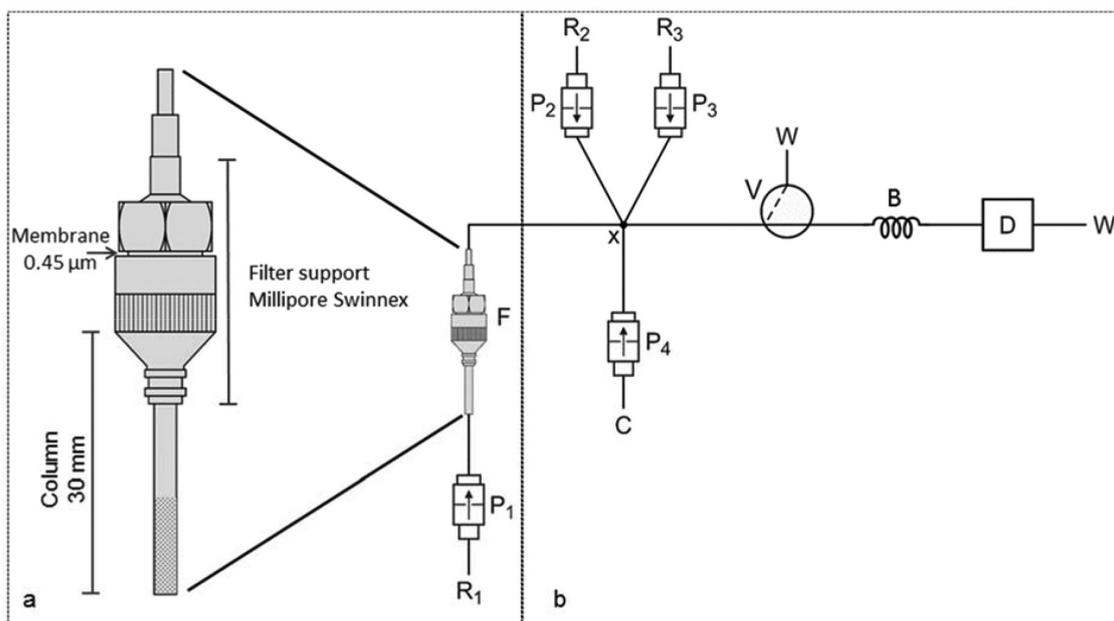
Figure 1. Flow diagram of the system for P-rem determination in soils. (a) Phosphorus adsorption module, filter and column highlight; (b) phosphorus determination module. R_1: $1.0\ mg\ L^{-1}$ P in $10.0\ mmol\ L^{-1}\ CaCl_2$; R_2: $2.0\ mmol\ L^{-1}\ (NH_4)_6Mo_7O_{24}$ in $1.0\ mol\ L^{-1}\ HNO_3$; R_3: $4.0\ mmol\ L^{-1}\ SnCl_2$ in $0.3\ mol\ L^{-1}$ HCl; C: $10.0\ mmol\ L^{-1}\ CaCl_2$; P_1-P_4: solenoid micro-pumps operated at 2.5 Hz; V: three-way solenoid valve; F: fluidized bed column containing soil sample, (different column for each sample); x: confluence point; B: 100-cm reaction coil; D: spectrophotometric flow cell; W: waste vessel. Arrows indicate the actuation of the micro-pumps.

The effects of parameters affecting the reaction development were evaluated: volumes of R_2 (1 to 6 pulses), R_3 (1 to 6 pulses) and R_1, 1.0 mg L^{-1} phosphorus (1 to 10 pulses); sampling cycles (1 to 7); reactor coil length (50, 75, 100, 150, 200 cm) and stop period (0, 15, 30, 45, 60 s).

The limit of detection (LOD) was estimated from 20 measurements of the analytical blank and the linear regression of analytical curve using the equation: LOD = 3.3σ / s; where σ = estimated standard deviation; s = slope of the analytical curve. The limit of quantification (LOQ) is estimated as LOQ = $5.0 \times$ LOD.[20]

Flow diagram and procedure

The flow manifold, showed in Figure 1, was operated according to the micro-pump and valve switching course specified in Table 2.

The R_1-R_3 reagents were introduced in the analytical path by micro-pumps P_1-P_3 with nominal volumes of 20 μL. The column packed with soil was filled with R_1 to the confluence point x and then four pulses of reagent containing phosphorous for adsorption were applied through the fluidized bed column, then one pulse of the other reagents to form the molybdenum blue complex, then five sampling cycles (steps 1, 2 and 3) and 15 s of stopped-flow (step 4). The sample carrier stream was delivered by the P_4 micro-pump, with a nominal volume of 40 μL. For each soil sample, 20 replicated determinations of P-rem were accomplished to obtain the adsorption profile. The measurements were performed in quadruplicate.

Results and Discussion

Adsorption kinetics

The P-rem index is normally determined under equilibrium conditions. In order to check the P adsorption kinetics in the soils, P-rem was determined by the conventional procedure with different shaking times (1, 5, 10 min) and results were compared with those obtained by the conventional procedure involving chemical equilibrium (shaking time = 60 min).

It was found that phosphorus adsorption was fast in all samples, with P-rem values related to the first minute similar to values obtained after 60 min of shaking. This was confirmed by the angular coefficients of the linear regression equations that compare the results of P-rem after 60 min, which approached unity: y = 0.9553x + 6.352 (r = 0.9724, n = 3, 1 min shaking time), y = 1.0050x + 3.554 (r = 0.9869, n = 3, 5 min shaking time) and y = 0.9824x + 5.554 (r = 0.9787, n = 3, 10 min shaking time).

It was observed that phosphorous adsorption occurred in two stages. The first stage is probably due to surface adsorption or precipitation with Fe, Al and Ca, and takes place rapidly (on the order of seconds), whereas the second one is slower, probably due to adsorption at anionic exchange sites or sites located in interstices of restricted access.[1] The second step represents less than 5% of the total P adsorption during the first minute of shaking. A similar tendency was noted for all tested samples, demonstrating the feasibility of determining the phosphorous adsorption capacity for soils in flow systems.

Optimization of phosphorus adsorption module

The fluidized bed resembles a fluid due to the agitation acquired in systems with the irregular trajectory of the particle,[12] allowing for rapid phosphorous transfer to the adsorption sites. The solenoid micro-pumps provide a pulsed flow within the column, and thus is more appropriate to be classified as pulsed fluidization.

The volume dispensed by the solenoid micro-pump was collected after passing through the soil column to investigate the flow system impedance using different soil mass. With a mass greater than 75 mg, the dispensed volume decreased by 18.0% on average, indicating the increased hydrodynamic impedance in the system. This occurred because the soil in the column does not establish the fluidization conditions and takes on characteristics similar to a packed column, such as clogging of the channel and the establishment of preferential paths, which affect

Table 2. Switching courses of the solenoid micro-pumps to evaluate phosphate adsorption in soils

Step	Description	Micro-pump	Stroke / μL	Pulse/time
1[a]	phosphorus insertion	P1	21.7	4/–
2[a]	$(NH_4)_6Mo_7O_{24}$	P2	22.8	1/–
3[a]	$SnCl_2$ insertion	P3	22.7	1/–
4	stopped-flow (s)	–	–	–/15
5	transport and detection	P4	43.4	40/–

[a]Five sampling cycles.

the adsorption process. Soil samples with a mass of 50 mg were selected due to the lower coefficient of variation 0.54%, considering the volume dispensed by the solenoid micro-pump.

The use of columns with a length of 10 mm provided 20.0% higher P-rem values, indicating the lower capacity of the soil to adsorb phosphorous. In this condition, the sample fills the whole column, limiting the liquid-solid interaction due to the establishment of preferential paths. The results obtained with lengths of 20 to 50 mm columns showed no changes in P-rem concentrations. In terms of convenience, operability and better precision, and considering the volume dispensed by the solenoid micro-pump (relative standard deviation (RSD) 0.54%), the 30 mm column was selected.

The results of P-rem using the adsorption module, Figure 2, were proportional with the conventional procedure. The comparison between the systems was performed using the first fraction, equivalent to the first 100 pulses of the solenoid micro-pump. The soils with higher clay contents and Fe and Al oxides (RH) had higher adsorption capacity, with lower P-rem values around of 0.24 mg L^{-1}. Conversely, sandy soils (TH) had lower adsorption capacity and higher P-rem, around 0.77 mg L^{-1}.
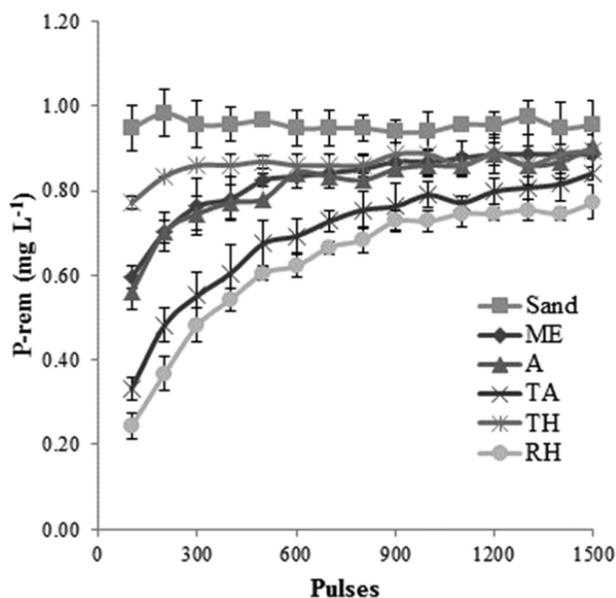
Figure 2. P-rem concentration in sand and in soil samples with different attributes. Values were collected every 100 pulses (2000 µL) of the solenoid micro-pump and analyzed by the conventional procedure.

The P-rem concentrations of the flow system (values between 0.0 and 1.0 mg L^{-1}) were converted into the P-rem concentration of the conventional procedure scale (0.0 to 60.0 mg L^{-1}) in order to use the established tables of doses and applications. The comparative scale was performed using the soil with the highest P adsorption capacity

(RH) as a lower limit and sand as the upper limit. For the conventional procedure, the scale comprises of 6.48 to 60 mg L^{-1} and for the flow system, from 0.24 to 0.95 mg L^{-1}. Thus, it was possible to correlate the values of the flow system with the P-rem values used to define phosphorous levels to be applied to the soil, according to the conversion in equation 1.

$$P\text{-rem(conventional)} = 75.38 \times P\text{-rem(flow)} - 11.59 \quad (1)$$

Comparing the flow system results with the conventional procedure,[3] the P-rem values follow a linear relationship ($y = 1.037x + 0.7678$; $r = 0.9422$) and do not show a difference (*t*-test, $p > 0.05$).

Optimization of the phosphorus determination module

The determination of remaining phosphorous was based on the classic reaction by the molybdenum blue method. The formation of a molybdenum blue complex is dependent on parameters such as pH, type and concentration of the reducing agent, and the molybdenum concentration,[21] which affects the reaction velocity for the formation of species.[22]

To obtain the maximum sensitivity, these parameters were evaluated using univariate analysis, alternating the number of pulses of the stannous chloride, ammonium molybdate and sample solutions, the reactor length and the time of stopped flow. Disrupted absorbance signals were observed due to the formation of Schlieren effects.[23] The binary sampling approach was used to obtain improved mixing[24] and the measurement at two wavelengths simultaneously (500 nm (Schlieren effect) and 700 nm (Schilieren effect + analytical signal)) was used to mathematically correct the signal.

For the $(NH_4)_6Mo_7O_{24}$ optimization volume, the best result was achieved with three pulses, but the increase in absorbance comparatively with the use of two pulses was only 4%. Considering the aim of reducing waste production and reagent use, two pulses (46 µL) were selected.

An increase in $SnCl_2$ pulses (from one to six) did not improve the sensitivity of the reaction; rather, it reduced the absorbance signal. The reduced sensitivity can be explained by an increase in the $SnCl_2$ volume in the sample zone and the decreased concentration of $(NH_4)_6Mo_7O_{24}$ and sample due to dispersion. Therefore, one pulse of $SnCl_2$ (23 µL) was selected as the best option.

The volume of the phosphorous solution was evaluated by inserting from one to ten pulses (22 to 220 µL) in each sampling cycle. Although higher sensitivity was achieved with ten pulses, eight pulses (176 µL) were selected because the difference between them was only 6%.

Given the optimization of the number of pulses of the reagents and sample, a 4:1:1 ratio (sample:stannous chloride:ammonium molybdate) was adopted for the optimization experiment regarding the number of cycles. Under these operating conditions, $(NH_4)_6Mo_7O_{24}$ was in excess of around 18,000-fold compared to the stoichiometric condition (the ratio is $1.7\,Mo_7O_{24}^{6-}:1.0\,PO_4^{3-}$). Stannous chloride also showed the best performance with an excess of the reagent, around 30-fold compared to the stoichiometric condition (1:1).

The reactor coil length was evaluated from 50 up to 200 cm. A 100-cm long reactor coil was selected because, with a shorter length, the sample zone exceeded the reactor volume. Beyond 100 cm, less sensitivity was obtained due to the greater sample zone dispersion in the analytical path.

The number of sampling cycles varied from one to seven, increasing linearly with the increase of the sample zone; however, with six and seven cycles, the reactor volume (500 µL) has been exceeded not influencing the sensitivity gain. Therefore, five cycles were selected.

The stopped-flow approach was exploited to favor complex formation. The highest sensitivity was observed for 30 to 60 s; however, as the difference between 15 and 30 s was only 8% in terms of increased sensitivity, 15 s was selected to improve the sampling rate.

Analytical features

A linear response was obtained between 0.0 and 1.0 mg L^{-1} phosphorous for the P-rem determination module, described by equation $A = 0.2422C + 0.0075$ ($r = 0.9999$), in which A is absorbance and C is P-rem concentration (mg L^{-1}). The coefficient of variation ($n = 20$; 0.4 mg L^{-1}) was estimated at 1.54%. The limit of detection (99.7% confidence level) was 17 µg L^{-1} and limit of quantification was 51 µg L^{-1}. The sampling rate was 87 determinations *per* hour. Each determination consumed 0.27 mg of $(NH_4)_6Mo_7O_{24}$ and 0.10 mg of $SnCl_2$, and generated 2.4 mL of waste.

Interference studies

The main interferences in the phosphorus determination by the molybdenum blue method in soil extracts are orthosilicate ions via the formation of silicic molybdic acid,[25] dissolved organic matter[26] and metallic ions that can precipitate as phosphates.

Signals of 0.4 mg L^{-1} of P were obtained in the absence and presence of concomitants in concentrations of at least five times greater than the maximum expected in the soil solution, after extraction with $CaCl_2$ 10.0 mmol L^{-1}.

There was no signal variation ($p > 0.05$) in the presence of 10 mg L^{-1} of Al, Ba, Fe, K, Mg and Mn, or with 4.0 mg L^{-1} of humic acid. Negative interference of 11% was observed in the presence of Si 10.0 mg L^{-1}. However, the addition of 10 mmol L^{-1} tartaric acid[27] in the $CaCl_2$ solution suppressed the interference.

Multi-pumping flow system for the determination of remaining phosphorus in soils

To determine P-rem in-line in soil samples, the adsorption and determination modules were coupled. Figure 3 shows the P-rem concentration in every 434 µL (20 pulses) of 1.0 mg L^{-1} P in 10.0 mmol L^{-1} $CaCl_2$, with four pulses of solution and five sampling cycles to establish an adsorption profile.

In Figure 2, the comparison of dynamic adsorption and the conventional procedure was performed with the first 100 pulses of the solenoid micro-pump. Likewise, in Figure 3 the average of the corresponding values to 100 pulses of the P solution was considered, represented by five fractions, with similar results to those previously obtained. However, the P-rem values of the first fraction, equivalent to 20 pulses of the micro-pump, may also be used for soil classification, and did not present differences from the results obtained with the average of five fractions.

The clayey soils, TA and RH, showed higher phosphorous adsorption capacity than the other samples. This was an expected result, based on studies of the adsorption module and as identified in the literature.[28] In most tropical soils, P adsorption is related to Fe and Al oxides and kaolinite groups.[29]

The P-rem values determined in soil samples and sand were compared with their respective textures and Fe and Al oxides percentages, as shown in Table 1. The influence of these parameters was observed in the phosphorous adsorption capacity in soil, in which the sandy soil (TH) with a lower content of Al_2O_3 (4.42%) and Fe_2O_3 (1.68%) had the highest P-rem (46.36 mg L^{-1}), and clayey soil (RH) with a higher content of Al_2O_3 (24.43%) and Fe_2O_3 (31.92%) had lower P-rem (6.48 mg L^{-1}). This feature is consistent with the results obtained by McDowell and Condron[30] that demonstrated a reduction in phosphorous sorption in soil with the removal of Fe and Al oxides, since these have the greatest impact on phosphorous sorption and desorption.

To validate the mathematical model and the adsorption results, sixteen soils samples with contrasting attributes were analyzed by the proposed procedure. The results (Figure 4) agree with those obtained with the conventional
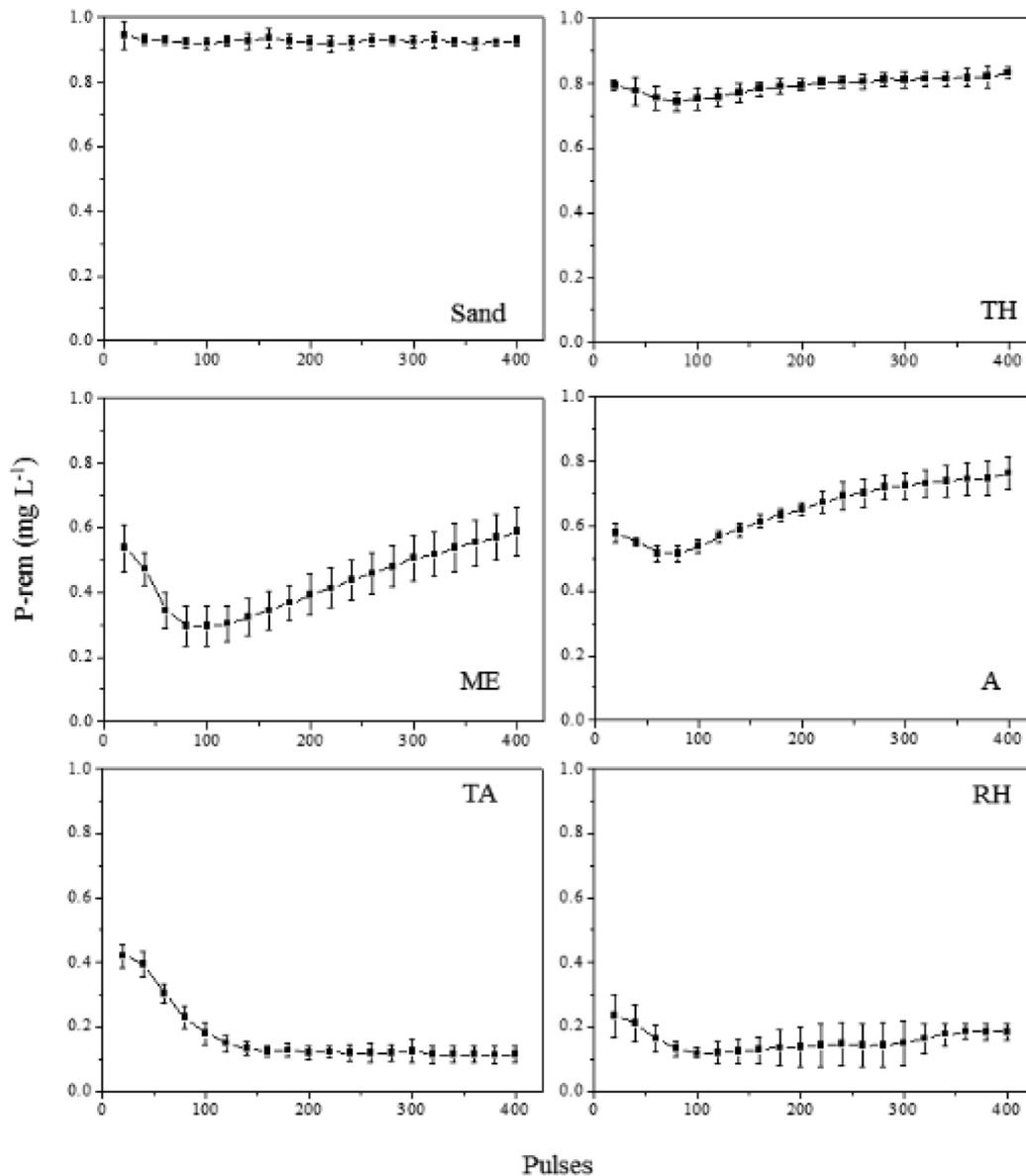
Figure 3. P-rem concentration in soil samples with different attributes and sand. Values were collected every 20 pulses (434 µL) of the solenoid micro-pump and analyzed by multi-pumping flow system. Coupling adsorption and determination module of P.

procedure at the 95% confidence level and had a linear relationship with a linear coefficient close to 100% (y = 0.9702x + 1.483; r = 0.9635). This determination also included previously used soil samples, with the exception of RH and sand, used as the upper and lower limit of the correlation.

The proposed flow system presents advantages such as a reduced sample mass, time of analysis, reagent consumption and, consequently, lower waste generation when compared to the conventional procedure. The mass of soil was reduced from 5 g to 50 mg, and the analysis time, including adsorption and determination, was reduced from 2 h to only 40 s. The generation of waste was reduced from 95 to 2.4 mL.

Conclusions

Phosphorus adsorption in soil using a multi-pumping flow system presented similar and consistent results with the conventional procedure for soil samples with contrasting attributes.

The highlights are the 100-fold decrease in the soil mass required, the 180-fold decrease in analysis time, and the 40-fold reduction in reagent consumption and waste production, demonstrating the potential of this system to be an environmentally friendly alternative.

The effects of pressure increase were not apparent for soil samples in the fluidized bed columns, even for those with clayey textures.
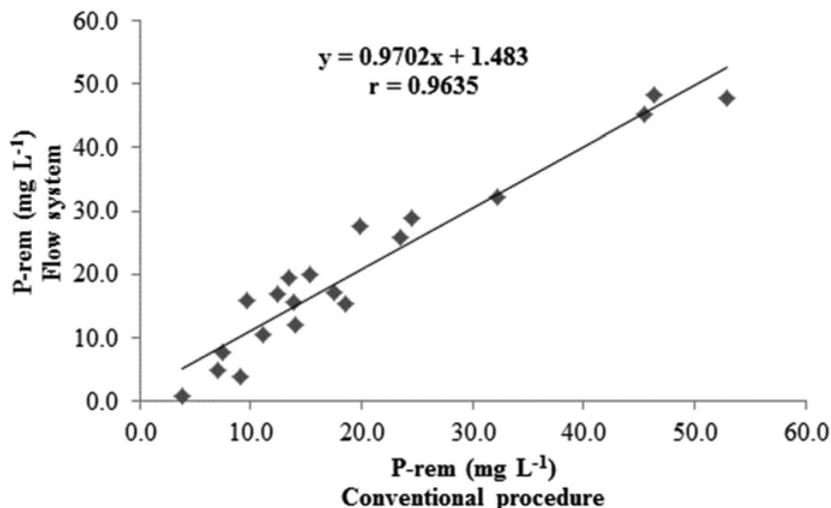
Figure 4. Correlation of P-rem values from the flow system and the conventional procedure using 20 soil samples.

Phosphorus is efficiently adsorbed by soil in a fluidized bed column with pulsed flow, which reduces the effects of pressure and does not change the volume dispensed by the solenoid micro-pump.

Acknowledgments

References

1. Van Raij, B.; *Fertilidade do Solo e Manejo de Nutrientes*; International Plant Nutrition Institute: Piracicaba, Brasil, 2011.

2. Bache, B. W.; Williams, E. G.; *J. Soil Sci.* **1971**, *22*, 289.

3. Venegas, V. H. A.; Novais, R. F.; Dias, L. E.; Oliveira, J. A.; *Boletim Informativo da Sociedade Brasileira de Ciência do Solo* **2000**, *25*, 24.

4. Melchert, W. R.; Reis, B. F.; Rocha, F. R. P.; *Anal. Chim. Acta* **2012**, *714*, 8.

5. Anastas, P.; Eghbali, N.; *Chem. Soc. Rev.* **2010**, *39*, 301.

6. Kolev, S. D.; McKelvie, I. D.; *Comprehensive Analytical Chemistry*, vol. 54; Barceló, D.; Elsevier: Amsterdam, Netherlands, 2008, p. 808.

7. Buanuam, J.; Miró, M.; Hansen, E. H.; Shiowatana, J.; Estela, J. M.; Cerdà, V.; *Talanta* **2007**, *71*, 1710.

8. Rocha, D. L.; Rocha, F. R. P.; *Microchem. J.* **2013**, *109*, 139.

9. Fortes, P. R.; Feres, M. A.; Sasaki, M. K.; Alves, E. R.; Zagatto, E. A. G.; Prior, J. A. V.; Santos, J. L. M.; Lima, J. L. F. C.; *Talanta* **2009**, *79*, 978.

10. Ribeiro, M. F. T.; Dias, A. C. B.; Santos, J. L. M.; Lima, J. L. F. C.; Zagatto, E. A. G.; *Anal. Bioanal. Chem.* **2006**, *384*, 1019.

11. Dixit, R.; Puthli, S.; *J. Pharm. Sci.* **2009**, *98*, 3933.

12. Rosende, M.; Miró, M.; Cerdà, V.; *Anal. Chim. Acta* **2010**, *658*, 41.

13. Rocha, D. L.; Rocha, F. R. P.; *Int. J. Environ. Anal. Chem.* **2013**, *93*, 1389.

14. Embrapa - Empresa Brasileira de Pesquisa Agropecuária; *Sistema Brasileiro de Classificação de Solos*, 1ª ed.; Embrapa: Brasília, Brasil, 2006.

15. Gee, G. W.; Or, D.; *Soil Sci. Soc. Am. J.* **2002**, *5*, 255.

16. Vettori, L.; *Métodos de Análise do Solo*; Ministério da Agricultura, Divisão de Pedologia e Fertilidade do Solo: Rio de Janeiro, Brasil, 1969.

17. Embrapa - Empresa Brasileira de Pesquisa Agropecuária; *Manual de Métodos de Análise do Solo*, 2ª ed.; Embrapa, Centro Nacional de Pesquisa de Solos: Brasília, Brasil, 1997.

18. Braga, J. M.; Defelipo, B. V.; *Rev. Ceres* **1974**, *21*, 73.

19. Miranda, J. C.; Kamogawa, M. Y.; Reis, B. F.; *Sens. Actuators B* **2015**, *207*, 811.

20. McNaught, A. D.; Wilkinson, A.; *IUPAC Compendium of Chemical Terminology ("Gold Book")*, 2nd ed.; Blackwell Scientific Publications: NC, USA, 1997.

21. Worsfold, P. J.; Gimbert, L. J.; Mankasingh, U.; Omaka, O. N.; Hanrahan, G.; Gardolinski, P. C. F. C.; Haygarth, P. M.; Turmer, B. L.; Keith-Roach, M. J.; McKelvie, I. D.; *Talanta* **2005**, *66*, 273.

22. Crouch, S. R.; Malmstadt, H. V.; *Anal. Chem.* **1967**, *39*, 1084.

23. Rocha, F. R. P.; Nóbrega, J. A.; *Quim. Nova* **1996**, *19*, 636.

24. Reis, B. F.; Giné, M. F.; Zagatto, E. A. G.; Lima, J. L. F. C.; Lapa, R. A.; *Anal. Chim. Acta* **1994**, *293*, 129.

25. Groudpan, K.; Ampan, P.; Udnan, Y.; Jayasvati, S.; Lapanantnoppakhun, S.; Jakmunee, J.; Christian, G. D.; Ruzicka, J.; *Talanta* **2002**, *58*, 1319.

26. McDowell, R. W.; Sharpley, A. N.; *Chemosphere* **2001**, *45*, 737.

27. Neves, M. S. A. C.; Souto, M. R. S.; Tóth, I. V.; Victal, S. M. A.; Drumond, M. C.; Rangel, A. O. S. S.; *Talanta* **2008**, *77*, 527.

28. Valladares, G. S.; Pereira, M. G.; Anjos, L. H. C.; *Bragantia* **2003**, *62*, 111.

29. Fontes, M. P. F.; Weed, S. B.; *Geoderma* **1996**, *72*, 37.

30. McDowell, R.; Condron, L.; *Commun. Soil Sci. Plant Anal.* **2001**, *32*, 2531.