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Evaluation of Five Extraction Methods for Available Phosphorus in Intensively Fertilized Greenhouse Soils

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Intensively fertilized lime receiving soils encased in greenhouses have complex nature. We collected 11 greenhouse soils and examined the chemical forms of inorganic phosphorus (P) by applying two kinds of sequential extraction methods. The results and the foregoing study on the same soils revealed that the method developed for calcareous soils is better suited for the greenhouse soils. Among the five availability testing methods including Truog method, Mehlich III method, Olsen method, water extraction and CaCl_2 extraction, the Truog and the Mehlich III methods gave good estimates for calcium phosphate minerals like dicalcium phosphate and octacalcium phosphate. In view of simplicity of the method, Truog method is considered to be the best method for P-enriched greenhouse soils.

INTRODUCTION

Phosphorus (P) is one of the most important nutrients in crop production. Crops uptake P in abundance but its supply from natural soils is not sufficient. Large amount of synthetic P fertilizers are applied annually to meet P requirement of crops. Since P is very reactive with soil minerals particularly with oxide and hydroxide minerals, substantial amount of P is adsorbed by some soil minerals and turned unavailable for crops. Farmers tend to apply excessive amount of P fertilizers in fear for P deficiency. The fixation of P by soil minerals makes it difficult to estimate the amount of available P in soils. For these reasons, enormous amount of P accumulates in intensively cultivated greenhouse soils.

In recent years, reflecting the rapid growth of agricultural production and increased fertilizer use in Asia, the depletion of phosphate rock resources has been receiving increasing interest. The fear for possible shortage of P resources induced a surge of P-fertilizer price in 2008. To avoid excessive application and economize P-fertilizers, reliable test methods for assessing available P in soils are indispensable. Traditionally simple extraction methods like dilute H_2SO_4 -, NH_4F - and NaHCO_3 extraction have been used to evaluate availability of P in soils (Olsen and Sommers, 1982; Kuo, 1996) and found to be useful.

Many researches have been focusing on the development of more reliable soil testing method for available P. One of the major shortcomings of the traditional testing methods, i. e. the chemical extraction (Thomas and Peaslee, 1973; Olsen and Sommers, 1982), is that the chemical conditions during P-extraction are not necessarily like those prevailing in the field soil. In addition,

slow diffusion process toward plant roots through interstitial pores cannot be evaluated. To overcome these drawbacks, van der Zee *et al.* (1987) proposed to use a filter paper that was impregnated with iron oxides and hydroxides (Pi-paper). Most commonly, a piece of the Pi-paper is immersed in a soil suspension for a certain period of time (Van der Zee, 1987). The iron oxide that strongly adsorbs dissolved phosphate ions acts as a P-sink and keeps the solution P concentration low, resulting in the enhanced dissolution of sparingly soluble P-compounds and desorption of adsorbed P. Thus, the amount of P extracted by the Pi-paper can be used as a conservative estimate for available P. The Pi-paper has also been used to study P diffusion by embedding a piece of Pi-paper in a soil column or *in situ* for a certain period of time (Menon *et al.*, 1990; Saarela, 1992). With these techniques, the availability of soil P can be evaluated considering both adsorption-desorption plus dissolution-precipitation and diffusion. A testing method based on the similar principle had also developed (Davison and Zhang, 1994) and applied to soil-P testing (Menzies *et al.*, 2005).

Nevertheless, the chemical extraction methods are still useful in that they can extract potentially available P fraction if the extracting chemicals are suitable for major chemical forms of P contributed to P availability in target soils. For example the extraction with NH_4F is believed to give a good measure for available P in acid soils where P is mostly retained on oxides and hydroxides of Al and Fe. On the other hand NaHCO_3 extraction is suitable for calcareous soils where Ca phosphates predominate. The Japanese soils encased in greenhouses are not calcareous but not acidic, receiving a lot of lime and other amendments annually. They have intermediate and complex nature with regard to the dynamics of nutrients and the criteria for selecting a suitable extracting reagent for available P have not been established. The objective of the present study was to identify the major chemical forms of P in some P-eutrophicated greenhouse soils for better selection of the extracting agent.

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Soils collected in greenhouses were subjected two kinds of sequential extraction to estimate the major chemical forms of P. In addition, five chemical extraction methods were applied and the results were compared.

MATERIALS AND METHODS

Soil samples

Soil samples were collected from the Ap horizon in 11 greenhouses for vegetable cultivation in Chikushino, Japan. The Y1, Y2 and Y3 soils had been used for eggplant cultivation at least for 3 years. The Y4 soil had been used for cucumber cultivation for 2 years. Tomato had been cultivated on the Y5 and Y6 soils for three consecutive years. The Y7 and Y8 soils had been used for rice paddy for more than ten years and newly encased in greenhouses and tomato was grown once. The Y10 soil had been used for strawberry at least for 10 consecutive years. Welsh onion had been harvested several times a year on the Y11 for 5 years. The Y12 soil had been used for eggplant and tomato cultivation.

The soil samples were air-dried after collection, passed through a 2-mm sieve and stored in polyethylene bags. The major chemical properties that are listed in Table 1 were determined following the methods described by the Editorial Boards of Methods for Soil Environment Analysis (1997) except for those otherwise specified. The total P content was determined by Na_2CO_3 fusion following the procedures described by Olsen and Sommers (1982). The carbonate content was determined by the method proposed by Than *et al.* (2010). All these analyses were carried out in duplicate and the averaged values were shown.

Sequential fractionation of inorganic P

Two sequential fractionation schemes were applied. One (Method A) is the method developed for calcareous soils (Adhami *et al.*, 2006) and the other (Method B) is the one for noncalcareous soils (Kuo, 1996). Extractants

used and procedure were briefly described in Table 2 and Table 3.

Available P extraction

So called available P was evaluated with five methods: Mehlich III (Sims, 2000), Olsen (Kuo, 1966), Truog (Editorial Boards of Methods for Soil Environment Analysis, 1997), CaCl_2 extraction (Kuo, 1996). For water extraction, 5 g of an air-dried soil sample was placed in a plastic bag, mixed with 50 mL of deionized water and agitated the mixture by scrubbing wet air through an inserted glass tube (Than *et al.*, 2010). A brief description of the procedure is given in Table 4.

RESULTS AND DISCUSSION

The soil samples contained about 1400 to nearly 3000 mg/kg of total P, indicating that they are extremely P-eutrophicated. These data and the history of the soils described in the materials and methods section suggests that the intensive fertilization after the soils were encased in greenhouses caused the P accumulation. The soil pH ranged from about 6.2 to 6.9 and the soils are nearly neutral. Some soils (Y3, Y4 and Y12) contained appreciable amounts of carbonate but the soil pH indicates that the soils are noncalcareous in nature. Than *et al.* (2009) concluded that the conventional procedure for soil pH measurement is not adequate for some greenhouse soils by showing that the measured pH value gradually approach 8 when the soil contained significant amount of carbonate ($>10 \text{ cmol kg}^{-1}$). The carbonate contents of the present soil samples are mostly $< 1 \text{ cmol kg}^{-1}$ and there would be no problem in the measured pH. The electric conductivity values show that there is no serious accumulation of soluble salts.

The results of the sequential fractionations were summarized in Table 5 and Table 6. The extraction reagents and procedures for method A and B are similar. One important difference is that the extraction step with

Table 1. Selected chemical properties of soil samples used

Sample No	pH	EC	Organic C	Total N	Exchangeable cations				Carbonate	Total P
					Ca	Mg	K	Na		
		dS m^{-1}	----- g kg^{-1} -----		----- cmolc kg^{-1} -----				cmol kg^{-1}	mg kg^{-1}
Y1	6.29	0.41	32.1	2.4	15.1	4.2	0.9	0.5	0.61	2893
Y2	6.28	0.52	30.6	2.3	14.6	4.4	0.9	0.6	0.92	2552
Y3	6.26	0.38	29.7	2.7	14.3	4.0	0.7	0.3	1.04	2779
Y4	6.42	0.67	18.6	1.9	13.8	3.5	0.7	0.6	1.63	2073
Y5	6.35	0.21	16.9	1.4	13.4	3.5	0.2	0.3	0.48	2273
Y6	6.21	0.26	17.5	1.5	12.0	3.2	0.2	0.2	0.55	1901
Y7	6.83	0.14	19.0	1.6	9.6	2.0	0.1	0.1	0.40	1408
Y8	6.71	0.14	17.7	1.4	8.7	1.9	0.2	0.1	0.35	1367
Y10	6.81	0.14	28.3	2.4	15.1	4.7	0.3	0.3	0.34	2366
Y11	6.89	0.09	22.2	1.5	11.3	2.9	0.3	0.1	0.93	1701
Y12	6.79	0.36	27.7	1.8	16.5	4.6	0.3	0.4	1.83	1925

acidic acetate buffer is missing in method B. This is because the presence of significant amounts of semi-stable calcium phosphate is not assumed in method B. The total amounts of inorganic P extracted by the two methods are surprisingly similar for all the soil samples but the extracted amounts in the corresponding steps

are quite different. Particularly the Ca-P fraction by method B nearly doubled that by method A. Accordingly, the estimated amount of calcium phosphate in method A (= Ca₂-P + Ca₈-P + Ca₁₀-P) was 2 to 3 times larger than that in method B (= soluble P + Ca₁₀-P). Than *et al.* (2010) extracted these soil samples successively with

Table 2. Sequential inorganic P fractionation procedure for method A

Step	Fractionation scheme	Typical P forms	Designation
1	0.25 M NaHCO ₃ , pH 7.5, 1 h shaking, wash twice with ethanol	Ca(H ₂ PO ₄) ₂ CaHPO ₄ ·nH ₂ O	Ca ₂ -P
2	0.5 mol L ⁻¹ NH ₄ OAc, pH 4.2, 4 h standing and 1 h shaking, wash twice with 1 mol L ⁻¹ MgCl ₂	Ca ₈ H ₂ (PO ₄) ₆ ·nH ₂ O	Ca ₈ -P
3	0.5 mol L ⁻¹ NH ₄ F, pH 8.2, 1 h shaking, wash twice with 1 mol L ⁻¹ MgCl ₂ , pH 8	Surface complex on Al hydroxide	Al-P
4	0.1 mol L ⁻¹ NaOH-0.1 mol L ⁻¹ Na ₂ CO ₃ , 2 h shaking, 16 h standing, 2 h shaking, wash twice with 1 mol L ⁻¹ MgCl ₂ , pH 8	Surface complex on Fe hydroxide	Fe-P
5	0.3 mol L ⁻¹ Na ₃ C ₆ H ₅ O ₇ , Na ₂ S ₂ O ₄ , 0.5 mol L ⁻¹ NaOH, 17 h shaking, wash twice with 1 M MgCl ₂ , pH 8	Incorporated in crystalline Fe oxides	Occluded P
6	0.25 mol L ⁻¹ H ₂ SO ₄ , 1 h shaking	Ca ₁₀ (PO ₄) ₆ ·(OH) ₂	Ca ₁₀ -P

Table 3. Sequential inorganic P fractionation procedure for method B

Step	Fractionation scheme	Typical P forms	Designation
1	1 mol L ⁻¹ NH ₄ Cl, pH 7.5, 1 h shaking , wash twice with ethanol	Ca(H ₂ PO ₄) ₂ CaHPO ₄ ·nH ₂ O	Soluble-P
2	0.5 mol L ⁻¹ NH ₄ F, pH 8.2, 1 h shaking, wash twice with 1 mol L ⁻¹ NaCl	Surface complex on Al hydroxide	Al-P
3	0.1 mol L ⁻¹ NaOH, 2 h shaking, 16 h standing and 2 h shaking, wash twice with 1 mol L ⁻¹ NaCl	Surface complex on Fe hydroxide	Fe-P
4	0.3 mol L ⁻¹ Na ₃ C ₆ H ₅ O ₇ , Na ₂ S ₂ O ₄ , 0.1 mol L ⁻¹ NaHCO ₃ , 17 h shaking, wash twice with 1 mol L ⁻¹ NaCl	incorporated in crystalline Fe oxides	Occluded P
5	0.5 mol L ⁻¹ H ₂ SO ₄ , 1 h shaking	Ca ₁₀ (PO ₄) ₆ ·(OH) ₂	Ca ₁₀ -P

Table 4. The five extraction methods for available P

Methods	Extraction procedure
Mehlich III	0.2 mol L ⁻¹ CH ₃ COOH-0.25 mol L ⁻¹ -NH ₄ NO ₃ -0.015 mol L ⁻¹ NH ₄ F, 0.013 mol L ⁻¹ HNO ₃ , 0.001 mol L ⁻¹ EDTA, 5 min shaking, soil : water volumetric ratio = 1 : 10
Truog	1 mmol L ⁻¹ H ₂ SO ₄ , 30 min shaking, soil : water volumetric ratio = 1 : 20
Olsen	0.5 mol L ⁻¹ NaHCO ₃ pH 8.5, 30 min shaking, soil : water volumetric ratio = 1 : 20
Water extraction	Equilibrated under continuous air bubbling for 5 days, soil : water volumetric ratio = 1 : 5
CaCl ₂ extraction	0.01 mol L ⁻¹ CaCl ₂ , 1 h shaking, soil : water volumetric ratio = 1 : 10

Table 5. The amounts of sequentially extracted inorganic P fractions by method A

Soil samples	Ca ₂ -P	Ca ₈ -P	Al-P	Fe-P	Occluded-P	Ca ₁₀ -P	Total
----- mg kg ⁻¹ -----							
Y1	198	511	866	606	232	155	2568
Y2	171	484	773	530	239	138	2335
Y3	209	808	551	523	210	155	2457
Y4	153	546	403	443	144	111	1800
Y5	173	237	696	457	145	108	1852
Y6	180	321	498	424	155	154	1731
Y7	48	63	546	312	119	101	1189
Y8	53	64	490	269	108	87	1071
Y10	135	219	464	692	228	158	1896
Y11	142	266	497	338	148	73	1463
Y12	173	613	345	295	149	104	1679

Table 6. The amounts of sequentially extracted inorganic P fractions by method B

Soil samples	Soluble-P	Al-P	Fe-P	Occluded-P	Ca ₁₀ -P	Total
----- mg kg ⁻¹ -----						
Y1	68	1203	722	433	134	2560
Y2	60	1039	546	373	101	2120
Y3	146	951	666	495	148	2407
Y4	119	770	602	362	129	1982
Y5	47	1050	546	248	66	1957
Y6	56	810	404	237	95	1602
Y7	8	654	298	122	82	1164
Y8	11	578	282	109	80	1059
Y10	47	685	903	216	89	1940
Y11	54	730	382	198	60	1425
Y12	153	657	410	344	114	1677

Table 7. Results of the five extraction methods for available P

samples	Extraction methods				
	Mehlich III	Olsen	Troug	Water	CaCl ₂
----- mg kg ⁻¹ -----					
Y1	735	174	662	20	20
Y2	631	160	658	14	17
Y3	904	196	950	44	50
Y4	713	138	694	18	16
Y5	577	164	388	21	21
Y6	575	151	381	21	18
Y7	180	55	155	3	3
Y8	195	54	144	3	3
Y10	482	145	415	21	23
Y11	597	120	474	34	39
Y12	641	157	690	28	30

1 mol L⁻¹ NaCl and 0.5 mol L⁻¹ NH₄OAc buffered at pH 4.2 and found large amount of Ca as well as P in the second extract. Since exchangeable Ca was removed in the first extraction, simultaneous dissolution of Ca and P showed that calcium phosphate dissolved in the second step. The amounts of dissolved P for Y1 to Y12 soils were 688, 634, 922, 646, 474, 431, 158, 173, 396, 385 and 610 mg kg⁻¹ and the Ca/P molar ratio ranged from about 1 to 3.5. They are approximate to the sum of Ca₂-P and Ca₈-P in Table 5, indicating that Ca₂-P and Ca₈-P fractions actually came from calcium phosphate. On the other hand, these amounts exceed the soluble P + Ca₁₀-P fraction in method B, suggesting that method B is not suitable for the present soil samples. Therefore, only the data from method A are used in the following discussion.

Table 7 shows the results of the five extraction methods for available P. The amounts of P extracted by Mehlich III method and Truog method were similar to each other and much higher than those by other methods. The comparison of the Truog-P and Mehlich III-P in Table 7 with the data in Table 5 indicates that they are very close to the sum of Ca₂-P and Ca₈-P. This is clearly seen from Fig. 1. These results and the foregoing discussion on the presence of calcium phosphate suggest that the major extraction mechanism of Truog and Mehlich III method in the present soil samples is the acid-mediated dissolution of calcium phosphates. In view of simplicity of the extracting reagent, the Truog method seems better and reasonable for these soil samples. The Olsen method extracted approximately 20% of calcium phosphates. In an experiment of the various P rates with and without liming on a same soil, Curtin and Syers (2001) stated that liming decrease the available Olsen P value with the effect being largest at the highest rate of P addition. Liming also tended to decrease water soluble P.

Water and CaCl₂ extractions gave almost identical results irrespective of different soil to water ratio, i. e.,

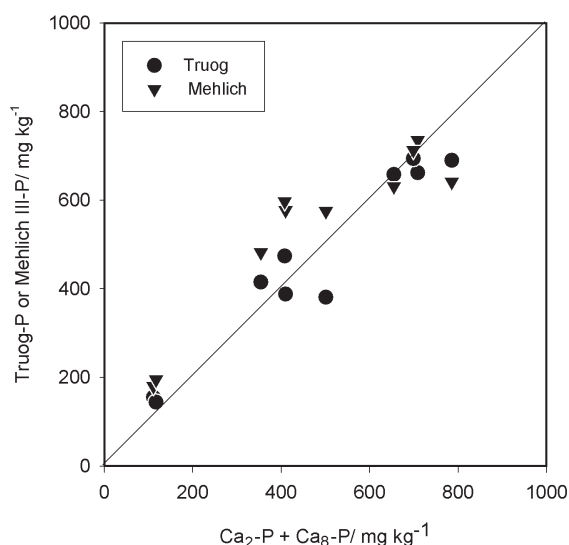


Fig. 1. Plot of Truog-P or Mehlich III-P versus Ca₂-P plus Ca₈-P from method A.

1:5 and 1:10 in the water extraction and CaCl₂ extraction, respectively. In the extraction with non-buffered, non-complex forming solutions, solution P concentration is governed by the solubility of existing phosphate minerals and higher amount of soluble P is expected in the extraction at wider soil to water ratio. The present results (Table 7) may be due to the mutual cancellation of the lower soil to water ratio and the suppression of calcium phosphate dissolution by increased Ca ion activity.

Oshima and Goto (2008) reported that the amount of P extracted with water at a soil to water ratio of 1:200 was highly correlated with the Truog-P with a correlation coefficient of 0.914. They concluded that the water extraction may be useful as a mean to evaluate the amount of available phosphorus. However, their observation is not compatible with the idea that the P concentration in the water extract is governed by the solubility of sparingly soluble calcium phosphates like mono or dicalcium phosphate and octacalcium phosphate. The highest amount of water-extracted P reported by Oshima and Goto (2008) was approximately 700 mg-P₂O₅ kg⁻¹ for a soil containing about 3500 mg-P₂O₅ kg⁻¹ of Truog P. Since the water to soil ratio was 1:200, the P concentration in the extract is calculated to be about 0.05 mmol L⁻¹. This concentration seems too low for the soil containing 3500 mg-P₂O₅ kg⁻¹ of Truog P. Their extraction may have not been at dissolution equilibrium because the extraction time was only 0.5 h and this would be the reason why the soil having larger Truog-P gave larger water extractable P.

Halvorson and Black (1985) applied 15 to 180 kg ha⁻¹ of P to a field on a Williams loam (Mollisol) and monitored the Olsen-P level for 16 crop years. The Olsen-P level decreased only gradually mainly due to the uptake by crop and maintained throughout the experimental period above the initial level. This indicates that the applied P was preserved as calcium phosphates and released gradually depending on the soil solution condition. Since Mollisols usually receive less precipitation and contain calcium carbonate, the applied P may have been converted to dicalcium phosphate, octacalcium phosphate etc. and preserved for the long period of time. It was earlier believed that dicalcium phosphate reverted to octacalcium phosphate and even to colloidal hydroxyl apatite and control the P solubility (Lehr and Brown, 1958; Larsen, 1967). In retrospect, Kurtz (1953) noted that phosphate with soils are not entirely irreversible and that for most soils the term fixation is an exaggeration. Mattingly and talibudeen (1967) suggested that the rate of P removal from solution by soil components in acid and neutral soils is faster than octacalcium phosphate can be formed, indicating that octacalcium phosphate, if formed, is unlikely to persist. Bache (1964) reached a similar conclusion for the stability of strengite and verisite in most soils.

The chemical conditions in Japanese greenhouses has some resemblance in that the soils are calcium saturated due to annual liming and subjected to weak leaching. In addition, large amounts of Al-P and Fe-P (Table

5) indicate that the reactive surfaces of oxide, hydroxide and noncrystalline minerals are nearly saturated with phosphate. Thus, the newly applied P can be preserved as phosphate minerals like dicalcium phosphate and octacalcium phosphate.

The data presented in Table 5 and Table 7 and the foregoing discussion suggest that the greenhouse soils used in the present study can be regarded as semi-calcareous soils and the major sources of available P are calcium phosphates like dicalcium phosphate and octacalcium phosphate. The Truog method applied to P-eutrophicated greenhouse soils extracts these phosphate minerals almost selectively and it is the best testing method among the five in terms of simplicity. Olsen method gives more conservative estimate for available P than Truog and Mehlich method. Combination of the Truog method and equilibrium water extraction will serve as a useful test scheme to evaluate extensive and intensive factors in availability of P.

The soil test P values in table 7 suggested that some long term experiment with or without P fertilizer application for a number of cropping years until crop yield start to lower than optimum, should be carried out to establish soil P "critical value" for a given soil and farming system in order to use nonrenewable P resources efficiently for economically and environmentally (Syers *et al.*, 2008).

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