CHAPTER 6*

Short cut approach alternative to the step-by-step conventional soil phosphorus fractionation method

6.1 INTRODUCTION

Conventional soil P tests, which usually consist of single extraction procedures, are used to estimate fertilizer requirements and represent an index of plant available P (Indiati et al., 2002). Since plant available P in soil is not a single entity, a "complete account or budget" of the P forms present in the soil have to be obtained in order to determine the fate of applied P fertilizers. This can be achieved by characterizing both labile and less labile inorganic and organic P pools (Solomon et al 2002).

The sequential extraction procedure developed by Hedley et al. (1982) has been applied to determine the different forms of P in the soil. The underlying assumption in this approach is that readily available soil P is removed first with mild extractants, while less available P can only be extracted with stronger acids and alkali. The overall advantage of the fractionation of soil phosphate in to discrete chemical forms permits the quantification of different P pools, their chemical status in native or cultivated soils, and to study the fate of the applied P fertilizers (Hedley et al. 1982; Tiessen and Moir, 1993). This method has recently been employed in long-term P desorption studies (Schmidt et al., 1997; Du Preez and Claassens, 1999; Araujo et al., 2003).

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This method has undergone several modifications, few of which are explicitly detailed (Guppy et al.2000). For instance, the original fractionation (Hedley et al. 1982) left between 20 and 60% of the P in the soil unextracted. This residue often contained significant amounts of organic P (Po) that sometimes participated in relatively short-term transformations. On relatively young, Ca-dominated soils this residual Po can be extracted by NaOH after the acid extraction, while on more weathered soils, hot concentrated HCl extracts most of the organic and inorganic residual P. Tiessen and Moir (1993) included the hot concentrated HCl step to enhance the percent recovery of the extraction by extracting more P_o than the original Hedley et al. (1982) procedure. The result of Tiessen and Moir (1993) was also supported by the results of Condron et al., (1990), as they extracted nearly all Po and P_i from tropical soils using hot concentrated HCl reflecting the importance of this particular step to further characterize the residual P.

Modifications made on the initial step of the Hedley et al (1982) procedure also have been made. Van der zee et al., (1987) proposed the use of Fe-oxide impregnated filter paper strips (Fe-oxide strips) as a promising method to study the P release kinetics of soils. Acting as a sink for P, the Fe-oxide strips have a sounder theoretical basis than the chemical extractants in estimating available soil P (Sharpley, 1996). However, this method was found to be not well applicable for long-term desorption studies as it may lead to errors due to adhesion of fine P-rich particles to the paper strips and due to the mechanical instability of the paper when used for long desorption studies (Freese et al., 1995; Lookman et al.; 1995). Recently, use of DMT-HFO in place of resin/Fe-oxide paper strip in the initial stage of fractionation for studying long-term P dynamics has been proposed (De Jager and Claassens, 2005; Ochwoh et al. 2005).

Plant P availability of residual P in soils can be quantified by successive cropping experiments carried out in field or green house studies, where P is taken up until P deficiency occurs or a response to added P is measured (Indiati, 2000). To deplete the soil P in this approach usually takes decades (Johnston and Poulton, 1976; McCollum. 1991). It is possible, however, to simulate the plant mode of action by artificially depleting the soil by successive desorption experiments using ion sink methods such as Fe-oxide impregnated paper strips or DMT-HFO. By making use of these methods, one can accomplish the above task in days instead of years and yet capable of obtaining reasonably comparable information on the types of P pools involved in replenishing the soil solution P. Consecutive extraction procedures carried out by these ion sink methods (McKean and Warner, 1996; de Jager and Claassens, 2005) combined with subsequent fractionation procedure (Hedley et al. 1982; Tiessen and Moir, 1993) previously termed as a *combined method* may, therefore, constitute a convenient laboratory method to characterize the P supplying capacity of a soil and to understand the dynamics of soil P.

Successive desorption of P by DMT-HFO followed by subsequent fractionation method as described by Hedley et al. (1982)/Tiessen and Moir (1993) have been recently employed in South Africa to study the P dynamics of incubated soils. This combined methodology helps to identify, which P pool, serves as a major sink/source of P in studying the P dynamics of soils during P addition/depletion. For instance, De Jager (2002) investigated the desorption kinetics of residual and applied phosphate to red sandy clay soils using this combined method. It was found that the total amount of phosphate desorbed during a 56 day period of extraction was virtually equal to the decrease in the NaOH extractable inorganic phosphate fraction that was ascribed to the active contribution of NaOH (moderately labile) fraction in the desorption process. Ochwoh et al. (2005) also studied the chemical changes of applied and residual phosphorus (P) in to different pools for two South African soils. They found that between 30-60% of the added P was transformed to the less labile P pools in 1 day and 80-90% of the added P after 60 days of incubation. A major portion of the P was transformed to the NaOH-extractable P pool. In the same study, Ochwoh (2002) attempted to determine the P desorption rates by successive DMT-HFO extractions followed by sequential extraction for the same soils. The results revealed that the so-called un-labile soil P pools contributed to the labile P pool by different proportions. As reported in chapter 3, the C/HCl-P_i was found to be the fraction that decreased most suggesting the importance of this fraction in replenishing the labile P fractions for the soils we investigated.

Although this combined methodology helps in understanding the P dynamics of soil in relatively shorter time as compared to successive cropping experiments, it is still too time consuming and expensive. For example, most of the P fractions are determined after 16 hrs shaking and it takes usually one week to finish the successive P extractions and determinations when using the following extractants: DMT-HFO, $NaHCO_3$, NaOH, D/HCl, C/HCl, H_2SO_4+H_2O_2. The process even becomes too cumbersome when the soil testing is made at a large scale. The major objective of this paper, therefore, was to propose a short cut method as an alternative approach to the combined fractionation method. However, it is important to identify the major P fraction that contributed in replenishing the labile fraction (plant available P) using the conventional step-by-step fractionation method. Once the major source of P for the labile fraction is identified, we can use the selected extractant to run the desorption experiment immediately following the initial fractionation step (DMT-HFO step), instead of going through all the steps as depicted above, which makes the alternative method less time consuming and more economical than the conventional approach. C/HCl-P_i has been identified as the major P pool that acted as a source for the labile fraction using the combined method for some South African long term fertilized soils from previous experiment and we compared this data with the data obtained using the short cut approach and the information extracted from both was intern compared with maize grain yield.

6.2 MATERIALS AND METHODS

The sampling procedure and experimental site history of the soil samples used in this experiment are also detailed in Sections 3.2.1 and 3.2.2. Table 3.2 shows some selected physical and chemical properties of the different treatments.

6.2.1 Long-term desorption study

A long term desorption study was carried out using dialysis membrane tubes filled with hydrous ferric oxides similar to that described by Freese et al. (1995), the detail of which is presented in Section 3.2.3.

6.2.2 Fractionation procedure

Soil samples were sequentially extracted for P using Tiessen and Moir (1993) method with a slight modification made on the initial step where by the resin in the Tiessen and Moir (1993) procedure was replaced by the DMT-HFO (De Jager & Claassens 2005; Ochwoh et al. 2005). The detail of this particular step is also presented in Section 3.2.2.

6.2.3 Short cut approach to a modified fractionation procedure

The short cut approach consists of a two-step fractionation procedure. Firstly by DMT-HFO followed by a single concentrated HCl extraction as follows. A 1.0 g soil sample in 80 ml 2 *mM* $CaCl_2$ and 0.3 *mM* KCl solution was successively extracted for soluble P with dialysis membrane tube filled with ferric hydrous solution for different times (1, 7, 14, 28 & 56 days). This was followed by extraction with C/HCl. A slight modification was also made on this particular step based on a preliminary investigations carried out previously (data not shown here). Instead of following the procedure as stipulated by Tiessens and Moir (1993) for this particular step, 15 ml of the C/HCl extractant was added to the 1 g sample after the DMT-HFO extraction and then shaken for 16h on an end-over-end shaker instead of using a water bath. The major reason for this modification was that many more samples could be done simultaneously than with the water bath where space was limiting and time consuming.

6.2.4 Field data

Maize (*Zea mays* L.) was grown in summer (November to March) since the establishment of the long-term experiment (1939). Field data for grain yield (t ha^{-1}) was collected from the experimental station. Since there was no plant analysis to evaluate plant P uptake, soil analysis data was correlated to dry maize grain yield (12% moisture content).

6.2.5 Data analysis

The data obtained were statistically analyzed by using Statistical Analysis System (SAS Institute 2004). Analysis of variance was done using the General Linear Model (GLM) procedure. The Tukey test was used to determine the least significant differences at $\alpha = 0.05$. The regression equations and correlation coefficients were determined with exponential fits to the data. Correlation with the plant yield parameter was done using Pearson linear correlation, PROC CORR (SAS Institute 2004).

6.3 RESULTS AND DISCUSSION

6.3.1 Modification made on the C/HCl step of Tiessen and Moir (1993) method

According to the method of Tiessen and Moir (1993), the C/HCl extract, which is the 5th step in the fractionation process, is determined following the extraction by dilute HCl step as stipulated below.

- Add 10 ml conc. HCl to the soil left after the D/HCl extraction step and vortex well
- Heat the soil sample on a water bath at 80 ^{0}C for 10 min.
- Add additional 5 ml Conc. HCl
- Cool to room temp by shaking every 15 minutes
- Centrifuge at 2500 RPM for 10 min and filter in to 100 ml volumetric flask
- Wash the soil with 10 ml water, centrifuge and add the supernatant solution to the previous filtrate
- Determine P_i and P_t

In the present study this step was modified by adding 15 ml C/HCl to the soil and the solution was shaken for 16h on an end-over-end shaker immediately after the DMT-HFO step. The amount extracted by both methods was compared with the sum of inorganic fractions obtained by the conventional step-by-step fractionation procedure of Tiessen and Moir (1993) that was done before and presented here as depicted in Table 6.1 below. This modified extraction procedure was tested on the 24h DMT-HFO-P_i extraction.

In general, the average C/HCl P_i extracted by the modified short cut approach was greater than the non-modified short cut approach (Table 6.1). These values were nearly similar to the amount of P extracted as described by Tiessen and Moir (1993) method that was used as a reference. This close relationship with the reference was also supported by the slightly higher correlation the modified (R^2 = 0.999) showed than the non-modified short cut approach (R^2 = 0.969) as illustrated in Figure 6.1.

The correlation observed above (Figure 6.1) was also verified by taking in to account all the replicates of trial 1 instead of average values to check if the relationship holds true. The modified short cut method correlated better in this regard too (Figure 6.2). Based on these results, the modified short cut approach was selected for running the extraction with C/HCl extractant.

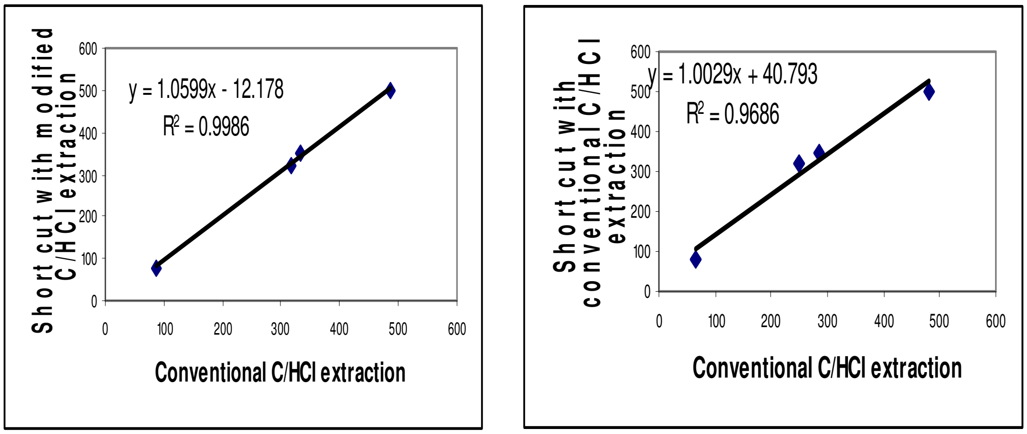
Table 6.1 Amount of C/HCl extracted P_i (mg kg^{-1}) for the different treatments according to the modified methods and the conventional approach of Tiessen and Moir (1993)

	Short cut with conventional C/HCl extraction			Short cut with modified C/HCl extraction			†Conventional approach
	Amount of C/HCl (mg kg-1) extracted by direct methods						‡$\sum P_i$ (mg kg^{-1})
Treatment	Trial 1	Trial 2	*Average	Trial 1	Trial 2	*Average	
NK	69.39	59.75	64.57	87.74	86.97	87.36	77.95
NPK	331.83	239.45	285.64	315.58	348.66	332.12	349.6
MNK	275.64	223.05	249.35	312.93	322.73	317.83	321.61
MNPK	553.34	409.51	481.43	516.56	454.39	485.48	498.16

†Conventional approach refers the method of Tiessen and Moir (1993)

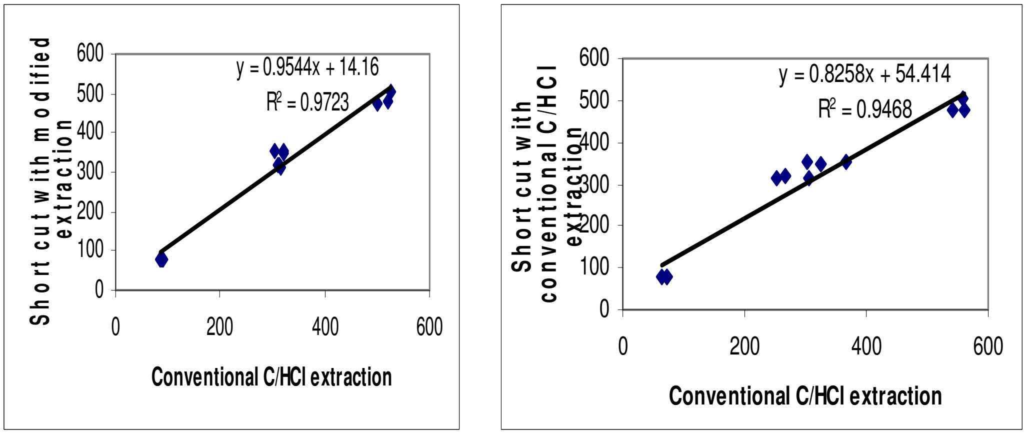
‡$\sum P_i$ refers to $\sum$DMT-HFO-P_i+$NaHCO_3$-P_i+NaOH-P_i+D/HCl-P_i+C/HCl-P_i

* Average result of two trials each performed in triplicates



a) b)

***Figure 6.1*(a-b)** Simple linear correlation between the conventional approach and short cut with (a) modified C/HCl extraction and (b) conventional C/HCl extraction. Average values of trial 1 and trial 2.



a **b**

***Figure 6.2*(a-b)** Simple linear correlation between the conventional approach and short cut with (a) modified C/HCl extraction and (b) conventional C/HCl extraction for the whole triplicates of trial 1.

6.3.2 DMT-HFO-extractable P_i

The amount of P_i extracted by DMT-HFO was significantly influenced ($P \leq 0.05$) both by P level and extraction time (Table 6.2). The amount of P desorbed ranged from 1.47 – 1.74, 4.59 – 23.61, 6.15 – 21.48 and 18.87 – 132.81 mg kg^{-1} for NK, NPK, MNK, and MNPK treatments respectively. Averaged over all P rates, the amount of DMT-HFO extracted P_i in general followed the order: MNPK>>NPK≈MNK>>NK. Application of P, in the form of fertilizers and/or manure therefore, increased DMT-HFO-P_i compared to the unfertilized control. In this study, NPK and MNK treated soils resulted in a comparable amount of extracted P at all levels of extraction time. This is possibly because in soils treated with large amounts of animal manure, like the case of MNK, most organic P might have been transformed to inorganic P (Sharpley, et al., 1993; Koopmans et al., 2003) as reported previously.

The cumulative P released with time followed the same pattern for all P treatments, with an initial rapid release of P with in the first two weeks (14 days), followed by a slower release that was still continuing after 56 days of extraction. This is attributed to the presence of two distinct pools of soil P, one with rapid release kinetics and the other with slower desorption kinetics (Lookman et al., 1995, De Jager and Claassens, 2005). The fast P pool presumably represents primarily P bound to the reactive surfaces, which are in direct contact with the aqueous phase (Hingston et al., 1974, Madrid and Posner, 1979). The slow P release rate from the second pool is either the result of slow dissolution rates or from slow diffusion from interior sites inside oxyhydroxide particles (McDowell and Sharpley, 2003). The fact that the control has very little DMT-HFO extractable P might be resulted from the low amount of available P (Table 3.1) as reported in the previous chapters.

Table 6.2 Effect of P levels and extraction time on soil P extracted by DMT-HFO

Desorption time (days)	NK	NPK	MNK	MNPK
	(mg P kg^{-1})			
1	[§]**x**1.47[†]**a**[‡]	**x** 4.59**a**	**x** 6.15**a**	**y** 18.87**a**
7	**x**1.54**a**	**y**11.31**ab**	**y** 11.6**ab**	**z** 85.8**b**
14	**x**1.57**a**	**y**15.69**bc**	**y** 15.76**bc**	**z** 103.97**c**
28	**x**1.62**a**	**y**19.44**bc**	**y** 17.55**bc**	**z** 108.85**c**
56	**x**1.74**a**	**y**23.61**c**	**y** 21.48 **c**	**z** 132.81**d**

[§] Mean values in rows with different letters x, y, and z are significantly different ($\alpha = 0.05$)

[†] Mean values of three replicates

[‡] Mean values in columns with different letters a, b, c, and d are significantly different ($\alpha = 0.05$).

6.3.3 C/HCl-Extractable P_i

The concentrated HCl extractable P_i after successive DMT-HFO-extraction periods declined over extractions time but was significant ($P \leq 0.05$) only for treatment MNPK (Table 6.3). This was contrary to the result obtained by using the conventional approach. In the conventional approach (Tiessen and Moir (1993) method), the change in the amount of this fraction with time was statistically significant for all P

Table 6.3 Effect of P levels and DMT-HFO extraction time on soil P extracted by C/HCl using the short cut approach and conventional approach

Adsorptions time (days)	NK	NPK	MNK	MNPK
		(mg P kg^{-1})		
		Short cut approach		
1	[§]x98.8[†]**a**[‡]	**y** 350.01**a**	**y**323.67**a**	**z** 516.56**a**
7	x97.83**a**	**y** 349.48**a**	**y** 319.5**a**	**z** 463.20**b**
14	x98.35**a**	**y** 338.91**a**	**y** 311.45**a**	**z** 433.67b**c**
28	x97.69**a**	**y** 323.01**a**	**y** 308.67**a**	**z** 420.97b**c**
56	x95.71**a**	**z** 315.11**a**	**y** 287.13**a**	**w**395.78**c**
		Conventional approach		
1	[§]x52.3[†]**a**[‡]	**z** 110.9**a**	**y**96.9**a**	**yz** 106.7 **a**
7	x49.78**a**	**z** 93.70**b**	**y** 75.19**b**	**z** 95.31**ab**
14	x50.31**a**	**yz** 78.90**c**	**y** 68.84**b**	**z** 85.87**b**
28	x44.80**a**	**yz** 73.07**c**	**y** 62.48**bc**	**z** 75.72**bc**
56	x42.63**a**	**yz** 67.78**c**	**y** 61.6**c**	**z** 72.54**c**

[†] Mean values of three replicates

[§] Mean values in rows with different letters x, y, z and w are significantly different ($\alpha = 0.05$)

[‡] Mean values in columns with different letters a, b, and c are significantly different ($\alpha = 0.05$).

treated soils (Table 6.3). The general declining trend (Figure 6.4) of this fraction with increasing time of extraction by DMT-HFO, however, was similar with the trend shown by the conventional approach (table 6.3). The amount of P extracted by this fraction followed the order NK<MNK<NPK<MNPK and agreed with the conventional approach too. The amount extracted by this extractant (mg kg^{-1}) varied from 98.8-95.71, 350.01-315.11, 323.67-287.13 and 516.56-395.78 for treatments NK, NPK, MNK and MNPK respectively after day 1 and 56 days of extraction as illustrated in Table 5.3.

The sum of DMT-HFO-P_i, NaHCO3-P_i, NaOH-P_i, D/HCl-P_i and C/HCl-P_i extracted by modified Tiessen and Moir (1993) method with the sum of DMT-HFO-P_i and C/HCl-P_i extracted by the short cut approach were compared as depicted in Table 6.4 for all extraction periods (1, 7, 14, 28 and 56 days). Figures 6.4(a-d) illustrate the correlation between the two methods for the stated extraction periods. In all periods of extraction, the correlations were very strong. Despite this relationship, the difference in the $\sum P_i$ between the short cut and the conventional methods seemed increasingly larger with enhanced period of extraction. The reason for these disparities especially at the later period of extraction could be ascribed to the differences in the steps these two approaches involved. In the step-by-step approach, all the inorganic fractions decreased and almost all the organic fractions increased with increased time of extraction by DMT-HFO as reported in Chapter 4. The reason for the decline of the inorganic fractions was partly due to the P removal from the soil solution by the DMT-HFO and partly as the result of P immobilization (Stewart and Tiessen, 1987). The fact that the organic fractions increased with increased time of extractions as has

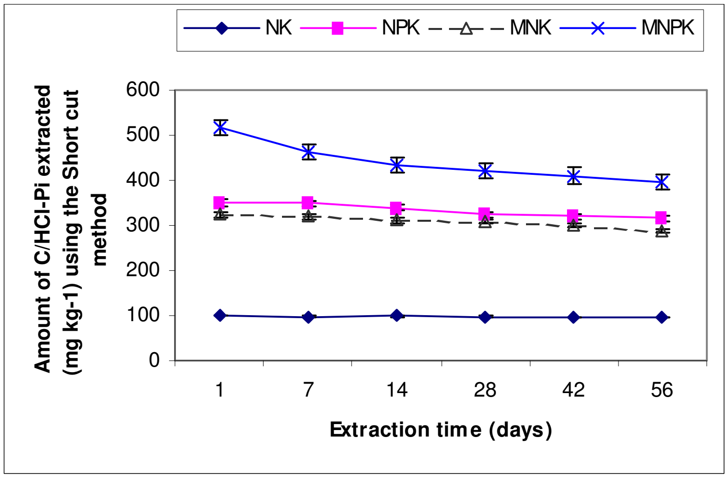


Figure 6.3 Changes in the C/HCl-extractable P_i with time. The values in the figure are means of three replicates. Vertical bars represent the standard error.

been reported in the previous experiment could offset the observed decreasing trend for the inorganic fractions. This is evidenced by having nearly similar average sum of inorganic and organic P fractions ($\sum P_i+P_o$) for both approaches (156.20, 498.81, 474.63 and 640.13 mg kg $^{-1}$ in the short cut and 131.89, 418.94, 419.62 and 654.46 mg kg^{-1} in the conventional step by step method for NK, NPK, MNK and MNPK treatments respectively). The slightly greater extract for NK, NPK and MNK treatments could be that some residual P from the more refractory pool might have been extracted in the short cut methodology than the same fraction extracted by the conventional Tiessen and Moir (1993) method. Besides, there could be a possibility of loss of some P (Table 4.1) in the step-by-step extraction method that might contribute for the relatively smaller extracted P compared with the short cut. This result is similar to the result reported previously emphasizing the need of modifying the C/HCl step at the beginning of the discussion in this particular chapter.

Table 6.4 Comparison of the sum of inorganic P fractions extracted by Tiessen and Moir (1993) method and the short cut approach

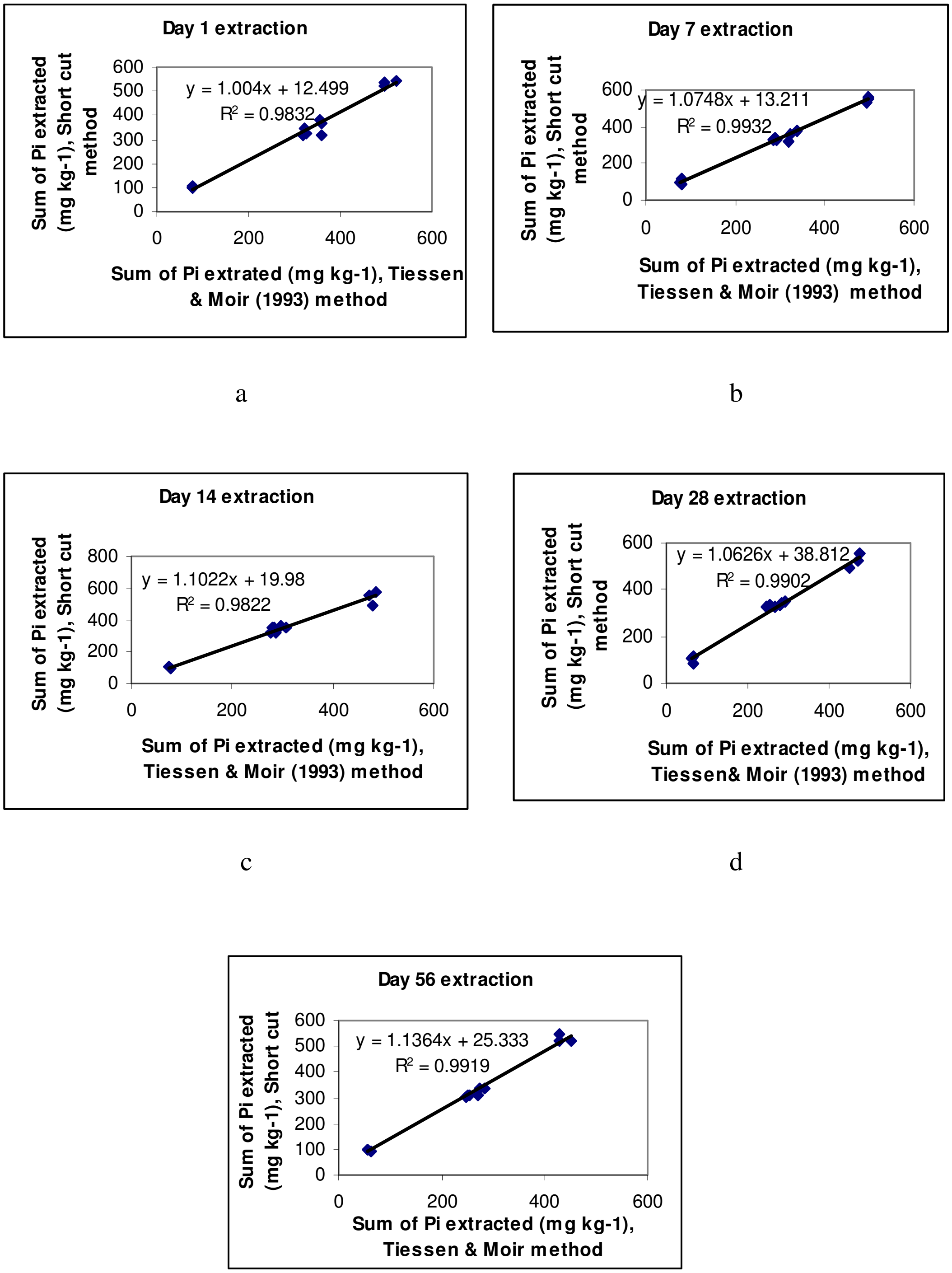
Treatment	Method	Pi fractions	Extraction time (days)				
			1	7	14	28	56
NK	Tiessen &Moir	HFO-Pi	0.08	1.05	1.07	1.08	1.13
	(1993)	HCO_3-Pi	6.33	4.11	5.46	2.29	0.88
		NaOH-Pi	17.75	16.58	15.68	14.47	11.09
		1M HCl-Pi	5.65	6.33	3.64	3.64	2.29
		C/HCl-Pi	52.3	49.78	50.31	44.48	42.63
		[♦]∑ Pi	**82.11**	**77.85**	**76.16**	**65.96**	**58.02**
	[‡]Short cut	HFO-Pi	1.48	1.55	1.58	1.62	1.74
		C/HCl-Pi	98.8	97.83	98.35	97.69	95.71
		[§]∑Pi	**100.28**	**99.38**	**99.93**	**99.31**	**97.45**
NPK	Tiessen &Moir	HFO-Pi	4.87	9.29	11.84	13.5	19.34
	(1993)	HCO_3-Pi	77	69.4	65.92	55.73	52.72
		NaOH-Pi	116.03	117.03	105.88	114.17	110.67
		1M HCl-Pi	41.75	37.97	32.86	26.67	25.67
		C/HCl-Pi	110.9	93.7	78.9	73.07	67.78
		[♦]∑ Pi	**350.55**	**327.39**	**295.4**	**283.14**	**276.18**
	Short cut	HFO-Pi	4.59	11.31	15.62	19.43	23.61
		C/HCl-Pi	350.01	349.48	338.91	323.01	315.11
		[§]∑ Pi	**354.6**	**360.79**	**354.53**	**342.44**	**338.72**
MNK	Tiessen &Moir	HFO-Pi	5.85	10.46	11.91	12.91	18.76
	(1993)	HCO_3-Pi	66.53	55.86	58.46	51.51	46.78
		NaOH-Pi	122.82	121.8	116.59	106.6	100.28
		1M HCl-Pi	29.99	26.23	25.76	24.69	23.33
		C/HCl-Pi	96.9	75.19	68.84	62.48	61.6
		[♦]∑ Pi	**322.09**	**289.54**	**281.56**	**258.19**	**250.75**
	Short cut	HFO-Pi	6.15	11.55	15.94	17.55	21.48
		C/HCl-Pi	323.67	319.5	311.45	308.67	287.13
		[§]∑ Pi	**329.82**	**331.05**	**327.39**	**326.22**	**308.61**
MNPK	Tiessen &Moir	HFO-Pi	19.83	60.72	73.33	87.62	103.47
	(1993)	HCO_3-Pi	108.5	97.84	91.25	85.9	70.17
		NaOH-Pi	167.83	160.03	154.6	150.8	145.14
		1M HCl-Pi	100.72	81.62	73.33	65.65	44.58
		C/HCl-Pi	106.7	95.31	85.87	75.72	72.54
		[♦]∑ Pi	**503.58**	**495.52**	**478.38**	**465.69**	**435.9**
	Short cut	HFO-Pi	18.87	83.8	103.97	108.85	132.81
		C/HCl-Pi	516.56	463.2	433.67	420.97	395.78
		[§]∑ Pi	**535.43**	**547**	**537.64**	**529.82**	**528.59**

[♦]∑ P_i refers the sum of all inorganic P fractions in the table above
[‡] refers the direct extraction of soil by C/HCl after the DMT-HFO extraction
[§]∑ Pi refers the sum of DMT-HFO-Pi and C/HCl-Pi

6.3.4 Plant growth as related to phosphorus extracts by DMT-HFO and C/HCl

The cumulative DMT-HFO-P_i extracted over 56 days of extraction for both approaches were correlated with maize yield (Table 6.5). Both results showed no significant correlations with yield for the same reason reported previously. The similar results observed for this fraction by both methods was as anticipated since this fraction is the initial step in both methodologies and no modification was involved in this step.

Unlike the correlation between DMT-HFO-P_i and yield, the correlation of the former with Bray was highly significant in both cases. This observation probably indicates the ability of these extractants to extract the labile P as reported previously. The amount of C/HCl-P_i was also correlated with maize grain yield. Highly significant and strong correlations were observed between maize grain yield and C/HCl-P_i both for the short cut approach ($r = 0.95^{**}$) and the method of Tiessen and Moir (1993) ($r = 0.95^{**}$). A significant correlation was also observed between maize grain yield and Bray1P ($r = 0.84^{*}$). The correlation between Bray1P and the C/HCl extracted P was stronger and highly significant for both short cut approach ($r = 0.96^{**}$) and the conventional Tiessen and Moir method (1993) ($r = 0.93^{**}$). This observation indicates that Bray-1P might have extracted P from the less labile portion too. Significant correlation between C/HCl-P_i and Bray-1P was also reported by Cajuste et al., (1994) for oxisol and alfisol soils they studied under laboratory conditions.



a

b

c

d

e

Figure 6.4 (a-d). Simple linear correlations between the Tiessen and Moir (1993) method and the short cut approach for the sum of P_i over different extraction periods

Table 6.5 Correlations between cumulative DMT-HFO-Pi and C/HCl-P_i (mg kg^{-1}) with maize grain yield (t ha^{-1}) both for the method of Tiessen and Moir (1993) and the short cut approach, N=4

	Short cut approach			Tiessen and Moir (1993) method	
	Yield	Bray 1P		Yield	Bray 1P
HFO-Pi	0.57	0.91^{**}	HFO-Pi	0.58	0.92^{**}
C/HCl-Pi	0.95^{**}	0.96^{**}	C/HCl-Pi	0.95^{**}	0.93^{*}
Bray 1P	0.84^{*}	-	Bray 1P	0.84^{*}	-

**Significant at 0.01 probability level *Significant at 0.05 probability level

6.4 CONCLUSIONS

In this chapter, we proposed a short cut method alternative to the conventional step-by-step method. The method was employed to understand the P dynamics of long-term fertilized soils especially for soils where the P pool acting as a source in replenishing the labile P is already identified. In the previous study made on the same soils the C/HCl-P_i was identified as a major source in replenishing the labile P pool. Comparison of the sum of DMT-HFO-P_i, NaHCO3-P_i, NaOH-P_i, D/HCl-P_i and C/HCl-P_i extracted by modified Tiessen and Moir (1993) method with the sum of DMT-HFO-P_i and C/HCl-P_i extracted by the short cut approach for all extraction periods resulted in a very strong and significant correlations. Correlation between C/HCl-P_i and maize grain yield was also strongly significant for both methods. This study revealed that the short cut approach could be a simplified and economically viable option to study the P dynamics of soils especially for soils where the P pool acting as a source is already identified. But data from a wider range of soils is also needed to evaluate the universality of this method.

CHAPTER 7*

Long-term phosphorus desorption using dialysis membrane tubes filled with hydrous iron oxide and its effect on phosphorus pools for Avalon soils

7.1 INTRODUCTION

The amount of P taken up by crops during the first year after their spreading in general varies from 3-33% of applied P fertilizer (Csatho et al., 2002; Aulakh et al., 2003; Zhang et al. 2004; Kamper & Claassens, 2005). Many agricultural fields that have received long-term applications of P, therefore, often contain levels of P exceeding those required for optimal crop production. Knowledge of the effect of the P remaining in the soil (residual effect) is of great importance for fertilization management.

Plant P availability of residual P in soils can be reliably estimated using successive cropping experiments carried out in field or green house studies, where P is taken up until P deficiency occurs or a response to added P is measured (Indiati, 2000). To deplete the soil P in this approach, however, takes many years (Halvorson and Black, 1985; Wagar et al., 1986). Although this approach is useful to estimate the time frame by which the residual P could be available, the process is time consuming and expensive. Besides it doesn’t indicate which P pool involves in replenishing the soil solution P. Therefore, it would be useful to have a laboratory method that would allow an estimate of phosphate desorption from the soil over time and the subsequent changes on the P dynamics that would result from successive P desorption.

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The kinetics of P release can be approximated using successive extraction of soil by ion-sink methods (Lookman et al. 1995; McKean and Warner, 1996; Indiati, 2000; De Jager and Claassens, 2005). Characterizing the residual P by employing this method could help to estimate the time frame by which these residual P could be available for plant use in a reasonably short time but lacks to indicate which P pools involve in replenishing the labile P pool.

The sequential extraction procedure developed by Hedley et al. (1982) has been applied to determine the different forms of P in the soil. Characterizing the residual P by making use of this method could solve the problem of identifying which P pool can involve in replenishing the P uptake by plants but doesn't indicate the time frame by which these residual P could be available for plant use. The problems mentioned above could be solved if the two methods are combined. Successive extraction procedures carried out by these ion sink methods combined with subsequent fractionation procedure (Hedley et al. 1982; Tiessen and Moir, 1993) termed as a *combined method* may, therefore, constitute a convenient laboratory method to investigate the kinetics of residual P release and to understand the dynamics of soil P.

Successive desorption of P by DMT-HFO followed by subsequent fractionation method as described by Hedley et al., (1982) or Tiessen and Moir (1993) have been recently employed in South Africa to study the P dynamics of incubated soils (De Jager 2002; Ochwoh et al. 2005). However, information regarding the effectiveness of this modified method on soils which have a long term fertilization history is limited. There is also a lack of information trying to relate such information with plant yield parameters. The objectives of this research were: 1) to study the changes in labile, non-labile and residual P using the combined method and 2) to investigate wich P pools contributed to the P requirements of maize for some soils with a long term fertilization history.

7.2 MATERIALS AND METHODS

7.2.1 Fertilization history and soil analyses

Topsoil samples (0-25cm) were collected from the long-term fertilizer trial initiated in 1976 by the Nooitgedacht Agricultural Development Center in Ermelo, Mpumalanga, South Africa. The experiment was conducted on an Avalon soil type. The samples were air-dried and ground to pass through a 2 mm sieve. Soil samples were collected from selected P treatments. The samples were taken from different locations of each treatment and mixed. Composite samples were used for the subsequent analyses.

The soil samples collected were differentially P treated soils. The control P_0L_0 received no P since the inception of the trial. The P_1L_1 and P_2L_1 treatments received P only for two seasons during the initiation of the trial. Double superphosphate (19.5%) was applied at the rate of 177 and 354 kg ha^{-1} in the year 1977/78 and 1979/80 for treatments P_1L_2 and P_2L_2 respectively. Potassium was band placed annually at a rate of 50 kg K ha^{-1} year^{-1} as potassium chloride (KCl). Limestone ammonium nitrate was applied annually at rates determined by the climatic conditions of the season. Since then there hasn't been any P applied to these soils despite the continuous maize production for more than 20 years. All the treatments except the control considered here were also limed to ensure the pH of the soil at an acceptable range ($pH \geq 6$)

suitable for maize production. Du Preez and Claassens (1999) have provided a detailed fertilization history of these soils. Table 7.1 shows some selected physical and chemical properties of the different treatments. The pH (KCl) of the samples was determined by dispersing 20g of dried soil in 50 ml of 1*M* KCl. After 2 h of end-over-end shaking at 20 rpm, the pH was determined in the soil suspension (Freese et al., 1995). Particle size analysis was determined by the hydrometer method after dispersion of the soil with sodium hexametaphosphate. Organic C was determined by dichromate oxidation technique while exchangeable Ca, Mg and K were determined by neutral 1 *M* ammonium acetate extraction. Total soil P (P_T) was determined on sub samples of 0.5 mg soil with the addition of 5 ml concentrated H_2SO_4 and heating to 360 ^{0}C on a digestion block with subsequent stepwise (0.5 ml) additions of H_2O_2 until the solution was clear (Thomas et al., 1967). The available phosphorus was determined using Bray and Kurtz (Bray-1P) method (0.03 *M* NH_4F + 0.025 *M* HCl). Details of analytical methods are described in Kuo (1996) and the Handbook of Standard Soil Testing Methods for Advisory Purposes (The Non-Affiliated Soil Analysis Work Committee, 1990).

7.2.2 Long-term desorption study

A long term desorption study was carried out using dialysis membrane tubes filled with hydrous ferric oxides similar to that described by Freese et al. (1995) the detail of which is presented in Section 3.2.3.

7.2.3 Fractionation procedure

Soil samples were sequentially extracted for P using Tiessen and Moir (1993) method with a slight modification made on the initial step where by the resin in the Tiessen and Moir (1993) procedure was replaced by the DMT-HFO (De Jager & Claassens 2005; Ochwoh et al. 2005). The detail of this particular step is also presented in Section 4.2.2.

7.2.4 Greenhouse experiment

A greenhouse experiment was carried out to generate dry matter yield and P uptake data for the same soil-P level combinations used in the laboratory study. Maize grain was planted and grown in pots containing 6 kg of soil for 56 days. Each pot was seeded with 6 maize grains and was thinned to 4 seedlings a week after emergence. 50 mg kg^{-1} N was applied before planting and another 50 mg kg^{-1} N was applied two weeks after emergence. Each treatment had three replicates. Shoot dry matter yield was determined at harvest, after drying fresh samples at 68 ^{0}C for 48 h in an oven. The P content of shoot dry matter was determined on 0.5 mg samples with the addition of 5 ml concentrated H_2SO_4 and heating to 360 ^{0}C on a digestion block with subsequent stepwise (0.5 ml) additions of H_2O_2 until the solution was clear (Thomas et al., 1967).

7.2.5 Data analysis

The data obtained were statistically analyzed by using Statistical Analysis System (SAS Institute 2004). Analysis of variance was done using the General Linear Model (GLM) procedure. The Tukey test was used to determine significant differences at $\alpha = 0.05$. The percent P extracted by each fraction was calculated by dividing the P extracted by the respective extractants with the total P obtained by direct determination of P and multiplying the ratio by 100%. Correlation with the plant yield parameter was done using Pearson linear correlation, PROC CORR (SAS Institute 2004).

7.3 RESULTS AND DISCUSSION

7.3.1 Percent P distribution

The average percent P extracted according to this fractionation method from treatments P_0L_0, P_1L_1 and P_2L_1 was 95.98, 99.24 and 106.32 of the total P as exhibited in Table 7.2. The different fractions/pools of P were grouped according to Tiessen and Moir (1993) as labile (DMT-HFO-P_i +$NaHCO_3$-P_i + $NaHCO_3$-Po), less labile (NaOH-P_i +NaOH-Po + D/HCl-P_i) and stable P pools (C/HCl-P_i +C/HCl-Po + C/H_2SO_4-P). According to the above classification, the percent P extracted from the stable P fractions varied from 74.93-85.67 of the total soil P. The percentage contributions of labile and less labile fractions represented 2.49-4.55 and 10.96-20.41 of the total extracted P respectively. These results showed that the largest portion of the total soil P, for all treatments, was the stable P fraction. These results concur positively with the results of Du Preez and Claassens (1999) and Ochwoh et al. (2005) carried out on some South African soils at the field and laboratory levels respectively. Similar result was also obtained for soils collected from one of the oldest long-term fertilizer trial in South Africa as reported in Section 4.3.1.

The proportion of this fraction was largest in all the treatments indicating the depletion of the more labile pools due to continuous cropping (>20 years). The fact that there was a decline of P after the 56-day extraction period indicated that the stable P pool might have contributed to the P extracted over the 56 day of extraction. Long-term application of P fertilizer changed the fractional distribution of P in the P treated soils compared to the control which becomes evident with increased amount of P. Hence, the labile and less labile fractions increased and the stable form decreased in the P treated soils. This indicated that the largest portion of the added P was transformed to the more labile P forms and less to the stable P form. However, the total P of the stable P pool also increased indicating that some of the excess applied P was transformed to the stable P pool.

The gain/loss of each fraction for all treatments between day1 and 56 days of extraction was compared as shown in Table 7.2. The gain/loss was calculated by subtracting the value of day 1 from day 56 for each fraction. The sum of the differences resulted in a value less than zero, revealing the loss of some P during the process. The percent P lost, as the result of analytical error was on average <1%. That means, on average, about 99% of the variation was resulted from P redistribution due to consecutive P extraction by DMT-HFO.

Table 7.1 Selected physical and chemical properties of the soil samples studied

Sample Types §	pH (KCl)	P_{total}	Bray-1P	Ca‡	Mg‡	K ‡	Texture			Organic C
		mg kg^{-1}					%Clay	%Silt	%Sand	%
P_0L_0	3.90	303.47	2.54	73	23	89	5.8	9.3	83.0	0.48
P_1L_1	5.40	333.83	2.26	423	74	163	9.0	6.0	82.2	0.63
P_2L_1	5.24	363.98	13.71	452	80	138	5.8	9.3	82.8	0.67

§ P_0L_0 = received no phosphorus and lime since the inception of the trial and served as a control;

P_1L_1 = treated with phosphorus and lime

P_2L_1= received both phosphorus and lime

‡Extractable Ca, Mg and K: Determined using 1 *M* Ammonium acetate at pH 7

7.3.2 Changes in inorganic P

7.3.2.1 DMT-HFO-extractable P_i

The amount of P_i extracted by DMT-HFO was significantly influenced ($P \leq 0.05$) both by the P content and extraction time (Table 7.3). The change of this fraction, however, was not significant between P_1L_1 and the control. The cumulative P desorbed was higher in the P_2L_1 treatment (0.72-5.71 mg kg $^{-1}$) and lower in the control (0.06-1.67 mg kg $^{-1}$) at all levels of extraction time (1 –56 days).

Cumulative P released with time followed, in general, the same pattern for all treatments, with an initial rapid release of P, roughly with in the first two weeks (14 days), followed by a slower release that was still continuing after 56 days of extraction though the degree of increment was very slow. This is attributed to the presence of two distinct pools of soil P, one with rapid release kinetics and the other with slower desorption kinetics (Lookman et al., 1995, De Jager and Claassens, 2005) as reported previously (Section 4.3.2.1). This can be explained by P desorbing quickly on to the surface of Fe and Al oxides, followed by relatively slow diffusion in to the matrix of sesiquioxides (Pavlatou and Polyzopoulos, 1988). No desorption maximum was reached by the end of the 56 day (1344h) period. Similar reports have also been reported by other researchers (Lookman et al., 1995; Maguire et al., 2001; Koopmans et al., 2001; De Jager and Claassens, 2005; Ochwoh et al., 2005).

Table 7.2 Phosphorus content (mg kg^{-1}) in different inorganic (P_i) and organic (Po) fractions for the differentially P treated soils

		P_0L_0[‡]		P_1L_1			P_2L_1		
P fractions	Day 1	Day 56	Difference[§]	Day 1	Day 56	Difference	Day 1	Day 56	Difference
DMT-HFO	0.06[†]	1.67	1.61	0.13	2.65	2.25	0.72	5.74	4.92
HCO_3Pi	0.89	0.49	-0.4	1.23	0.92	-0.31	7.34	3.37	-3.97
HCO_3Po	7.55	8.71	1.16	6.91	7.71	0.8	9.57	9.75	-0.18
Labile	8.50	10.87		8.27	11.01		17.63	14.79	
%Labile	**2.89**	**3.77**		**2.49**	**3.33**		**4.55**	**3.93**	
OH-Pi	5.97	6.65	0.68	6.32	8.15	1.83	29.69	32.3	2.61
OH-Po	28.6	24.67	-3.93	30.83	30.3	-0.53	34.22	34.9	0.68
1M HCl-Pi	0.27	0.3	0.03	2.21	1.31	-0.9	15.55	8.75	-6.8
Less-labile	34.84	31.62		39.36	39.76		79.46	75.95	
%Less-labile	**11.84**	**10.96**		**11.84**	**12.04**		**20.52**	**20.41**	
C/HCl-Pi	21.61	21.32	-0.29	33.65	24.33	-9.32	48.9	37.15	-11.75
C/HCl-Po	3.26	3.68	0.42	9.99	16.06	6.07	7.99	13.96	5.97
C/H_2SO_4-P	225.93	220.54	-5.39	241.19	238.98	-2.21	233.34	230.34	-3
Stable	250.8	245.54		284.83	279.37		290.23	281.45	
%Stable	**85.27**	**85.13**		**85.67**	**84.62**		**74.93**	**75.62**	
$\sum P_i$+Po	294.14	288.42		332.46	330.14		387.32	376.16	
[◆]P_{total}	303.47			333.83			363.98		
($\sum P_i$+Po)/ P_{total} (%)	96.93	95.04	-1.89	99.59	98.89	-0.7	106.41	106.22	-0.19

[§] Values are cumulative P differences between 56 days and 1 day of extractions for the different P fractions (mg kg^{-1}), total P extracted (mg kg^{-1}), percent P recovered, negative values signify decreases and positives, increases

[†] Mean values of three replicates

[‡] Plots treated with different amount of P [◆] Total P obtained by direct determination of P

The percentage distribution of DMT-HFO-P_i fraction ranged from 0.02 –0.58, 0.04 – 0.80 and 0.19 – 1.54 for PoLo, P_1L_1 and P_2L_1 treatments respectively from day 1 to 56 days of extraction time (calculated from table 7.2). The percent P extracted in all cases was very low as compared to the total P. In this regard, the results are found to be similar to the previous experiments as reported in Chapter 4. Similar results have also been reported by other researchers (Koopmans et al., 2001; De Jager and Claassens, 2005; Ochwoh et al. 2005). In this study the last time the soils received any P was in the season 1979/80, which means the soils were incubated on average for nearly 25 years. Cropping did continue after P application discontinued, which means, at the same time, that P in the soil was also depleted. It was therefore expected that, as a result of the longer equilibration time and P depletion, the easily available P would be lower.

7.3.2.2 0.5M $NaHCO_3$- extractable P_i

The amount of P_i extracted by 0.5M $NaHCO_3$ was significantly influenced ($P \leq 0.05$) both by the P content and extraction time (Table 7.3). The effect of P level on this fraction, however, was not significant between P_1L_1 and the control. The temporal change of the 0.5M $NaHCO_3$ extractable $P_{i,}$ as the result of successive DMT-HFO extraction, was also not statistically significant for treatments PoLo and P_1L_1. The amount of this fraction ranged from 0.89-0.5, 1.23 –0.92, and 7.34-3.37 mg kg^{-1} between 1 and 56 days of extraction for PoLo, P_1L_1 and P_2L_1 treatments respectively. This fraction decreased with increasing time of extraction (Table 7.3). Ochwoh et al., (2005) and De Jager (2002) also reported similar results for some South African soils, which have been incubated for 6 and 5 months respectively and subjected to the same successive extraction by DMT-HFO from 1-56 days. The reduction in this fraction was more pronounced in plots where relatively high P was added than the control. This result is in agreement with Du Preez and Claassens (1999) made on the same soils at a field level. According to this study, the resin extract was replaced by the DMT-HFO and it is presumed that the P extracted by both DMT-HFO and $NaHCO_3$ was assumed to represent the plant available (labile) P (Ochwoh et al., 2005). The labile fraction accounted for a small percentage of the total soil P taken by the plants. This suggests that the less labile fractions have also contributed to the P taken up by the plants.

The percentage distribution of this fraction was 0.30-0.16, 0.37-0.28, and 1.89-0.90 for treatments PoLo, P_1L_1 and P_2L_1 respectively from day 1 to 56 days of extraction time. Du Preez and Claassens (1999) reported that the percentage contribution of this fraction to be in the range from 4.3 to 8.8%. The reason for the much lower fractional contribution in this study revealed the depletion of this pool as the result of continuous cropping.

7.3.2.3 0.1M NaOH- extractable P_i

The changes in 0.1M NaOH extractable P_i after the successive DMT-HFO extraction showed significant difference ($P \leq 0.05$) due to the influence of applied P and extraction time (Table 7.3). However, the effect of P level was not significant between PoLo and P_1L_1. Besides, temporal change of this fraction showed no significant difference for the control. This fraction decreased until the 14th day and increased at the later time of extraction, the amount extracted being nearly the same between day 1 and 56 days of extraction for PoLo and P_1L_1. This finding was contrary to the results obtained by De Jager (2002), Ochwoh et al. (2005) and Section 4.3.3.1 of this study. They observed a consistent decreasing trend with increased extraction time for some South African soils and subjected to successive desorption by DMT-HFO between 1 and 56 days of extraction. The reason for this anomaly could be attributed to the replenishment of this fraction from the more resistant pools such as C/HCl-P_i as this is the fraction that decreased most according to this study.

The percentage distribution of this fraction was 2.03-2.30, 1.90-2.46 and 7.67-8.68 for treatments PoLo, P_1L_1 and P_2L_1 respectively. PoLo and P_1L_1 resulted in a similar amount of extractable NaOH-P_i. The percentage distribution of this fraction was in general very low as compared to the results reported in Section 4.3.3.1. De Jager (2002) reported that the 0.1M NaOH extractable P_i was ranged from approximately 15-16% of the total P for control and the high P incubated soils after 1 day and 56 days of extraction by DMT-HFO. In a similar work done by Ochwoh et al., (2005), the percentage of this fraction ranged from 12-14% after 1 day and 56 days of extraction by DMT HFO for the control and high P incubated soil. The lower fractional contribution in this study could be the inherently lower inorganic fractions due to P depletion over time and transformation of P in to more stable forms due to long equilibration time.

Table 7.3 Effect of P levels and extraction time on soil P desorption

P fractions (mg kg^{-1})	Treatment	Extraction time (days)					
		1	7	14	28	42	56
HFO-P_i	PoLo	[‡]x0.06[†]a[§]	x0.12a	x0.93ab	x1.04ab	x1.41b	x1.67b
	P_1L_1	x0.13a	x0.23a	x1.33b	x1.50bc	x2.53c	x2.65bc
	P_2L_1	x0.72a	y1.72a	y3.63b	y4.66bc	y5.54c	y5.71c
LSD $_{(0.05)}$ = 1.12, CV = 18.67							
HCO3-P_i	PoLo	x0.89a	x0.73a	x0.6a	x0.53a	x0.53a	x0.5a
	P_1L_1	x1.23a	x1.19a	x1.02a	x0.73a	x0.57a	x0.92a
	P_2L_1	y7.34a	y6.19b	y4.43c	y4.45c	y4.45c	y3.37d
LSD $_{(0.05)}$ = 0.98, CV = 14.48							
OH-P_i	PoLo	x5.97a	x5.78a	x5.61a	x5.47a	x6.57a	x6.65a
	P_1L_1	x6.32a	x5.99a	x5.61a	y8.6b	x8.11ab	X8.51a
	P_2L_1	y29.67ab	y28.61a	y27.41a	z32.26b	y33.3b	y32.31ab
LSD $_{(0.05)}$ = 2.70, CV = 6.07							
D/HCl-P_i	PoLo	x0.26a	x0.31a	x0.31a	x0.45a	x0.36a	x0.31a
	P_1L_1	y2.21a	y2.24a	y2.25a	x2.07a	x1.39a	x1.31a
	P_2L_1	z15.55d	z12.61c	z10.49b	z9.94ab	z9.28a	z8.75a
LSD $_{(0.05)}$ = 1.83, CV = 13.44							
C/HCl-P_i	PoLo	x21.61a	x28.01a	x30.63a	x21.47a	x20.81a	x20.31a
	P_1L_1	y33.65b	x29.02a	xy33.7b	x24.02a	x27.88a	x23.33b
	P_2L_1	z48.9b	y40.08a	y40.43a	y40.33a	y38.99a	y37.15a
LSD $_{(0.05)}$ = 9.08, CV = 9.52							
HCO_3-Po	PoLo	x7.55b	x6.39b	x5.91b	y11.04a	x5.77b	x8.71ab
	P_1L_1	x6.91ab	x5.92ab	x4.63b	x7.15ab	xy7.31ab	x7.72a
	P_2L_1	x9.57a	x9.24a	x8.36a	xy9.75a	y9.62a	X9.75a
LSD $_{(0.05)}$ = 2.97, CV = 12.69							
OH-Po	PoLo	x28.60b	x28.88b	x19.63a	y28.58b	x22.79a	x24.67ab
	P_1L_1	xy30.83b	y36.72c	xy24.12a	y33.97bc	z42.93d	x30.30b
	P_2L_1	y34.22c	x26.69b	y25.35ab	x20.30a	y30.38bc	y34.89c
LSD $_{(0.05)}$ = 5.60, CV = 6.28							
C/HCl-Po	PoLo	x3.26a	x4.61a	x4.03a	x6.21a	x6.02a	x3.55a
	P_1L_1	x9.99ab	y17.18b	xy8.21a	x12.31ab	y21.37b	y16.05b
	P_2L_1	x7.99a	z27.20b	y14.44a	x13.31a	y23.04b	y13.95a
LSD $_{(0.05)}$ = 7.58, CV = 20.96							
C/H_2SO_4-P_i	PoLo	x225.93a	x214.58a	x206.73a	x218.95a	x212.4a	x220.74a
	P_1L_1	x241.18a	y244.71a	y232.47a	x239.07a	x233.67a	x238.29a
	P_2L_1	x233.34a	x228.83a	y239.54a	x225.92a	x233.34a	x230.48a
LSD $_{(0.05)}$ = 25.15, CV = 3.59							

[†] Mean values of three replicates

[§] Mean values in rows with different letters a, b, c, d and e are significantly different ($\alpha = 0.05$)

[‡] Mean values in columns with different letters x, y, z and w are significantly different ($\alpha = 0.05$).

7.3.2.4 1M HCl- extractable P_i

This fraction also showed a significant difference ($P \leq 0.05$) with respect to variations in P levels and extraction time with DMT-HFO (Table 7.3). Extraction time did not influence significantly the extractable P_i for both PoLo and P_1L_1 treatments. However, the effect of P level on the amount of extractable 1M HCl-P_i was significant between PoLo and P_1L_1 though only for the first 14 days. This fraction represents the apatite-type (Ca-bound) minerals (Ottabong & Persson, 1991; Hedley et al., 1982) in the soil and the reason for the significant difference of this particular fraction between PoLo and P_1L_1 could be attributed to the difference in the pH between these two treatments resulted from liming as shown in Table 7.1. In all treatments the 1M HCl-extractable P_i decreased with time of successive extraction by DMT-HFO and the effect of time on the extractability of this fraction was more pronounced on the treatment with high P content (P_2L_1).

The percent 1M HCl-P_i extracted ranged from 0.09-0.11, 0.66-0.39 and 0.04-0.02 for PoLo, P_1L_1 and P_2L_1 respectively. The contribution of this fraction is on average <1% for all treatments. This is in consonant with the results of Du Preez and Claassens (1999). They reported <1% contribution of this fraction to the total P for the same soil done previously. While other similar studies revealed 5-8% contribution of this fraction to the total P (Hedley et al., 1982; Sattell and Morris, 1992; Ochwoh et al. 2005). The percent of this fraction was also reported to be about 6% for the soils considered in the previous experiment (Section 4.3.3.3).

7.3.2.5 C/HCl-extractable P_i

The change in concentrated HCl extractable P_i after successive DMT-HFO-extraction showed a significant difference ($P \leq 0.05$) both with respect to applied P levels and extraction time (Table 7.3). The amount extracted by this extractant (mg kg $^{-1}$) varied from 21.61-20.31, 33.65-23.33 and 48.90-37.15 for PoLo, P_1L_1 and P_2L_1 respectively after day 1 and 56 days of extraction. The C/HCl-P_i is the fraction that decreased most especially in the high P treatments indicating that this fraction contributed significantly to the P extracted by DMT-HFO. This suggests that this fraction may be a buffer to more labile P fractions. The P sources that act as a buffer for soil available P varied from soil to soil and include: organic P (Zhang and Mackenzi, 1997b), NaOH-P_i for soils receiving repeat applications from fertilizer and/or manure (Schmidt et al., 1996; Zhang and Mackenzi, 1997b; Guo et al., 2000) and HCl-P and/or residual P (Guo et al., 2000). Most studies made on highly weathered tropical soils revealed the importance of NaOH-P_i in replenishing the labile P fractions (Du Preez and Claassens, 1999; Ochwoh et al., 2005; De Jager and Claassens, 2005). The present investigation, however, resulted contrary to the above reports but positively concurs with the report of Araujo et al. (2003). The latter researchers reported the importance of acid P (equivalent to C/HCl-P in our study) in replenishing the labile P fractions for latosols. The reason for this apparent contrast especially as compared to the previous report made on the same soil by Du Preez and Claassens (1999) could be the shifting of the source of P fraction from the NaOH to the C/HCl fraction resulted from exhaustion of the former due to continuous cropping for over 20 years.

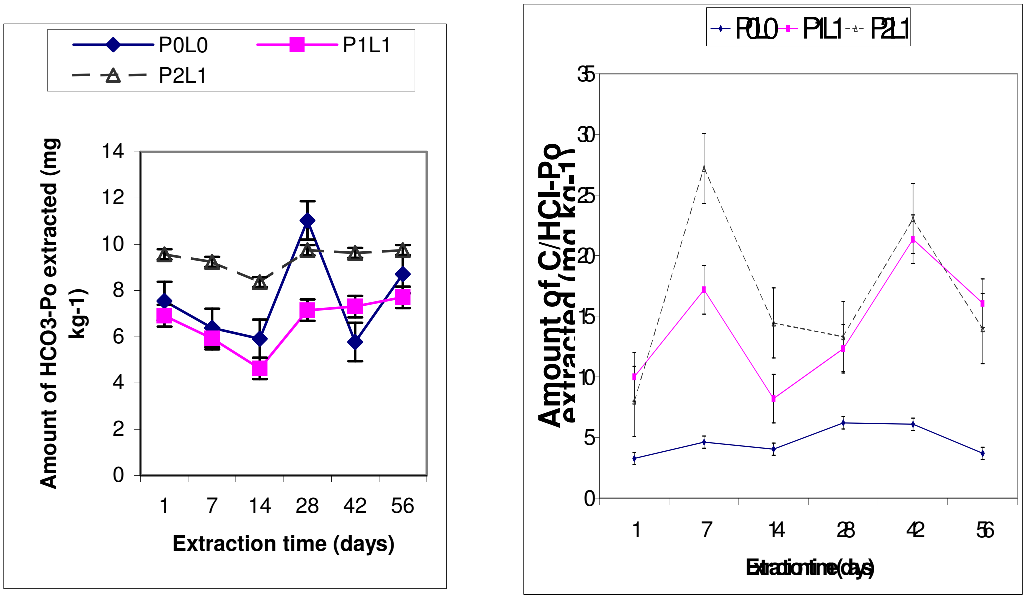
As an average of all extraction time, the percent C/HCl-P_i constituted 7.32, 8.75 and 11.31 for PoLo, P_1L_1 and P_2L_1 respectively. The contribution of this fraction is on average 9.12% for all treatments. The average percentage contribution of this fraction was reported to be about 12% for the soils investigated in Section 4.3.4.1. Ochwoh (2002) reported between 15-25% contribution of this fraction to the total P for Loskop and Rustenburg soils of South Africa. The contribution of this fraction is relatively lower in this study possibly because of the long equilibration time as opposed to the literature reports made on P incubated soils.

7.3.3 Changes in organic P

7.3.3.1 0.5M HCO_3-extractable P_o

The change in the 0.5M $NaHCO_3$-extractable organic P after successive DMT-HFO extraction was significant for all treatments ($P \leq 0.05$). The effect of P level variation on the extractability of this fraction was not significant between the control and P_1L_1 (Table 7.3). The change of this fraction with time showed a similar pattern for the different treatments (Figure 7.1a) despite some irregularities. The amount extracted decreased with increasing time of extraction up to the 14th day but increased at the latter time of extraction .The increased extractable Po after 14 days successive extraction by DMT-HFO could probably be attributed to microbial immobilization of P (Stewart and Tiessen, 1987).

The percentage distribution of HCO_3-extractable P_o was 2.56-3.02, 2.08-2.34 and 2.47-1.55 for PoLo, P_1L_1 and P_2L_1 respectively between 1 day and 56 days of extraction. As an average of all extraction time and P levels, the percent 0.5M NaHCO3- extractable P_o was about 2.34. Hence, the percentage contribution of this fraction to the total P was generally very low and in consonant with the results of Du Preez and Claassens (1999) and Ochwoh et al. (2005) and the results obtained for the soil collected from the long-term fertilized trial mentioned in the previous experiment (Section 4.3.2.3).



a

b

Figure 7.1 a-b: The change in extractable (a) HCO_3-Po and (b) C/HCl-Po over time. The values in the figures are means of three replicates. Vertical bars represent the standard error

7.3.3.2 0.1M NaOH-extractable P_o

The change in the 0.1M NaOH-extractable P_o showed a significant difference ($P \leq 0.05$) with respect to changes in P levels and extraction time (Table 7.3). The amount of this fraction ranged from 28.60- 24.67, 30.83-30.30 and 34.22-34.89 mg kg $^{-1}$ for PoLo, P_1L_1 and P_2L_1 respectively after 1 day and 56 days of extraction by DMT-HFO. This fraction is the second largest fraction for the control and the third largest fraction for P received plots. There were significant increases in extractable NaOH Po due to increasing of P application compared to the control. In all treatments the OH-P_o extracted increased with time of extraction. The reason for the increased amount of this fraction could be due to microbial immobilization of P (Stewart and Tiessen, 1987).

The percentage distribution of NaOH-extractable P_o was 9.72-8.55, 9.27-9.17 and 8.84-9.37 for PoLo, P_1L_1 and P_2L_1 respectively between 1 day and 56 days of extraction. There seemed to be no big difference on the percent recovery of this fraction from P treated soils as compared to the control. Averaged over all extraction time and treatments, the contribution of this fraction to the total P was 9.15%. The percentage contribution of this fraction from the previous experiment was found to be about 11% (Section 4.3.3.2). Du Preez and Claassens found 12.1% and 9.2% contribution of this fraction to the total P for Avalon and Clovelly soils respectively. On a similar study Ochwoh et al. (2005) reported 6.31% and 5.39% contribution of this fraction for two soils having different P fixing capacity from South Africa. Hedley et al. (1982) however reported an average of 15% contribution of this fraction to the total P.

7.3.3.3 C/HCl-extractable P_o

The change in concentrated HCl extractable P_o as the result of successive DMT-HFO-extraction showed a significant difference ($P \leq 0.05$) with respect to P levels and extraction time (Table 7.3). The amount extracted by this extractant (mg kg $^{-1}$) varied from 3.26-3.55, 9.99-16.05 and 7.99-13.95 for treatments PoLo, P_1L_1 and P_2L_1 respectively after 1 day and 56 days of extraction. This fraction showed a general increasing trend with increased extraction time despite some fluctuations in between (Figure 7.1b). The reason for this inconsistency could be due to microbial immobilization and mineralization that may be induced during prolonged desorption process (Barros et al., 2005).

Averaged over all extraction time and treatments, the contribution of this fraction to the total P was 2.67%. Du Preez and Claassens (1999) reported 6.4-8.5% contribution of this fraction to the total P on a similar experiment made on these same soils. The reason for decreased contribution of this fraction in the present study is the long equilibration time and continuous cultivation as reported before. In the previous experiment the fractional contribution of this fraction was reported to be about 4.2% (Section 4.3.4.2). Ochwoh et al. (2005) reported 2-4% contribution of this fraction to the total P. The C/HCl-P_o extracted by Hedley et al. (1982) was also found to be 3%. Bashour et al. (1985) however reported 26.7% contribution of this fraction to the total P.

7.3.4 C/H_2SO_4 + H_2O_2 -extractable P

This fraction showed no statistically significant difference with extraction time. However, the decrease in this fraction with increased time of extraction indicates that it might contribute very little to the labile P pool. This fraction was the largest fraction of all fractions for both the control and P treated soils. Similar reports have been made by Du Preez and Claassens (1999) carried out on the same soils and Clovelly soils too. Percentage contribution of this fraction was found to be larger in the present study as the result of P transformation to the most refractory form due to the long equilibration time and also due to the exhaustion of the labile and less labile P pool due to continuous cropping.

7.3.5 Plant growth as related to phosphorus fractions

The amount of P extracted by the different extractants (including total P) was correlated with dry matter yield and plant P uptake as illustrated in Table 7.4. This comparison was made between the different P extracts extracted after 56 days of extraction by DMT-HFO and maize yield. Comparison was also made between Bray1P and maize yield. Highly significant correlations were observed between dry matter yield and the P pools extracted by HFO-P_i (0.997^{**}), HCO_3-P_i ($r = 0.994^{**}$), OH-P_i ($r = 0.969^{**}$), OH-Po (r = 0.944**), D/HCl-P_i (0.991^{**}) and C/HCl-P_i (r = 0.997**). Strongly significant correlations were also observed between the different P fractions and plant P uptake (Table 7.4). In general

Table 7.4 Correlation among the cumulative P desorbed over 56 day period, the subsequent fractions shoot dry matter yield, P uptake and Bray 1P; N=3

P fractions	Dry matter yield	P uptake	Bray 1P
		mg kg^{-1}	
HFO-P_i	0.997**	0.999**	0.982**
HCO_3-P_i	0.994**	0.999**	0.988**
HCO_3-P_o	0.728	0.778	0.883*
OH-P_i	0.969**	0.985**	0.999**
OH-P_o	0.944**	0.916*	0.823*
D/HCl-P_i	0.991**	0.998**	0.982**
C/HCl-P_i	0.997**	0.999**	0.982**
C/HCl-P_o	0.574	0.511	0.338
C/H_2SO_4	0.304	0.231	0.042
Total P	0.304	0.231	0.042
Bray 1P	0.965**	0.982**	-

*Significant at 0.05 probability level **Significant at 0.01 probability level

the correlation of the different P fractions with P uptake was better than the dry matter yield. However, results contrary to this finding was reported in the previous experiment (Section 4.3.5) carried out at the field level especially for the DMT-HFO-P_i. Cajuste et al., (1994) reported strong and significant correlations among the different P fractions, dry matter yield and plant P uptake for a green house experiment carried on oxisol and alfisol soils planted with maize. They found a strong correlation between dry matter yield and the fractions: resin-P_i, HCO_3-P_i, D/HCl-P_i, Residual P and total P. The correlation between P fractions and plant P uptake was also found to be significant with all except sonicated inorganic hydroxide P. Similar reports were also reported by Vazquez et al., (1991) on soils with long-term cultivation and significant correlations were obtained among the fractions resin-P_i, HCO_3-P_i, HCO_3-Po and OH-P_i, with both dry matter and P uptake.

A significant correlation was also observed between Bray1P and dry matter yield ($r=0.965^{**}$) and plant P uptake ($r=0.982^{**}$). The correlation between the different P fractions and Bray1P was very strong and significant for all fractions except C/HCl-Po, C/H_2SO_4 and total P. Cajuste et al., (1994) also reported highly significant correlations between Bray-1P and the different P fractions for oxisol and alfisol soils they studied under laboratory conditions.

7.4 CONCLUSIONS

In the present study the involvement of the labile and non-labile P_i fractions in replenishing the solution P_i was significant except the residual fraction. The organic fraction appeared to have limited contribution in replenishing the solution P at least for the duration of the experiment considered in the present study. The amount of P extracted by the different fractions in general followed the order P_2L_1>P_1L_1>P_oL_o. P_1L_1 and P_oL_o

showed significant difference on the organic and residual fractions. From the inorganic fractions only D/HCl-P_i showed significant difference between these two treatments. The C/HCl-P_i is the fraction that decreased most especially for the high P treatments indicating that this fraction contributed significantly to the P extracted by DMT-HFO. This suggests that this fraction may be a buffer to more labile P fractions instead of NaOH-P_i. Correlation between the different fraction and maize yield was highly significant for most of the P pools. The combined method we employed here can act as an analytical tool to approximate successive cropping experiments carried out under green house conditions. But data from a wider range of soils is also needed to evaluate the universality of this method both at the green house and field levels.