Preliminary validation of a sequential fractionation method to study phosphorus chemistry in a calcareous soil

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HIGHLIGHTS

- Two types of Ca-Ps can be distinguished by the sequential fractionation method.
- Three types of Ca-Ps cannot be distinguished by the method as previously proposed.
- Including a weak acid extractant can prevent overestimation of Ca-P.
- The method can quantify both inorganic and organic P fractions in a calcareous soil.

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ABSTRACT

A sequential fractionation method proposed by Jiang and Gu (1989) distinguished three types of calcium phosphates (Ca-P) according to their different plant availabilities. Three extractants, $NaHCO_3$, NH_4Ac, and H_2SO_4 were used to extract Ca_2-P, Ca_8-P, and Ca_{10}-P types, respectively, from soil. This sequential fractionation method was tested and modified for analyzing the P chemistry of a calcareous soil. The solubility test and the model diagrams of the stability of the major Ca-P minerals showed that $NaHCO_3$ was able to extract brushite (Ca_2-P type), and NH_4Ac extracted brushite and β-tricalcium P (Ca_8-P type) as well as hydroxyapatite (Ca_{10}-P type). Therefore the P forms targeted by extraction with NH_4Ac should include both Ca_8-and Ca_{10}-P types. The sum of the P extracted by all extractants in the sequential fractionation method in the calcareous soil was in agreement with the total P measured by the perchloric acid digestion method. A proportion of organic P measured by the sequential fractionation method was in agreement with the result from solution ^{31}P NMR spectroscopy. This study showed that the modified sequential fractionation method and its target P forms would be useful for quantifying and characterizing inorganic and organic P in a calcareous soil, even though it should be used in combination with other techniques, such as solution ^{31}P NMR spectroscopy.

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1. Introduction

Jiang and Gu (1989) introduced a phosphorus (P) fractionation method for calcareous soils based on the methods by Chang and Jackson (1957) and Hedley et al. (1982). In their method, Ca-P minerals was divided into three types; (i) Ca_2-P, including monetite and brushite, extractable with a weak alkaline extractant, $NaHCO_3$, (ii) Ca_8-P, including octacalcium P (OCP) and β-tricalcium P (TCP), extractable with a weak acid extractant, NH_4Ac (ammonium acetate), and (iii) Ca_{10}-P, such as hydroxyapatite (HAP)

extractable with a strong acid extractant, H_2SO_4. An additional three P fractions, including Al-P extractable with NH_4F, Fe-P extractable with $NaOH-Na_2CO_3$, and occluded P extractable with CD (sodium citrate—dithionite - sodium hydroxide), are distinguished in their method. There was no significant difference of the total amounts of P extracted by this method compared to the method proposed by Chang and Jackson, and the sum of P fractions extracted with NH_4Cl and H_2SO_4 in the method proposed by Chang and Jackson was equivalent to the sum of P fractions extracted with $NaHCO_3$, NH_4Ac and H_2SO_4 in the method proposed by Jiang and Gu (Jiang and Gu, 1989). The method proposed by Jiang and Gu has been tested by several researchers (Guo et al., 2008; Ma et al., 2009; Shen et al., 2004, 2011; Song et al., 2007; Than and Egashira, 2008; Wang et al., 2010) and has been modified (Adhami et al., 2006;

Ruttenberg, 1992; Samadi and Gilkes, 1998).

In the sequential fractionation method proposed by Jiang and Gu (1989), P fractions are extracted in the order of labile to stable P, beginning with weaker base/acid extractants and followed by stronger base/acid extractants as well as a reductant. The first extractant is 0.25 M NaHCO$_3$ with pH adjusted to 7.5. The expected inorganic P forms extracted by this extractant are adsorbed P onto the surface of clays and mineral oxides, and easily dissolved Ca-Ps, such as brushite and monetite (Adhami et al., 2006; Chang and Jackson, 1957; Jiang and Gu, 1989; Mostashari et al., 2008). This labile P fraction is comparable to the P fraction extracted by Olsen P (Jiang and Gu, 1989; Samadi and Gilkes, 1998; Wang et al., 2010). The extractant is also known to extract easily mineralizable organic P, such as orthophosphate diesters including nucleic acids and phospholipids (Negassa and Leinweber, 2009; Turner et al., 2005). The second extractant is a weak acid, 0.5 M NH$_4$Ac with pH adjusted to 4.2. The target P forms extracted include moderately labile inorganic Ca-Ps, such as OCP, TCP (Adhami et al., 2006; Jiang and Gu, 1989b; Samadi and Gilkes, 1999), and acid soluble organic P, such as Ca(Mg)-phytates (Celi et al., 2000). Many of the common sequential fractionation method do not include a weak acid extractant, such as NH$_4$Ac, thus most Ca-Ps are often extracted with a strong acid, such as 1.0 M HCl or 0.5 M H$_2$SO$_4$, after extracting with a strong alkaline extractant. Re-adsorption and/or precipitation of Ca-Ps, as well as hydrolyzation of organic P can occur during extraction with strong alkaline extractants, such as 0.1 M NaOH (Barbanti et al., 1994) and this would lead to an underestimation of Fe-P or organic P, and to overestimation of Ca-Ps (Barbanti et al., 1994). Therefore, the inclusion of an extraction with weak acid prior to the extraction with a strong alkaline extractant can prevent both underestimation of Fe-P and organic P, as well as overestimation of Ca-Ps. The third step, the extraction with MgCl$_2$ recovers P re-adsorbed to the unattached substrates by means of a dual action, competition by Cl$^-$ and complexion by Mg^{2+} (Barbanti et al., 1994; Ruttenberg, 1992). This step is critical, especially when it is followed by acidic extractants, such as NH$_4$Ac (Barbanti et al., 1994; Ruttenberg, 1992). Analysis of re-adsorbed P after the extraction steps with NH$_4$Ac, NH$_4$F, NaOH−Na$_2$CO$_3$ and CD was further studied by Adhami et al. (2006). They found that a significant amount of P was detected only after extraction with NH$_4$Ac, because NH$_4$Ac removes CaCO$_3$ in solution and thus eliminates re-adsorption of P onto CaCO$_3$ in the subsequent extraction steps (Adhami et al., 2006). The fourth step, the extraction with NH$_4$F, targets Al-P. The fluoride ion reacts with Al or Ca ions to form AlF$_3$ or CaF$_2$ (Pierzynski et al., 2005). However, CaF$_2$ strongly sorbs P, which will not be recovered until extraction with the reductant soluble or acid soluble P forms, and thus resulted in these two P fractions being overestimated, and as a consequence, an underestimation of Al-P (Pierzynski et al., 2005). If the previous extractants (NH$_4$Ac and MgCl$_2$) are able to extract the readily soluble Ca, an overestimation of both reductant and acid soluble P fractions, as well as an underestimation of Al-P can be prevented. The fifth and sixth steps are the extraction with NaOH-Na$_2$CO$_3$, and CD, which target Fe-P and occluded P, respectively. Occluded P refers to P fractions associated with crystalline Fe oxides such as goethite (Adhami et al., 2006). Citrate is a chelating agent and dithionite is a strong reducing agent that reduces amorphous Fe(OH)$_3$ to soluble Fe^{2+} ion, and therefore releases Fe-bound P (Barbanti et al., 1994; Ruttenberg, 1992; Zhang et al., 2010). These strong alkaline extractants also extract organic P associated with humic acids and orthophosphate monoesters, such as phytic acids (Negassa and Leinweber, 2009). The seventh and final step is extraction with H$_2$SO$_4$, targeting stable apatite P types that are considered to be not available for plants.

In this study, the sequential fractionation method proposed by Jiang and Gu (1989) and modified by Adhami et al. (2006) was tested and modified for analyzing P chemistry of a calcareous soil. Also this method was tested for its ability to extract three types of inorganic Ca-P (brushite as Ca$_2$-P type, TCP as Ca$_8$-P type and HAP as Ca$_{10}$-P type) using the extractants, NaHCO$_3$, NH$_4$Ac and H$_2$SO$_4$, respectively, and two types of organic P, phytic acid and Ca(Mg)-phytate, using acid and alkaline extractants, NH$_4$Ac (pH 4.2) and CD (pH 13.0), respectively.

2. Materials and methods

Topsoil (~15 cm) was collected from an organically-managed farm field located near Scotland, Ontario (43 00′18.5"N, 80 25′02.1"W). The sandy loam soils in this area are classified as Brunisolic Gray Brown Luvisol and belong to the Breton series. The Gray Brown Luvisol is a typical soil around southern Ontario, which occurs typically under deciduous or mixed forest vegetation on calcareous materials in areas of mild, humid climate (Agriculture and Agri-Food Canada, 2013). The field moist soil was passed through a 2 mm sieve and stored at ~5 °C until the experiment was conducted. The filed moist soil was not air-dried to minimize the potential increase in dissolved organic P caused by rewetting of dry soils (Turner et al., 2005). The sequential fractionation method proposed by Jiang and Gu (1989) and modified by Adhami et al. (2006) was tested and modified accordingly (Table 1). Each P fraction was extracted as follows; 1.25 g (oven-dry weight) of sample soil was weighed out into a 50 mL polyethylene centrifuge tube. A volume of 25 mL of the first extractant, NaHCO$_3$ (Table 1) was added and placed in an automated mechanical shaker at 25 °C with 110 rpm for 1 h to allow time for the solution to equilibrate. The tubes were then centrifuged at 11,000 × g for 15 min and the supernatant was carefully filtered through a 0.45 μm Millipore polycarbonate membrane with minimum loss of soil. This extraction procedure was repeated sequentially with seven different extractants with their required shaking/standing time as in Table 1. The amount of P in each extract was stored at ~5 °C until being analyzed by inductively coupled plasma optical emission spectrometer (ICP-OES) (Perkin Elmer 5300 DV), and colorimetrically using the molybdate blue method (Murphy and Riley, 1962) on a Technicon Autoanalyzer II set at 880 nm to determine the concentration of total P and inorganic P present in each extract, respectively. The amount of the organic P pool was calculated by subtracting the amount of the molybdate blue reactive orthophosphate from total P. In addition, the organic P in the soil was characterized using solution ^{31}P NMR spectroscopy in the NaOH-EDTA extract followed the method reported by Cade-Menun (2015) except using 5 mm probes in 600 Hz magnets, which was performed by NMR center at University of Guelph.

The amounts of dissolved cations including Ca, Al, and Fe in each

Table 1
A modified sequential fractionation method based on the method proposed by Jiang and Gu (1989) and modified by Adhami et al. (2006).

Step	Inorganic P fractions	Extractants	pH	Shaking time
1[a]	Ca$_2$-P type	0.25 M NaHCO$_3$	8.0	1 h
2	Ca$_8$-P type	0.5 M NH$_4$Ac[b]	4.2	16 h stand, 1 h
3	Prevent re-adsorption	1.0 M MgCl$_2$	8.0	2 h
4	Al-P	0.5 M NH$_4$F	8.2	1 h
5	Fe-P	0.1 M NaOH-Na$_2$CO$_3$	12.0	2 h, 16 h stand, 2 h
6	Occluded P	0.3 M CD[c]	13.0	16 h
7	Ca$_{10}$-P type	0.25 M H$_2$SO$_4$	1.0	1 h

[a] Ethanol wash after step 1.
[b] Ammonium acetate.
[c] 0.3 M Sodium citrate (20 mL) − dithionite (1.0 g) - 1.0 M sodium hydroxide (5 mL).