Determination of total nitrogen and phosphorus in soil by ion chromatography with conductivity detector following persulfate digestion method

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ABSTRACT

This study introduces a new method to determine both TP and TN in one process, reducing the analytical cost and time. During this procedure, soil samples were digested in alkaline potassium persulfate ($K_2S_2O_8$) at 150 °C for two hours in a reactor to convert organic N (including NH_3/NH_4^+) and P species to the highest oxidation states NO_3^-, PO_4^{3-}. After that, H_2O_2 removes the color of the digest, which could influence the efficiency of the analytical process. Finally, ions NO_3^- and PO_4^{3-} were separated on the anion-exchange column and quantified by a conductivity detector. According to the spiked samples, which were made by mixing Bovine serum albumin (BSA) with organic nitrogen and triphenyl phosphate containing organic phosphorus with real soil matrix, the recovery of TN and TP was 85% and 97%, respectively. Both TN and TP linear concentration ranges were between 0.50 and 50 µg.mL^{-1}. The results of relative standard deviation and limit of detection for TN were from 4.2%, and 0.31 mg.kg^{-1}; and for TP were from 3.5%, and 0.25 mg.kg^{-1}.

Keywords: Total nitrogen; Total phosphorus; Alkaline potassium persulfate; Ion chromatography.

1. INTRODUCTION

Nitrogen (N) and phosphorus (P) are essential nutrients for plant growth and development. However, too much N and P can be toxic to plants and hurt crops and animals. Analysis and assessment of N and P content in soil and crops will help build a balanced and effective fertilizer plan for agricultural production. N and P come in two forms organic nitrogen compounds and inorganic compounds. N and P content in the soil varies with depth, soil type, and conditions required for the natural cycle of nitrogen and phosphorus [5, 7, 9, 11].

Some standard methods are used widely for measuring N and P but only address one or two N and P species [10, 14, 15]. Kjeldahl methods can be used to analyze total nitrogen [TKN]) [16] and phosphorus [TKP]) [17], broadening the scope of measurable compounds but may use toxic mercury catalysts, mainly for organic species. The TKN/TKP digestions do not recover all compounds of interest and suffer from interferences that require speciation to be performed and results mathematically combined. P species exist as inorganic orthophosphate and polyphosphate, and N species as NO_3^-n NO_2^- and are known to interfere with TKP/TKN, resulting in a negative bias and not providing an actual value. Therefore, it requires an additional analytical technique to achieve an actual total N (TN) and P (TP) value. Consolidating all of these processes into a single safe preparatory step combined with a single mutually expressive analysis would be necessary while reducing complexity, the potential for inaccuracy, and exposure to hazardous materials.

One of the methods used to treat samples for TN and TP recently is the alkaline persulfate digestion technique. The sample matrix is mainly water and plant samples [2, 6, 8]. The alkaline

persulfate digestion technique has not been mentioned in the USEPA and ISO standard systems for analyzing TP and TN in soil samples. However, the U.S. Geological Survey (USGS) concluded that the alkaline persulfate digestion technique for determining TN and TP in water samples is more sensitive and accurate and uses less toxic reagents than the Kjeldahl digestion method [1]. Therefore, in this topic, we boldly apply the advantages of the alkaline persulfate digestion technique to apply the analysis for TN and TP in soil samples. The ion chromatography with conductivity detection, as described in U.S. Environmental Protection Agency (EPA) methods 300.0 and 300.1, is one of the most common approaches used to determine nitrite and nitrate, and Phosphate for soil sample digest solution [3, 18].

2. METHODS AND MATERIALS

2.1. Chemical

The standard NO_3^- 1000 µg.mL^{-1}, and KH_2PO_4 (Merck, Darmstadt, Germany). ACN, BuOH (HPLC grade, Germany). $K_2S_2O_8$, NaOH, H_2O_2, NH_4Cl, Ethylenediaminetetraacetic acid (EDTA), Nitrophenol (NP), Triphenyl phosphate (TPP), acid oxalic, sodium gluconate, sodium tetraborate decahydrate, boric acid and *Bovine serum albumin (*BSA) (Merck, Germany). Deionized water purified by Thermo Scientific D7031 (USA) was used to prepare mobile phases and solutions of standards and samples. All of the chemicals were PA grade. The mature BSA protein contains 583 amino acids in amino acid sequence, an average mass of 66433 Dalton, and the elemental composition of C_{2932} H_{4614} N_{780} $O_{898}S_{39}$. In this structure, the percentage of N was 16.4 % w/w.

^{1}DTHKSEIAHRFKDLGEEHFKGLVLIAFSQYLQQCPFDEHVKLVNELTEFAKTCVADESH
AGCEKSLHTLFGDELCKVASLRETYGDMADCCEKQEPERNECFLSHKDDSPDLPKLKPD
PNTLCDEFKADEKKFWGKYLYEIARRHPYFYAPELLYYANKYNGVFQECCQAEDKGAC
LLPKIETMREKVLASSARQRLRCASIQKFGERALKAWSVARLSQKFPKAEFVEVTKLVT
DLTKVHKECCHGDLLECADDRADLAKYICDNQDTISSKLKECCDKPLLEKSHCIAEVEK
DAIPENLPPLTADFAEDKDVCKNYQEAKDAFLGSFLYEYSRRHPEYAVSVLLRLAKEYE
ATLEECCAKDDPHACYSTVFDKLKHLVDEPQNLIKQNCDQFEKLGEYGFQNALIVRYTR
KVPQVSTPTLVEVSRSLGKVGTRCCTKPESERMPCTEDYLSLILNRLCVLHEKTPVSEKV
TKCCTESLVNRRPCFSALTPDETYVPKAFDEKLFTFHADICTLPDTEKQIKKQTALVELL
^{533}KHKPKATEEQLKTVMENFVAFVDKCCAADDKEACFAVEGPKLVVSTQTALA

Figure 1. *Amino acid sequencing of BSA [4].*

2.2. Instrument

Waters (USA) 's ion chromatography system includes pump 515, column oven and temperature controller, manual sample injection with loop volume 20 µL.

Ion chromatography parameters including anion exchange column IC-PAK Anion HR (4.6 × 75 mm) with guard column was set up with a flow isocratic of 1 mL/min and oven temperature at 30 ^{o}C and sample injection with loop volume of 20 µL. The mobile phase sodium borate/gluconate eluent was prepared with components containing sodium gluconate 0.032% w/v, boric acid 0.036% w/v, sodium tetraborate decahydrate 0.05% w/v, glycerine 0.5% v/v, n-butanol 20% v/v and acetonitrile 12% v/v, fill to the 1 L with DI water and mix thoroughly. After that, filter through a 0.45 µm membrane filter by a low-pressure filtration system and transfer in a plastic bottle. The new mobile phase was prepared daily, and sonication took 30 minutes to remove air bubbles before use [21].

2.3. Soil digestion

Soil samples were collected at three locations: red basalt soil at Bao Loc - Lam Dong, sandy soil at Son Tra - Da Nang, and alluvial soil at Ba tri-Ben Tre in Vietnam. The sampling was performed according to VietNam Standards 4046-85 and 7538-2:2002 [19, 20]. In detail, the

sample's soil was collected in the dry season. Each soil sample is a mixture of 20 separate soil samples, with cultivation depth from topsoil to a depth of 30 cm. Then, collect a mixture sample with a mass of about one kilogram.

Soil samples were homogenized through a 1 mm sieve and stored in the dark. Take 0.5 g soil into a beaker 100 mL, add 2.5 mL NaOH 10% w/v, 2.5 mL $K_2S_2O_8$ 10% w/v, and put glass surface on top and then place on the hot plate at around 100 ^oC during time 60 min. The solution was kept not boiling in digest time. Fortified samples were prepared with inorganic and organic species of N and P. Salts inorganic for spiked NH_4Cl, KNO_3 for TN, and KH_2PO_4 for TP at 0.1% N w/w into the soil sample. In contrast, organic species were EDTA, NP, and protein BSA for TN and TPP for TP at a 0.1% w/w in the soil sample. After digestion, the sample was adjusted to pH 7 with oxalic acid. If the solution sample has color, H_2O_2 was added until colorless and put into a water bath at 60 ^oC to remove residue H_2O_2. Finally, filtration of the solution into a flask of 100 mL, then filtrate 2 mL through 0.45 μm membrane before being injected into IC system.

3. RESULTS AND DISCUSSION

3.1. NO_3^- and PO_4^{3-} identification by ion chromatography couple with conductivity detector

The following method is mentioned in the US. EPA 300, NO_3^- and PO_4^{3-} were separated on IC-PAK Anion HR and borate/gluconate eluent. The chromatogram Fig.1a shows that NO_3^- and PO_4^{3-} retention time was 5.1 min and 7.2 min, respectively. The mixture of 10 $\mu g.mL^{-1}$ containing NO_3^- and PO_4^{3-} was prepared in the mobile phase and compared to the response signal with a blank sample treated the same way as the soil sample. The results showed a difference of 3.8% in the two above conditions (Fig. 2a and Fig. 2b).

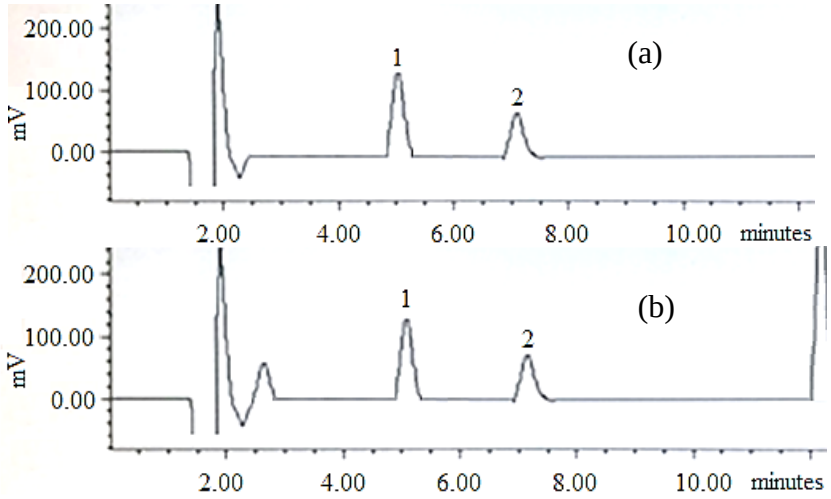

Figure 2. *The chromatogram separation of a mixed (1) NO_3^--N and (2) PO_4^{3-}-P standard and comparison between mixed standard prepared in (a) mobile phase (B) digested blank water.*

3.2. Optimal Alkaline Persulfate Digestion Technique

The various levels of $K_2S_2O_8$, alkaline, time, and temperature were evaluated. Pipet 2.5 mL of $K_2S_2O_8$ from 1% w/v to 20% w/v in the red basalt soil sample spiked with NP and compared efficiency recovery. The results were shown at level 10% w/v $K_2S_2O_8$ gets the highest efficiency recovery and is stable in the range of 10% w/v to 20% w/v (Fig. 3a). Furthermore, the noise background on chromatography at level 10% w/v smoothy and lower than 20% w/v. The signal has trended increasing when time and temperature digestion together. Time digestion in the range of 60 and 180 min has a 6.4% difference in efficiency recovery (Fig. 3b). In contrast, temperature digestion in the range of 150 and 250 ^oC has a 5.1% difference in efficiency

recovery (Fig. 3c). The alkaline had a role in cleaving the organic chain into smaller molecules, and we did not see the difference when pipetting 2.5 mL of NaOH varies in the range of 5% w/v to 20% w/v (Fig. 3d). However, NaOH of 10% w/v was chosen to guarantee efficient recovery in the case of samples containing more than organic materials. In sum, the soil samples have optimal alkaline persulfate digestion set 60 min at 150 ^{o}C with pipet 2.5 mL NaOH and $K_2S_2O_8$ of 10% w/v. Overall, the procedure for determining TN and TP in soil was summarized in Fig. 4.

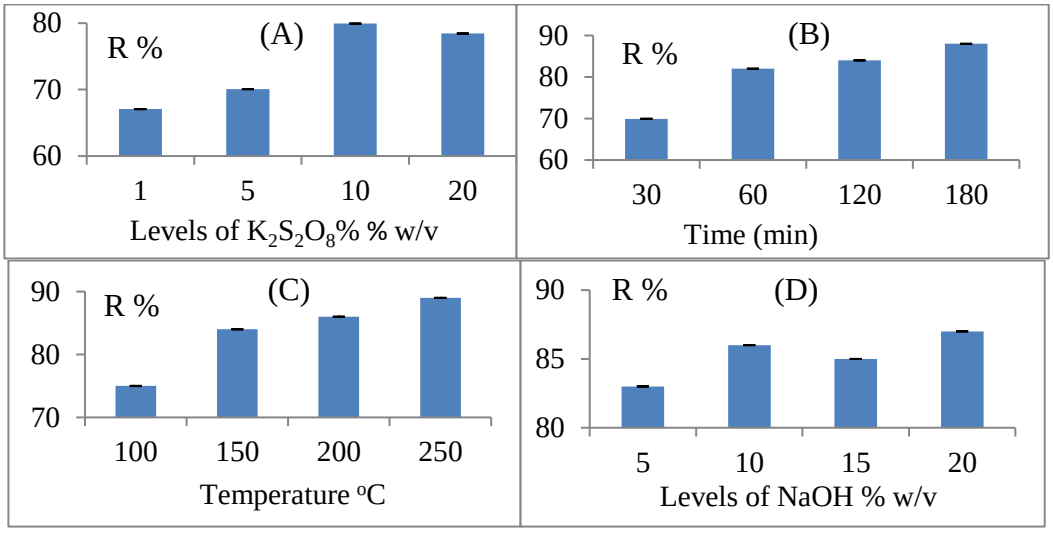

Figure 3. *Optimal alkaline persulfate digestion technique (a) levels of $K_2S_2O_8$, (b) time, (c) temperature, and (d) levels of NaOH. Survey order from (A) to (D), the optimal condition of the previous survey was selected for the next w/v stand for weight/volume.*

Table 1. *IC-CD validation results.*

Analyte	Linear in range (µg.mL^{-1})	Calibration Curve	R^2	RSD%	LOD (mg.kg^{-1})
TN	0.5-50	y = 167307x + 63135	0.9995	4.2	0.31
TP	0.5-50	y = 130789x - 1154.6	0.9998	3.5	0.25

Table 2. *Criteria for evaluating the TP and TP content in the soil [12, 13] w/w stand for weight/weight.*

Soil types	TN (N% w/w)		TP (P% w/w)	
	Range	Average	Range	Average
Red soil	0.065 to 0.530	0.177	0.022 to 0.262	0.131
Alluvial soil	0.095 to 0.270	0.141	0.022 to 0.131	0.044
Sandy soil	Trace to 0.120	0.068	0.013 to 0.022	0.017
Criteria for evaluation				
% TN < 0.08		% TP < 0.0218		Poor
% TN: 0.08 – 0.15		% TP: 0.218 to 0.0437		Medium
% TN 0.15-0.2		% TP: 0.0437 to 0.0655		High
% TN > 0.2		% TP > 0.0655		Rich

Table 3. *Results for TN and TP determinations in digested soil samples w/w stand for weight/weight.*

Sample	Red basalt soil	Sandy soil	Alluvial soil
TN (% N w/w)	0.170 ±0.006	0.070 ±0.008	0.186 ± 0.030
TP (% P w/w)	0.136 ±0.005	0.015 ±0.006	0.076 ± 0.007

Table 4. *Recovery of TN and TP in the red basalt soil sample spiked nitrogen and phosphorus-containing compounds.*

Compounds	Spiked (% w/w)	Results (% w/w)	Found (% w/w)	Recovery (%)
NH_4Cl	0.1 % N	0.273±0.013	0.103	103
KNO_3	0.1% N	0.2704 ±0.0046	0.100	100
EDTA	0.1% N	0.2589 ± 0.0028	0.089	89
NP	0.1% N	0.2567±0.0018	0.087	87
BSA	0.1 % N	0.2598±0.005	0.090	90
KH_2PO_4	0.1 % P	0.233±0.005	0.097	97
TPP	0.1 % P	0.2301±0.0022	0.094	94
Mix of NP + BSA + TPP	0.2 % N	0.306 ±0.012	0.170	85
	0.1 % P	0.233±0.08	0.097	97

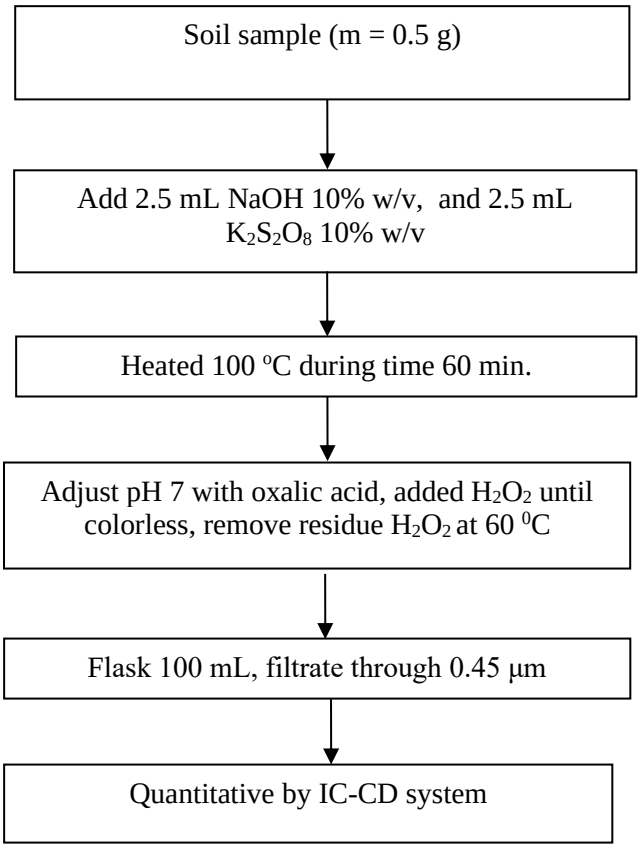

Figure 4. *Flow chart of TN and TP analysis in soil samples by IC with a conductivity detector by persulfate decomposition method.*

3.3. Validation of Method

The soil sample was spiked with species of N and P compounds to evaluate TP and TN actual values. Salts inorganic for spiked NH_4Cl, KNO_3 for TN, and KH_2PO_4 for TP at 0.1% w/w into the soil sample. In contrast, organic species were minor compounds such as EDTA, NP, and macro compounds such as protein BSA for TN and TPP for TP at a 0.1% w/g in the soil sample. The results show in table 4 that the recovery efficiency in all cases gets more than 80%. This procedure was applied to protein BSA to recover more than 90%. Both TN and TP linear

concentration ranges were between 0.50 and 50 µg.mL^{-1}. The results of relative standard deviation and limit of detection for TN were 4.2%, and 0.31 mg.kg^{-1}; and for TP were 3.5%, and 0.25 mg.kg^{-1}.

In soil samples containing N and P in compounds, organic and inorganic species. The species of N in small organic compounds had high-efficiency digestion illustrated in Tab. 4. However, are there macromolecule compounds, such as proteins, that the alkaline persulfate digestion technique can digest? We used the BSA protein to verify the results for this question. The result in Tab. 4 illustrated this process of high-efficiency digesting protein, with the recovery percentage reaching 90 %.

3.4. Soil samples

The soil samples were alkaline persulfate digestion techniques at optimal conditions—the result was shown in Tab. 3. The result shows that TN and TP contained in the soil types are consistent with the range of values promulgated by Vietnamese standards (Tab. 2). Comparable criteria for evaluation for the TP and TP content in the soil, red basalt soil at Bao Loc - Lam Dong and alluvial soil at Ba tri - Ben Tre have TN at a high level and TP at a rich level, sandy soil at Son Tra - Da Nang has the same poor level for TN and TP.

4. CONCLUSIONS

The procedure for determining simultaneous TN and TP with a single safe preparatory step was easy and accurate. The TN and TP in soil have high-efficiency recovery with various compounds fortifier species inorganic and organic, both organic small and large compounds. The soil samples were analyzed TN and TP in the range suitable for plant growth. We can build a balanced and effective fertilizer plan for agricultural production by analyzing the results of TP and TN in soil.

The research team investigated the optimal conditions of the persulfate method of sample decomposition, validated the method on an IC device with a conductivity detector, and achieved a linear range of TN and TP, both in the range from 0.5 to 50 µg.mL^{-1}. Furthermore, we have investigated the results of the relative standard deviation, and the detection limit for TN is between 4.2% and 0.31 mg.kg^{-1}, and for TP is between 3.5% and 0.25 mg.kg^{-1}, respectively. The recovery efficiency for all surveyed cases for TN and TP reached over 80%.

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TÓM TẮT

Xác định tổng nitơ và photpho trong đất bằng sắc ký ion với máy dò độ dẫn điện theo phương pháp phân hủy persulfate

Nghiên cứu này giới thiệu một phương pháp mới trong quá trình để xác định cả TP và TN, giảm chi phí và thời gian phân tích. Trong quy trình này, các mẫu đất được phân hủy trong kiềm kali persulfate ($K_2S_2O_8$) ở 150 °C trong hai giờ trong lò phản ứng để chuyển hóa N hữu cơ (bao gồm NH_3/NH_4^+) và P thành các trạng thái oxy hóa cao nhất NO_3^-, PO_4^{3-}. Sau đó, H_2O_2 loại bỏ màu của dung dịch phân hủy, điều này có thể ảnh hưởng đến hiệu quả của quá trình phân tích. Cuối cùng, các ion NO_3^- và PO_4^{3-} được tách trên cột trao đổi anion và được định lượng bằng máy dò độ dẫn. Theo các mẫu phân loại được thực hiện bằng cách trộn albumin huyết thanh Bò (BSA) với nitơ hữu cơ và triphenyl photphat có chứa photpho hữu cơ với nền đất thực, hiệu suất thu hồi của TN và TP lần lượt là 85% và 97%. Cả hai khoảng nồng độ tuyến tính TN và TP đều nằm trong khoảng từ 0.50 đến 50 $\mu g.mL^{-1}$. Kết quả của độ lệch chuẩn tương đối và giới hạn phát hiện đối với TN là từ 4.2% và 0.31 $mg.kg^{-1}$ và đối với TP là từ 3.5% và 0.25 $mg.kg^{-1}$.

Từ khóa: Tổng nitơ; Tổng photpho; Alkaline potassium persulfate; Sắc ký ion.