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Declaration

I, Abi Taddesse Mengesha, hereby declare that this dissertation for a PhD degree at the University of Pretoria is my own work and has never been previously submitted by myself at any other university

Abi Taddesse Mengesha

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DEDICATION

To my wife, Rahel, with much love, admiration and appreciation.

Characterizing phosphate desorption kinetics from soil: An approach to predicting plant available phosphorus

By

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ABSTRACT

Many agricultural fields that have received long-term applications of P 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. In order to characterize P forms in soils, a wide variety of methods have been proposed. The use of dialysis membrane tubes filled with hydrous ferric oxide (DMT-HFO) has recently been reported as an effective way to characterize P desorption over a long-term in laboratoty studies. However, there is little information on the relationship between kinetics of P release using this new method and plant P uptake. This method consist of a procedure of shaking a sample for a long period of time there by exploiting the whole volume of the soil which is in contrast to the actual plant mode of uptake. This method has also practical limitations in employing it for a routine soil analysis, as it is very expensive and time consuming. The objectives of this study were (i) to study the changes in labile, non-labile and residual P using successive P desorption by DMT-HFO followed by a subsequent fractionation method (combined method) (ii) to assess how the information gained from P desorption kinetic data relates to plant growth at green house and field trials

(iii) to investigate the effect of varying shaking time on DMT-HFO extractable P and (iv) to propose a short cut approach to the combined method.

The release kinetics of the plots from long term fertilizer trials at the University of Pretoria and Ermelo were studied. P desorption kinetics were described relatively well by a two-component first-order model ($R^2 = 0.947$, 0.918, & 0.993 for NPK, MNK, & MNPK treatments respectively). The relative contributions of both the labile pool (SP_A) and the less labile pool (SP_B) to the total P extracted increased with increased P supply levels. Significant correlations were observed between the rate coefficients and maize grain yield for both soil types. The correlation between the cumulative P extracted and maize yield ($r = 0.997^{**}$) however was highly significant for Ermelo soils.

This method was also used to determine the changes in the different P pools and to relate these P fractions with maize yield. Highly significant correlations were observed between maize grain yield and the different P fractions including total P. In both soil types the contribution of both the labile and non-labile inorganic P fractions in replenishing the solution P_i was significant where as the contributions from the organic fractions were limited. The C/HCl-P_i is the fraction that decreased most in both cases as well.

Investigation was carried out to evaluate the effect of varying shaking periods on the extractable DMT-HFO-P_i for UP soils of varying P levels. Four shaking options were applied. Significant difference was observed for the treatment of high P application. Shaking option 2 seemed relatively better than the others since it showed the strongest correlation. Thus for soils with high releasing kinetics and high total P content,

provided that the P release from the soil is a rate limiting step, reducing the length of shaking time could shorten the duration one needs to complete the experiment with out influencing the predicting capacity of the methodology.

The other objective of this thesis was also to present a short cut method alternative to the combined fractionation method. Comparison of the sum of DMT-HFO-P_i, $NaHCO_3$-P_i, NaOH-P_i, D/HCl-P_i and C/HCl-P_i extracted by a conventional step-by-step method with the sum of DMT-HFO-P_i and a single C/HCl-P_i extraction as a short cut approach for all extraction periods resulted in strong and significant correlations. The C/HCl-P_i fraction extracted by both methods was correlated with maize grain yield and it was found to be highly significant. This study revealed that this 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 in replenishing the labile portion of P is already identified.

The method employed here therefore could act as an analytical tool to approximate successive cropping experiments carried out under green house or field condition. However, data from a wider range of soils is needed to evaluate the universality of this method. More work is also required in relating desorption indices of this method with yield parameters especially at field level.

Key words: desorption of P, dialysis membrane tubes, phosphorus, phosphorus dynamics, phosphorus fractionation, phosphorus release rate, shaking time optimization, short cut methodology, soil test methods, successive desorption of P, two component first order model

CHAPTER 1

GENERAL INTRODUCTION

Phosphorus is commonly a limiting nutrient for plant growth in many soils arround the world (McDowell and Stewart, 2006). The amount of available soil P has been more frequently evaluated than the rate of its release when studying the P nutrition of plants. The availability of a nutrient to plants depends, among others, on the rate at which it is released to replenish the soil solution (Raven and Hossner ,1994). There can be a significant residual effect due to desorption of phosphate from the soil of long term fertilization history and this can lead to an underestimation of the benefit of phosphate fertilizer if not taken in to account (Mckean and Warren, 1996).

Soil tests for plant available P are used world wide to determine the current P status of soils so as to estimate fertilizer P requirements for specific yield goals. The current P status is due to indigenous (native) P present in the soil and P from previous fertilizer P application (residual P) (Indiati, 2000). Since the actual plant available P is composed of solution P plus P that enters the solution as the result of desorption/dissolution from a solid phase, the conventional soil test methods have been unsatisfactory in predicting the plant P uptake (Beck and Sanchez, 1994).

Plant P availability of residual P in soils can be reliably estimated by successive cropping experiments carried out in field or green house conditions, where P is taken up until P deficiency occurs or a response to added P is measured (Indiati, 2000). As this approach is very expensive and time consuming, soil extractions with P sink methods have been proposed to estimate residual P. Contrary to the conventional soil

P test methods , these P-sink methods may be considered nondestructive methods as they do not react with soil and have minimal effect on the soil physicochemical properties that influence the release of P. Furthermore, extraction with these sink methods prevents solution P from increasing to levels where further P release is prohbited and hence one can make a series of extractions from a soil sample (Indiati, 1998, Mckean and Warren, 1996). Consecutive extraction of soils by these methods may therefore be a convenient laboratory method to characterize the capacity of soil to supply P, and to investigate the kinetics of residual P release. Such methods use anion exchange resins (Abrams and Jarrel, 1992), iron oxide impregnated paper strips (Indiati, 2000) or dialysis membrane filled with hydrous ferric oxide solution (DMT-HFO) (Freese et al., 1995; Lookman et al., 1995; Koopmans et al., 2001) Characterizing the residual P by employing these methods could solve the time frame by which these residual P become 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) and modified by Tiessen and Moir (1993) 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 involves in replenishing the P uptake by plants but doesn't indicate the time frame by which these residual P become available for plant use. The problems mentioned in this and the above paragraph could be alleviated if the two methods mentioned above are combined. Thus, successive extraction procedures carried out by these ion sink methods combined with subsequent fractionation procedure (Hedley et al. 1982; Tiessen and Moir, 1993) hereafter 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. This combined method simulates the successive cropping experiment carried out either in the field or green house conditions. In addition to this, it indicates which P pool serves as a major source for buffering the solution P depleted as the result of continuous desorption.

This combined method has been recently employed in South Africa to study the desorption kinetics and P dynamics of incubated soils. De Jager and Claassens (2005) investigated the desorption kinetics of residual and applied phosphate to red sandy clay soils. They reported that no desorption maximum was reached after 56 days of shaking revealing that desorption could possibly continue for a longer period. They also reported that application of P increased the desorption rate of P from the labile pool (SP_A) where as the P applied had less impact on the desorption rate of P from the less available pool (SP_B). In the same study De Jager (2002) reported 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. Ochwoh et al. (2005) also studied the chemical changes of applied and residual phosphorus (P) in to different pools in two soils [Alfisols], a red sandy clay soil [Haplo-Palcustafs] and a red sandy loam soil [Pale-Xerults] after P application and incubation using the same procedure. 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.

However, there is little information on the relationship between kinetics of P release using this new method and plant P uptake for soils with long-term fertilization history.

Methods like this follow the procedure of shaking for a long period of time there by exploiting the whole volume of soil. However plants exploit only a limited amount of the soil volume ranging from 3-4 % (Kamper and Claassens 2005). The other problem with regard to this is its impracticality to use it for a routine soil analysis, as it is very expensive and time consuming. Accordingly, the objectives of this study were:

i) To determine the desorption characteristics of soils of long-term fertilization history using successive DMT-HFO extraction method

ii) To assess how the information gained from P desorption kinetic data relate to plant growth at green house and field trials

iii) To study the changes in labile, non-labile and residual P using successive P desorption by DMT-HFO followed by a subsequent fractionation method (combined method)

iv) To investigate the effect of varying shaking time on DMT-HFO extractable P.

v) To propose a short cut approach to the combined method.

CHAPTER 2

2. LITERATURE REVIEW

Phosphorus deficiency in soils is a wide spread problem in the world (Harrison 1987). It is believed to be the second most important soil fertility problem through out the world next to nitrogen (Warren 1992) and often the first limiting element in acid tropical soils (Buehler et.al., 2002).

Also in the Sub-Saharan Africa, P is a limiting nutrient in many soils of the semi-arid tropics and in acid, highly weathered soils of the sub-humid and humid tropics (Buresh et al., 1997). Oxisols and andisols are major soils in the sub-humid and humid tropics of Africa (Deckers, 1993) and are characterized by low total and available P content and high P retention capacity (Friesen et al.,1997). In addition, andepts and oxisols have a high P fixation capacity (Sanchez and Uehera, 1980).

In acid soils, P is fixed in to slightly soluble forms of precipitation and sorption reaction with Fe and Al compounds as well as crystalline and amorphous colloids (Sanchez and Uehera, 1980). Phosphorus sorption was highly correlated with the clay and total free Fe-oxide contents extracted by Dithionite-Citrate-Bicarbonate (DCB) in ultisols and alfisols derived from the savanna and rainforest zones of West Africa (Juo and Fox, 1977). Arudino et al., (1993) found that sorption capacity of acidic alfisols from South Africa were highly correlated with the DCB extractable iron oxides and with amorphous Fe and Al oxide content (Oxalate extractable). Based on P sorption isotherms for 200 soils from West, East and South Africa, Warren (1992) concluded that fertilizer requirements tend to follow the order andisols> oxisols> ultisols>

alfisols> entisols. With the exception of andisols, there is, in general, a direct relationship between P sorption by soils and the surface area of Fe and Al oxides. Clay content in soils also affects P sorption. For example, millet producing soils of West Africa in the Sudano-Sahellian agro-ecological zone are generally sandy in texture, have a low sorption capacity and only need low to medium inputs of P to maintain an adequate pool of labile P (Manu et al., 1991).

In calcareous alkaline soils, solid-phase $CaCO_3$ is the dominant factor affecting P availability. Data for 19 soils from different agricultural areas of West Asia and North Africa showed that $CaCO_3$, Fe-oxides, amount and reactivity of silicate clays as well as P fertilizer addition rate and time after application affect the availability of P in calcareous soils (Afif et al., 1993). Iron oxides particularly the more reactive forms have a modifying influence on P fractions in calcareous soils, despite the dominant influence of $CaCO_3$ (Ryan et al., 1985). With 20 calcareous soils in the USA, Sharpley et al., (1984) found a negative correlation between labile P and $CaCO_3$ content after six months of incubation.

2.1 Sorption and desorption of phosphorus

Phosphorus sorption is the removal of labile P from the soil solution, due to the adsorption on, and absorption into the solid phases of the soil, mainly on to surfaces of more crystalline clay compounds, oxihydroxides, or carbonates (Hollford and Mattingly, 1975). The term “labile P” is commonly used to represent mobile P, which is available (or rapidly becomes available by reactions with fast kinetics) as a nutrient for plant growth, including soluble P and that which has been deposited by the slow reaction (which is not readily available) (McGechan and Lewis, 2002). Although soil P sorption has been studied intensively, relatively less has been done on the P desorption in soils and sediments. Desorption refers to the release of P from the solid phase in to the solution phase. Desorption occurs in soils when plant uptake depletes soluble P concentrations to very low levels, or in an aquatic system when sediment – bound P interacts with natural waters with low P concentrations (Pierzynski et al., 1994). Interest in P desorption studies are rising due to the importance of P on soil fertility and pollution (Sharpley, 1985). Intensive animal husbandry in Europe has led to the production of large amounts of animal manures, and the disposal of manures on the agricultural land have led to increased soil P tests (Gerke, 1992). Many soils have become saturated and contributed to surface water eutrophication (Sharpley, 1985; Mozaffari and Sims, 1994; Penn et al., 1995; Sharpley, 1996; Pote et al., 1998). Similar problems also occur where sewage sludges has been disposed on land (Gerke, 1992; Sharpley and Sisak, 1997).

2.2 P sorption and desorption rates

Phosphorus sorption capacity is an important soil characteristic that affects the rate and plant response to P fertilizer application. (Fox and Kamprath, 1970; Hollford and Mattingly, 1975). Phosphorus sorption by soils is usually rapid at first but then slows with time (Dimirkou et al., 1993). The initial fast P sorption rates are presumably due to reaction with surface sites of metal oxides or hydroxide particles that are exposed to the solution phase. Slow P sorption that continues after the initially rapid sorption is ascribed to the slow diffusion in to the soil aggregates (Willet et al., 1988), or due to the slow formation of P containing minerals (Van Riemsdijk et al., 1984; Lookman et al., 1995; McGechan and Lewis, 2002).

The P desorption rate in the soils are of particular interests in respect to the bioavailability and the pollution risk as a result of P translocation to deeper layers and by surface runoffs (Pote et al., 1996; Li et al., 1999; Paulter and Sims, 2000). Desorption kinetics can also be classified in to fast and slow rates (Munns and Fox, 1976). The fast P pool presumably represents primarily P bound to the reactive surfaces that are in direct contact with the aqueous phase (Hingston et al., 1974, Madrid and Posner, 1979). The relatively higher surface coverage of soil with P and thus, easy replacement of the adsorbed phosphate may be attributed to a higher initial P desorption from the soil (McGechan and Lewis 2002). Other possible contribution to the fast desorbing pool may be the less soluble P salts originating from recent fertilizers applications that are not yet in equilibrium with reactive hydrous oxides (Lookman et al., 1995). Complexed P with organic material may also be part of the fast desorbing pool (Gerke, 1992). 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 particle (McDowell and Sharpley, 2003). The extent to which this slow reaction is then reversible (desorption) is fundamental in determining the residual effectiveness of added phosphate.

2.3 Phosphorus status of South African soils

Phosphorus deficiency is the most widespread and economically important nutrient deficiency in the higher rainfall areas of South Africa. The problem of satisfying the P requirements of plants is twofold. Firstly the soils are severely deficient in P and secondly, the plant availability of applied fertilizer P tends to be rapidly reduced through reactions with soil components (Bainbridge et al., 1995). The main reasons for the low plant availability of phosphate are presence of ferric Fe (III) - and aluminum (Al) oxyhydroxides (Sposito, 1989; Bainbridge et al., 1995) and low organic material content of South African soils (Applet et al., 1975; Stevenson, 1982; Iyamuremye and Dick, 1996; Baldock and Skjemstad, 1999).

The studies of Reeve and Sumner (1970) revealed a wide variation in the P sorption capacities of some oxisols in Kwa-Zulu-Natal province. Similarly McGee (1972), in evaluating P sorption in soils of Guateng, Mpumalanga, North West and Free State provinces found considerable variation in their sorption capacities. Bainbridge et al., (1995) determined the P-sorption isotherms of 50 soil samples from a number of localities in the Kwa-Zulu-Natal province. They reported that the amount of P sorbed ranged from 5-1174 mg kg $^{-1}$ and that the highest sorption occurred in the highly weathered red and yellow-brown clay soils with a high organic carbon content in the A horizons (Inanda, Kranskshop and Mgwa forms). This agrees with the findings of Haynes (1984) who had indicated that ferric and aluminum ions complexed with organic matter provide additional sites for P sorption. In an effort to identify soil properties responsible for P sorption, Henry and Smith (2002) constructed phosphorus isotherms for 21 selected soils from the Republic of South Africa and reached to the conclusion that the citrate bicarbonate dithionite- Al to be an important factor in P sorption although other soil constituents such as clay percentage, organic matter, citrate bicarbonate dithionite-Fe and Bray II P content also contributed to P sorption characteristics of the soils. Estimates of the phosphorus requirement of 20 selected soils of the South African tobacco industry were interpolated from phosphorus sorption isotherms and the results showed that the phosphorus required varied widely and is influenced by both the level of Bray II P content and the P fixation capacity of the soil (Henry and Smith, 2003). Although P sorption has been found to increase with increasing soil clay content, a considerable variation in sorption capacities have been obtained in different soils with similar clay contents (Johnston et al., 1991). It has been shown further that, soils with predominantly 1:1 type clay material (i.e. highly weathered red and yellow brown clay soils) sorp much more P than the soils with predominantly 2:1 type clays.

Van Zyl and Du Preez (1997 I) have tried to study the effect of farming practices such as tillage, fertilization and liming on the phosphorus fractions in soils from the summer rainfall area (25^0-30^0S; 24^0-30^0E) in South Africa by comparing the phosphorus level of selected virgin and cultivated areas. They found that P_T(total P) increased in the case of cultivation, which is attributed to use of fertilization as opposed to the virgin land. They also reported the influence of cultivation on the phosphorus fraction of the same soils and found that most of the inorganic fractions increased as the result of cultivation although the effect was not significant for the residual P_i fraction. $NaHCO_3$-P_O was found the most depleting organic fraction due to cultivation ascribing its easily minerlizable property as opposed to the other organic fractions (Van Zyl and Du Preez, 1997II). In a long-term experiment (>15 years) on yellowish brown sandy clay loam (Avalon) and a red sandy clay (Clovelly) soil in Ermelo, Mapumalanga province, Du Preez and Claassens (1999), concluded that the NaOH-extractable P (moderately adsorbed P) was mainly responsible for the replenishment of the labile soil P pool.

Relatively little information is available on areas pertaining to the long-term P desorption studies. Recently, studies related to the desorption kinetics of residual and applied phosphate to an acid sandy clay soils of Piet Retief, Mpumalanga were carried out over a 56-day period using hydrous ferric oxide in dialysis tubes (DMT-HFO) as a specific phosphate sink, followed by a sequential phosphate extraction. The total amount of phosphate desorbed during the stated period was reported to be virtually equal to the decrease in the NaOH (moderately labile) extractable inorganic phosphate fraction revealing the active participation of this fraction in the desorption process (De Jager, 2002). In an endeavor to investigate the fate of the applied P in soils, Ochwoh et al., (2005) also carried out the same experiment for sandy clayey soil (Ferric Luvisols) from Rustenberg (high P fixing) and a red sandy loam soil (Ferric Acrisols) from Loskop (low P fixing). The results showed that 30-60 % of the added P was transformed into the less labile P pools with in one day and 80-90% after 60 days. In the same study made by Ochwoh (2002), an attempt was made to determine the P desorption rates by successive DMT-HFO extractions after the transformation of the applied P followed by sequential extraction. They observed the transformation and redistribution of the applied P during incubation periods and proved that all the so-called unlabile soil P pools contributed to the labile P pool by different proportions.

2.4 Chemical extractants

Soil phosphate testing is used to predict plant yield from the amount of P already present in soil. This requires knowledge of the relationship between plant yield and soil P test values, where the yield measured later on in a season is related to soil P test values measured on soil samples collected earlier in season (Kumar et al., 1992). Soil testing for P is done using a chemical extractant. A large number of extractants have been suggested by various researchers (Tan, 1996) and the choice of appropriate soil test reagent depends on many factors, among which are the following:

- The soil and extractant type (Kleinman et al., 2001)
- The nature of the crop (Ibrikci et al., 1992) and
- The fertilizer type (Indiati et al., 2002).

The suitability of a specific soil P tests for soils is dependent on the pedogenic properties of the soils. For instance, Bray-1, Melich-1, and to a lesser extent, Melich-3, are not considered suitable for calcareous soils because soluble P may be precipitated by CaF_2, a product of the reaction between NH_4F and $CaCO_3$. Generally, acid extractants provide inconsistent measures of soil P in calcareous soils. Some extraction methods, however, such as Olsen, are considered suitable over a wide range of soils, from acidic to calcareous (Kleinman et al., 2001). Dilute acidic extractants such as Melich-1 (M-1) have been used on acidic soils. Investigations involving the M-1 test in Florida's acidic soils suggested excessive P recommendations for other crops such as watermelon [*Citrus lanathus Thunb*]. The M-1 dissolves Ca-P compounds in soils containing apatite and predicts high P values (Ibricki et. al., 1992). The Mehlich-3 (M-3) extractant was developed to predict nutrient requirements of plants over a wide range of soil chemical characteristics for macro- and micronutrients. The M-3 contains fluorides, which enhances the extraction of Al-phosphate through complexation reaction. According to Menon et al. (1990), acid extractants used in Bray-1 and 2 procedures, may extract more P from soils than the amount accumulated by plants. Acid extractants are capable to dissolve aluminum phosphate and calcium phosphate (Leal et al., 1994) giving high P values that do not reflect the level of available P. In general, acidic extractants have been found very effective in estimating available P in acidic soils. The same methods may not be appropriate when used in calcareous soils because of neutralization by the soil carbonates. In addition, acidic solutions may overestimate P from soils fertilized with water-insoluble fertilizer P such as phosphate rock (PR), by dissolving more P from PR than the plant could use.

Selection of appropriate soil test reagent also depends on the crop type. Crop species are known in their efficiency for utilization of nutrients from the soil. For instance, peanut [*Arachis hypogia L*] has been shown not to respond to phosphorus even in the soils testing low in Olsen extractable P where as wheat grown on the same field shown marked responses to residual as well as direct P application. Total P removed by peanut and wheat was comparable. It was, therefore, postulated that peanut perhaps taps some of the reserve P-fractions in the soil that are not readily available to other crops like wheat and mustard as the result of long-term fertilizer P application (Pasricha et al., 2002). A similar report was obtained on some soils of western Quebec (Canada), which were brought in to cultivation in the 1940s for some forage grasses. Grass grown on fine textured soils of the area did not respond to P fertilizer during the first two growing seasons during a 3-year *in situ* study (Ziadi et al., 2001). These soils initially had low Melich-3 extractable P contents and very high clay contents. Some studies using chemical extractions reported that the Melich-3 soil test might underestimate the P availability in clay soils (Cox, 1994). The lack of response of forage grass to P fertilizers suggests a significant contribution of the P reserves, which was not predicted by the Melich-3 extractant.

Identification of appropriate soil testing method is also influenced by the fertilization history of the soil that is whether the nature of fertilizer employed is consistent or not. Soil P testing has been developed for soluble P fertilizers, such as superphosphates and ammonium phosphate fertilizers. Recently, however, reactive rock phosphate (PR) and partially acidulated rock phosphate (PAPR), fertilizers are being advocated as alternative P fertilizers for super phosphate principally due to

i) Per kilogram of P, PR is usually the cheapest fertilizer and

ii) PRs can be more efficient than soluble fertilizers in terms of recovery of phosphate by plants, even from short-term crops in soils where soluble P is readily leached, as in sandy soils and possibly for long-term crops in other soils (Indiati et al., 2002).

Partially acidulated rock phosphates (PAPR) are prepared by treating the phosphate rock (PR) with less acid than would be required to convert the entire P content into superphospates (Menon et al., 1991). Application of the above fertilizers resulted in an increase in different soil P fractions. Phosphate rock fertilization resulted in an increase in the H_2SO_4- soluble Ca-P fraction (Steffens, 1994). After applying different P fertilizers there are still problems with soil testing methods in analyzing P availability for a P fertilizer recommendation. This is especially true after PR or PAPR fertilization. Acid extraction methods such as double lactate overestimate P and CAL method underestimates the plant availability of apatite P. This occurs because the soil test methods do not consider the release of adsorbed P or the dissolution of apatitic P in the soil (Steffens, 1994).

The information on proper fertilizer use emanating from the soil testing laboratories is primarily based on critical soil fertility limits of different nutrient elements and soils

(Sonar, 2002). However these soil tests give only a relative index of available P that can be supplied by the soil for plant growth, but do not measure actual available P quantitatively (Hedley et al., 1982; Tiessen and Moir, 1993). Plant available P is all P that is taken up by a plant during a specific period, such as a cropping season, year, or growth cycle (Tiessen and Moir, 1993). Since the actual plant available P is composed of solution P plus P that enters the solution as the result of desorption/dissolution from a solid phase, the conventional soil test methods have been unsatisfactory in predicting the plant P uptake. A possible explanation is that P from the less labile pools not measured by the common soil tests also contribute to plant uptake (Stevenson, 1986; Tiessen and Moir, 1993) as these fractions are in equilibrium with the P fractions extracted by the soil P tests.

2.5 The sequential extraction of phosphorus

The sequential extraction procedure of Chang and Jackson (1957) extracts various inorganic P pools and is widely used to study transformations of applied phosphate (Nurwakera 1991) and native phosphate forms (Williams et al., 1967). However this method extracts predominantly strongly retained P and is not appropriate for studying soil P dynamics that influence uptake by plants (Beck and Sanchez, 1994). The extraction procedure introduced by Hedley et al. (1982) fractionates the soil P into five inorganic P (P_i) pools, three organic P (P_o) pools, and one residual P pool. Sequential fractionation procedures are based on the assumption that chemical extractants selectively dissolve discrete groups of P compounds, and such operationally defined soil P fractions are subject to broad interpretations. Nevertheless, the information obtained from P fractionation schemes has been useful for interpretation of soil development (Cross and Schlinsinger, 1995) as well as plant availability of P (Tiessen and Moir, 1993). The overall advantage of the fractionation of soil phosphate into 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 fertilizer (Hedley et al., 1982, Tiessen and Moir, 1993).

In the fractionation procedure developed by Hedley et al., (1982) and modified by Tiessen and Moir (1993), the P fractions (in order of extractions) are interpreted as follows. Resin –P_i represents inorganic P (P_i) either from the soil solution or weakly adsorbed on (oxy) hydroxides or carbonates (Mattingly, 1975). Sodium bicarbonate 0.5 M at pH 8.5 also extracts weakly adsorbed P_i (Hedley, 1982) and easily hydrolysable organic P (P_o) (Buehler et al., 2002). Sodium hydroxide 0.5 M extracts P_i associated with amorphous and crystalline Al and Fe (oxy) hydroxides and clay minerals and P_o associated with organic compounds (fulvic and humic acids). Hydrochloric acid 1M extracts P_i associated with apatite or octacalcium P. Hot conc. HCl extracts P_i and P_o from more stable pools. Organic P extracted by conc. HCl may also come from particulate organic matter (Tiessen and Moir, 1993). The residue left from the HCl extraction is dissolved in hot concentrated H_2SO_4 plus H_2O_2 and assumed to be composed of occluded P_i associated with the remaining inorganic minerals, and non-extractable P_o (Tiessen and Moir, 1993).

2.6 Methods used to investigate and describe phosphorus desorption

2.6.1 Use of P-free solution

Among the many methods that have been used to examine the kinetics of P release is the use of water or P-free solutions such as $CaCl_2$ to induce desorption. Some researchers equilibrated soil or mineral samples with water at soil/water ratios ranging from 1:10 to 1:1000, and measured the P concentration in the equilibrating solution after given reaction periods to calculate the amount of P desorbed (Dimirkou et al., 1993). Other researchers have studied P desorption kinetics in a similar way using dilute solutions such as 0.01M $CaCl_2$ (which is designed to simulate soil solutions) instead of water as desorptioin media with soil /solution ratios in the range of 1:5 to 1:200 in single (Munns and Fox, 1976) or successive extractions (Hooda et al., 2000). The 0.01M $CaCl_2$ as a universal soil extractant was recommended by Houba et al., (1986). The advantage is that the other nutrients also could be measured in this extractant. The disadvantages are the analytical difficulties raised by some soils because of low levels of desorbed P. In earlier studies, significant relationship has been obtained between the 0.01M $CaCl_2$ desorbed P and P fertilizer dose and between $CaCl_2$-P and the estimated P balance (Jaszbereni and Loch, 1996). They also reported the importance of 0.01M $CaCl_2$ in predicting the P supply potential using the soil samples of long-term fertilization experiments. The result of the desorption investigations showed that beside characterizing the actual supply, the single time extraction P values in 0.01 M $CaCl_2$ can also express the P supply potentials. Not only plant available, labile soil-P can be characterized by the 0.01M $CaCl_2$ extractable P but also the excessive and environmentally undesirable P levels. Recent investigations on the use of 0.01 $CaCl_2$ have also revealed that this extractant can be used to characterize the potentially available P and the P in solution (McDowell and Sharpley, 2003). The disadvantage of these methods however is, they release small concentrations of P because the increase in solution concentration leads to the establishment of equilibrium. The process can, in principle, be repeated to desorb more P; however, experimental (analytical) errors tend to accumulate and still only a small percentage of the P present in the sample can be desorbed in this way (Freese et al., 1995). They also suggest that true release kinetics might be masked due to the resorption of P.

Leaching of soil columns with a P free solution is another option to study desorption (Van der Zee and Gjaltema, 1992). This is an excellent method for soils with relatively high P concentrations. Soils with low P concentrations however, require impractically high numbers of pore volumes due the strong non- linearity of the phosphate adsorption isotherm. Another disadvantage is that the experimental set up required is more complicated and rather expensive. This technique is, therefore, not very suitable to study large numbers of soil samples (Freese et al., 1995). The soil column leaching method, however, was found to be advantageous in experiments, which involve the stability of soil aggregates. It prevented the break up of soil aggregates resulting from the various shaking required by the other methods. Leaching soil columns also permitted the removal of desorbable P with time, which simulates nutrient removal by plant uptake more closely than batch equilibrations (Wang et al., 2001).

2.6.2 Use of materials that bind phosphate

Desorption can also be studied by adding materials that bind phosphate strongly, keeping the solution activity low so that the desorption from the soil particles can continue. The added material should have a high capacity to bind P. Another requirement is the possibility of separating the phosphate "sink" from the soil suspension in order to be able to assess the amount of P desorbed from the soil particles (Freese et al., 1995). Anion exchange resins (AER) have often been used for this purpose (Abrams and Jarrel, 1992; Sen Tran et al., 1992; Yang and Skogley, 1992).

Ion exchange materials can be viewed as competitive exchangers with those soil solids that are in dynamic equilibrium with the soil solution. In the case of P at a relatively acid pH range (4.3-5.0), $H_2PO_4^-$ is transferred via the soil solution from the soil solid phase to the ion exchange material. The reaction is simple exchange of adsorbed Cl^- for other anions in solution. In contrast, the equilibrium reaction of $H_2PO_4^-$ with metal-oxide-coated resin can be characterized as surface precipitation and adsorption via ligand exchange (Menon et al., 1990). This reaction is essentially irreversible, although anions like selenate, arsenate, and organic acids have been shown to compete with phosphate sorbed to Fe- and Al- oxyhydroxides (Traina et al., 1986). The resultant functional model for exchange resins relates to soil solution P dynamics. Since the mechanism for resin materials is ion exchange, there will be competition between $H_2PO_4^-$ and other anions at the resin sorption surface, particularly if other anion activities are high.

According to Cooperband and Logan (1994), over time, anion exchange materials will behave as either sinks or exchangers for P depending on: (i) the intrinsic anion exchange capacity of the resin material; (ii) the amount of time in contact with the soil; and (iii) the soil's P retention capacity. Throughout the literature, resin materials are described as infinite- sinks, probably because their exchange capacities remain large across the study period or the soil's P retention capacities are low enough to minimize competition for P between the resin and soil solid phase. In general, then, most anion-exchange resins react rapidly with $H_2PO_4^-$, and the rate of sorption is limited by the rate of desorption or dissolution in the case of agitated systems, and by pore and film diffusion in the case of *in situ* resin placement. Resin can be used to estimate instantaneous soil solution $H_2PO_4^-$ concentration by regression analysis. Resin-membrane-extractable P could also be calibrated with the labile P component of soils with differing P retention capacities. Once this relationship is established resin materials can be used in the field with time to estimate changes in net labile soil P (Cooperband and Logan, 1994). The resin extraction method is considered superior compared to chemical based soil tests for assessment of nutrient availability (Ibrikci et al., 1992).

Various researchers have modified this method using different soil/resin/solution ratios, equilibration times, forms of resins, and means to separate the resin beads from the soil after extraction (Yang et al., 1991). However, all the AER bead methods have disadvantage in that the soil must be finely ground so that it can be separated from the resin beads after extraction. Also, analytical errors can arise when fine roots and soil particles are trapped in the cloth, nylon, or polyester-netting bags often used to facilitate the separation process. Furthermore, the sealed edges of the bags may rapture through normal wear and tear resulting in the loss of resin beads into the soil suspension (Lee and Doolittle, 2002). The other problems with regard to the use of AER are their non-specific adsorption desorption of different anions and the incapacity of the resin to maintain low P concentrations and to act as infinite sink especially in the long-term studies (Freese et al., 1995)

The use of anion exchange resin membranes (Cooperband and Logan, 1994) provides a major improvement on the point of separability of P sink and soil suspension, the other disadvantages of the use of anion exchange resins as a P sink, however, remain. Apart from the drawbacks mentioned above, the capacity of an anion exchange resin to fix desorbing P depends on the chemical forms of the resin, e.g., Cl^-, HCO_3^-, or OH^- (Freese et al., 1995). Bacha and Ireland (1980) stated that the HCO_3^- form is better than the Cl^- form because the HCO_3^- form of the resin extracts a constant proportion of the isotopically exchangeable P from acid and calcareous soils. Besides, it stabilizes the extraction system in such a way that the resin type and soil/ water ratio only slightly affect the quantities of extracted P and the pH of the suspension (Sibbesen, 1978). The P extracted by HCO_3^- saturated resin is also better correlated with plant growth, apparently because it resembles the chemistry of the rhizosphere due to HCO_3^- accumulation in the medium (Sibbesen, 1978). Use of the bicarbonate form however, generally leads to an increase in the pH of the soil solution (Abrams and Jarrel, 1992), rendering HPO_4^{2-} species the dominant P ion in solution. The relatively weak specificity of a strong acid anion exchange resin for phosphate in an acid pH range of about 5 to 6 is based solely on the fact that a bivalent ion is preferred over monovalent ions in the ion exchange process. For these reasons the anion exchange method, although often used to assess plant available phosphorus, is not very suitable for studying P desorption of acid soil under conditions of natural pH.

Despite these disadvantages, anion exchange membranes (AEM) however are used as extracting agents. Saggar et al., (1990) reported that the AEM behaves similarly to AER beads and give an equally good estimate of soil phosphate. Schoenou and Haung (1991) reported that similar trends in predicting relative P availability were observed for AEM-extractable P, water extractable-P, bicarbonate extractable total P, and bicarbonate extractable organic P. Therefore, the AEM is well suited for routine soil P analysis. It is also low cost, simple, and consistent across all soil types. Lee and Doolittle (2002) showed that the AEM extracted more P than the AER from the soil-solution systems and the amount of soils phosphorus extracted by AEM and AER was significantly correlated in all the soil types tested.

Desorption studies of soil using Fe or Fe-Al oxide impregnated filter paper as a P sink, (P_i) became a better option than the resin approaches (Sharpley, 1991; Bramley and Roe, 1993; Sharpley, 1993). The two major drawbacks of this method however made it unsuitable for studying long-term P desorption from the soils. First, the paper strips are mechanically unstable during longer desorption times (weeks), leading to relatively large losses of the P sink in to the soil sample. Moreover, filter paper traps part of the soil material during every desorption step, affecting particularly the fine size fraction (Freese et al., 1995). These results in an overestimation of the amount of P desorbed, since any P associated with these particles is accounted for as desorbed after analyzing the filter paper.

Some investigations also reported on the use of cation anion exchange resin membranes (CAERM) (McKean and Warren, 1996; Indiati, 2000; Delgado and Torrent, 2000, and Delgado and Torrent, 2001) for extraction of soil P. The reports revealed that this method is in general effective in extracting more amounts of P than the other methods. The relative effectiveness of CAER method is probably due to promoted dissolution of metal phosphates. The cation exchange resin reduces cation activity in solution, thus decreasing the ionic activity product and favoring metal phosphate dissolution (Delgado and Torrent, 2000).

Recently, a new desorption technique has been developed that is also based up on the use of hydrous ferric oxide (HFO) as a sink for P (Freese et al., 1995). Instead of being impregnated in filter paper, the HFO is present inside dialysis tubing. Separation of P sink from the soil suspension thus becomes possible without extracting soil particles. This new system is found to be mechanically stable for very long reaction periods, provided that a microbial inhibitor, e.g., chloroform, is added to the soil suspension to prevent hydrolysis of the membrane. The pH of the soil solution during desorption remain almost constant. As such this technique has important advantages to the Fe- oxide impregnated filter paper extraction method. The system is capable of maintaining low P activity in solution necessary to study long term desorption kinetics of soils (Freese et al., 1995; Lookman et al., 1995; Koopmans et al., 2001; De Jager and Claassens 2005; Ochwoh et al., 2005). The disadvantage of using dialysis tubing is that P diffusion kinetics through the membrane may affect the soil P release kinetics. This is, however, only the case for the initial stage of desorption where the P release is relatively rapid. The DMT-HFO technique is therefore not as such useful to study short-term desorption kinetics (Lookman et al., 1995).

In summary, soil tests for plant available P are used world wide to determine the current P status of soils so as to estimate fertilizer P requirements for specific yield goals. The current P status is due to indigenous (native) P present in the soil and P from previous fertilizer P application (residual P). Plant P availability of residual P in soils can be reliably estimated by successive cropping experiments carried out in field or green house conditions, where P is taken up until P deficiency occurs or a response to added P is measured (Sahrawat et al., 2003). As this approach is very expensive and time consuming, soil extractions with P sink methods have been proposed to estimate residual P. Thus consecutive extraction of soils by these methods may be a convenient laboratory method to characterize the capacity of soil to supply P, and to investigate the kinetics of residual P release. Such methods use anion exchange resins (Abrams and Jarrel, 1992), iron oxide impregnated paper strips (Indiati, 2000) and DMT-HFO (Freese et al., 1995; Lookman et al., 1995; Koopmans et al., 2001; De Jager & Claassens, 2005; Ochwoh et al., 2005). This study focuses on the assessment of the efectiveness of succssive P desorption followed by subsequent extraction, termed as combined methodology, which is used to investigate the long-term desorption study of soils under green house and field trials.

CHAPTER 3

Kinetics of phosphate desorption from long-term fertilized soils of South Africa and its relationship with maize grain yield

3.1 INTRODUCTION

The amount of P removed from a field by crops in general varies from 3-33% of applied P fertilizer (Aulakh & Pasricha, 1991; Linquist et al., 1998; Csatho et al., 2002; Aulakh et al., 2003; Pheave et al. 2003; Zhang et al., 2004; Kamper & Claassens, 2005). Soils receiving successive applications of fertilizer P or manure over a long-term, therefore, can accumulate large amounts of residual P. This represents not only an uneconomic practice but also the risk of potential for P loss to surface waters via overland or subsurface flow and intern accelerate freshwater eutrophication (McDowell & Sharpley, 2002).

The P availability for plants is usually done using single chemical extraction methods. However, it is accepted that the plant acquires its P from the soil solution that has to be replenished over the growth period. The availability of P to plants therefore depends, among other things, on the rate at which it is released to replenish the soil solution (Raven and Hossner, 1994). Due to P build up in soils over a long period, a significant residual effect can be expected and this can lead to an underestimation of the available P if not taken in to account.

Plant P availability of residual P in soils can be reliably estimated by successive cropping experiments carried out in field or green house conditions, where P is taken up until P deficiency occurs or a response to added P is measured (Indiati, 2000). This approach, however, takes many years to realize which makes it very expensive and time consuming. Therefore, instead of attempting to tap the residual P by continually cropping till the plant responds, more rapid soil test methods that can approximate this biological measure have been designed. According to these methods, a given soil is subjected to successive P desorptions using materials that can act as P sinks. By employing these methods, one can study the P release rate of a given soil and for how long a given soil can supply P. This intern enables to know for how long it will take for soil P to deplete to a concentration where manure or fertilizer P can again be applied.

Recently, successive extraction procedure employing hydrous ferric oxide in dialysis membrane tubes (DMT-HFO) as a phosphate sink, has been used in assessing long-term phosphate desorption (Freese et.al., 1995). This method is similar to Fe-oxide impregnated filter paper strips but in this case the HFO is placed in a dialysis membrane tube instead of being impregnated in the filter paper.The fact that this system is capable of maintaining low P activity in solution for longer period of time, and its mechanical stabilty makes it appropriate for long-term studies (Freese et al., 1995). However, relatively little information is available on the literature related to the use of this method. Lookman et al. (1995) studied the kinetics of P desorption using this procedure. They concluded that P desorption could be well described by a two component first order model: $P_{R(t)} = SP_{Ao} (1- e^{-kAt}) + SP_{Bo} (1- e^{-kBt})$, with SP_{AO} and $SP_{BO,}$ the amounts of P initially present in the labile pool A and strongly fixed pool B respectively. They also reported that no desorption maximum was reached in the entire period of desorption (1600h). Research was also done which linked short-term soil P tests to long-term soil P kinetics (Koopmans et al., 2001; Maguire et al., 2001). Recently, studies were also made on some South African soils using DMT-HFO method as a phosphate sink. De Jager and Claassens (2005) investigated the desorption kinetics of residual and applied P to acid sandy clay soils from Mpumalanga, South Africa. They reported that no desorption maximum was reached after 56 days of shaking. They also reported that application of P increased desorption rate of P from the labile pool (SP_A) where as the P applied had less impact on the desorption rate of P from the less available pool (SP_B). However, there is still a paucity of information on the relationship between kinetics of phosphorus release using this new method and plant yield parameters for soils that received fertilizers over a long-term. The objectives of this research were 1) to study desorption of residual P from soils with a long-term fertilization history using successive P extractions by DMT-HFO and 2) to relate the kinetic data generated to maize grain yield.

3.1.1 Theory

Desorption kinetics of soil as determined by DMT-HFO can be schematically represented as

$$SP \xrightarrow{k_R} P_{sol} \xrightarrow{k_T} P_{HFO} \qquad (1)$$

Where SP is solid phase P, P_{sol} is P in solution, P_{HFO} is P adsorbed by HFO, k_T is the rate constant of P transport through the membrane ($0.09 \pm 0.01 h^{-1}$) (Freese et al., (1995) and k_R is the rate constant of P release (De Jager & Claassens (2005)).

The presence of two pools is assumed: the pool with the fast release kinetics is pool A (SP_A) and the pool with the slow release kinetics is pool B (SP_B). With this assumption, the mass balance equation for the total exchangeable solid phase soil P (SP_{total}) at time t = 0 is:

$$SP_{total\ 0} = SP_{A0} + SP_{B0} \qquad (2)$$

Where SP_{A0} is initial amount of P in pool A and SP_{B0} is initial amount of P in pool B.

The mass balance equation at time **t** will therefore be:

$$SP_{total\ (t)} = SP_{A(t)} + SP_{B\ (t)} \qquad (3)$$

Assuming the decrease in SP_A and SP_B follow first order kinetics, the integrated rate laws for the decrease of SP_A and SP_B will be:

$$SP_{A(t)} = SP_{A0}\, e^{-k_A t} \quad \text{and} \quad SP_{B(t)} = SP_{B0}\, e^{-k_B t} \qquad (4)$$

Where k_A and k_B are conditional first order rate constants (day $^{-1}$) for P desorption from pools A and B respectively.

The total solid phase soil P ($SP_{total\ (t)}$) remaining at time t will be given by:

$$SP_{total\ (t)} = SP_{A0}\, e^{-k_A t} + SP_{B0}\, e^{-k_B t} \qquad (5)$$

The total amount of P released at time t is expressed as:

$$
\begin{aligned}
P_{R(t)} &= SP_{A0} - SP_{A\,(t)} + SP_{B0} - SP_{B\,(t)} \\
&= SP_{A0} - SP_{A0}\, e^{-k_A\, t} + SP_{B0} - SP_{B0}\, e^{-k_B\, t} \\
&= SP_{A0}\,(1 - e^{-k_A\, t}) + SP_{B0}\,(1 - e^{-k_B\, t}) \qquad (6)
\end{aligned}
$$

It was further assumed that the rate constant of P release from the soil was equal to the rate constant of P adsorption (k_A) by the DMT-HFO. The rate constant of P adsorption (k_A) by the DMT-HFO was obtained from a plot of the natural logarithm (*ln*) of the P adsorbed by the DMT-HFO against time with the slope as k_A (De Jager and Claassens, 2005).

3.2 MATERIALS AND METHODS

3.2.1 Sampling procedure and experimental site history

Surface soil samples (0-20cm) were collected from one of the oldest long-term fertilizer trial in South Africa established in 1939. According to the USDA Soil Taxonomy System (Soil Survey Staff, 1990), the soil is a loamy, mixed, thermic Rhodic Kandiudalf. The soils were air-dried and ground to pass through a 2 mm sieve. Soil samples were collected from selected P treated plots. The samples were cored from three sites on each plot and four replications at each site. The samples were mixed and composite samples were used for the subsequent analyses.

The soil samples collected had the following fertilization history. The NK treatment received only N (ammonium sulphate) and K (KCl) fertilizers since the inception of the trial and acted as a control. The NPK and MNK treatments served as medium P level samples. They have nearly similar P contents (Table 3.1) but received different sources of P. The P source of the NPK treatment was inorganic (superphosphate) where as MNK treatment received a mixture of cattle dung and compost, here in this paper referred to as manure, as a P source. The MNPK treatment received both inorganic and organic P fertilizer and was considered as high P soils relative to the others. The inorganic P was applied from 1939 to 1985 and discontinued since 1985. Application of P in the form of manure was applied from 1939 to1990 and discontinued after 1990. The reason for discontinuing P application in both cases was due to the build up of P resulted from previous excessive application. Nel et al. (1996) has provided a detailed fertilization history (1939 to 1991) of these soils. Since then the plots received 125 and 80 kg ha $^{-1}$ year $^{-1}$ N and K respectively. Table 3.2 shows the fertilization history of the selected treatments.

3.2.2 Soil characterization

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 distribution of the soils was determined using a hydrometer method after dispersion of the soil with sodium hexametaphosphate. Organic C was determined by dichromate oxidation technique while extractable Ca, Mg and K were determined by extraction with neutral ammonium acetate solution (1*M*). Total soil P (P_T) was determined on sub samples of

Table 3.1 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	%
NK	5.36	367.16	1.35	453.28	148.17	110.67	23.70	6.30	66.90	0.69
NPK	4.85	600.00	51.37	405.41	122.47	91.42	24.65	7.65	64.75	0.84
MNK	5.04	623.43	45.10	551.52	140.25	93.94	21.15	10.65	66.20	1.14
MNPK	4.81	851.22	100.01	535.19	135.25	98.81	21.45	9.30	66.30	1.04

[§] NK= received only inorganic Nitrogen and Potassium, used as a control; NPK= Inorganic N, P K fertilizers applied to these soil types; MNK= the source of P is organic (cattle dung and compost) and MNPK= the source of P is both inorganic fertilizer and Cattle manure

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

0.5mg 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). Table 3.1 shows some selected physical and chemical properties of these treatments.

Table 3.2 N, P, K and manure (kg ha^{-1} y^{-1}) applied to NK, NPK, MNK and MNPK treatments

Year	N	P	K	Manure (dry)
1939-1966	42.5	34	31.5	4470
1966-1972	85	68	63	8940
1973-1983	205	100	100	8940
1984	205	0	100	8940
1985-1990	125+125[a]	0	80+100[b]	8940[c]
1991-2003	125	0	80	0

[a] Additional N topdresssed on NPK treatments

[b] Additional K applied to NPK treatments

[c] Applied annually up to year 1990

3.2.3 Long-term desorption study

A long-term desorption study was carried out using dialysis membrane tubes filled with hydrous ferric oxides as described by Freese et al. (1995). The hydrous ferric oxide-dialysis membrane tubes were placed in 200 ml polyethylene containers with 1g of soil and 80 ml of 2 *mM* $CaCl_2$ and 0.3 *mM* KCl solution. All the experiments were executed in triplicates. The polyethylene containers were continuously shaken for 56 days on an end-over-end shaker at 120 oscillations per minute (*opm*). It was found in a preliminary investigation (data not shown) that shaking at 120 *opm* created the required perturbance yet the tubes could be shaken for 14 days without physically damaging the dialysis tubes. On days of 1, 7, 14, 21, 28 and 42 days, the DMT-HFOs were replaced with new DMT-HFO. When they were replaced, a glass rod was used to remove any attached soil from the dialysis membrane tubes. At each time interval, three of the tubes were removed, opened and the contents transferred to glass bottles. The suspension was then dissolved by adding 1ml concentrated (98%) sulfuric acid. P in solution was colorimetrically determined with the molybdophosphoric blue (Murphy and Riley, 1962) method using ascorbic acid as a reductant. A standard series and blank were prepared with the same background Fe and sulfuric acid.

3.2.4 Field data

Maize (*Zea mays* L.) was grown in summer since the establishment of the experiment (1939). Field data for maize grain yield (t ha^{-1}) was collected from the experimental station. Since there was no similar data on the plant P uptake, the correlation was restricted only to dry (12% moisture content) maize grain yield.

3.2.5 Data analysis

The data obtained were statistically analyzed 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 regression equations and correlation coefficients were determined with the exponential fits of the graph. k_A and k_B values of equation [6] were determined by splitting the respective pools in to two pools (Pool A and Pool B), taking in to account the pattern of P released with time, and plotting the natural log of the P desorbed against time. Correlation of k_A, k_B and the total amount of P released with plant yield parameter (Maize grain yield) was done using Pearson linear correlation, PROC CORR (SAS Institute 2004).

3.3 RESULTS AND DISCUSSION

3.3.1 DMT-HFO extractable P

The amount of P_i extracted by DMT-HFO was significantly influenced ($P \leq 0.05$) both by the levels of P applied and extraction time (Table 3.3). Temporal change of this fraction, however, was not significant for the control. The cumulative P desorbed was higher in the MNPK treated soil (19.83-103.46mg kg $^{-1}$) and lower in the NK (0.08-1.13 mg kg $^{-1}$) at all levels of extraction time (1 –56 days). In this study, NPK (4.87 – 19.34 mg kg $^{-1}$) and MNK (5.85 – 18.76 mg kg $^{-1}$) 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, P might have been accumulated in inorganic forms in preference to organic forms (Sharpley, et al., 1993; Koopmans et al., 2003; Turner and Leytem, 2004). This is evidenced by having nearly similar amount of Bray and total P for both NPK and MNK treated soils (Table 3.1). The P source therefore seemed not to influence the amount of P extracted from both types of treatments.

Table 3.3. Effect of P levels and extraction time on soil P desorption

Desorption time (days)	NK	NPK	MNK	MNPK
	(mg P kg^{-1})			
1	[§]**x** 0.08[†] **a**[‡]	**x** 4.87 **a**	**x** 5.85 **a**	**y** 19.83 **a**
7	**x** 1.05 **a**	**y** 9.29 **a**	**y** 10.46 **ab**	**z** 60.72 **b**
14	**x** 1.07 **a**	**y** 11.84 **ab**	**y** 11.90 **ab**	**z** 73.33 **c**
28	**x** 1.08 **a**	**y** 13.50 **ab**	**y** 12.91 **ab**	**z** 87.62 **d**
42	**x** 1.11 **a**	**y** 15.65 **ab**	**y** 14.74 **b**	**z** 93.12 **d**
56	**x** 1.15 **a**	**y** 19.34 **b**	**y** 18.76 **b**	**z** 103.47 **e**

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

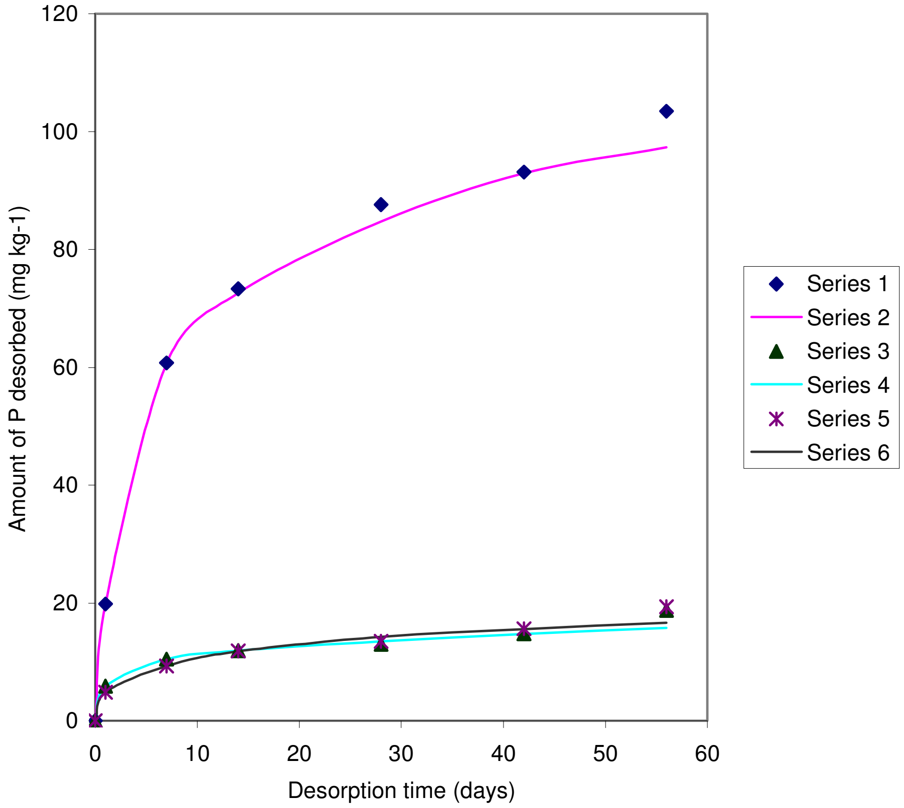
[†] Mean values of three replicates

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

When expressed as a percentage of the total P (Table 3.1), the percentage distribution of DMT-HFO-P_i fraction ranges from 0.02 –0.40, 0.81 – 3.22, 0.93 – 3.01 and 2.33 –

12.15 for NK, NPK, MNK and MNPK treated soils respectively from day 1 to 56 days of extraction time respectively. The percent P extracted was very low compared to the total P. Similar results were reported by other researchers (Koopmans et al., 2001; De Jager and Claassens, 2005; Ochwoh et al., 2005). Treatment MNPK however resulted in a relatively larger percent of extractable DMT-HFO-Pi especially at the latter time of extraction. This could be ascribed to the higher Bray and total P of this treatment (Table 3.1).

Cumulative P released with time followed, in general, the same pattern for all P treated soils, with an initial rapid release of P within the first two weeks (14 days), followed by a slower release that was still continuing after 56 days as illustrated on Figure 3.1. 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 P bound to reactive surfaces, directly in 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 and/or diffusion kinetics from interior sites inside oxyhydroxide particles (McDowell and Sharpley, 2003). The fact that the control had very little DMT-HFO extractable P might be the result of the low amount of available P. The amount of P extracted from the control during the 56 days of extraction was comparable with the Bray-1P. De Jager and Claassens (2005) however reported contrary to this, cumulative DMT-HFO extractable P extracted over 56 days was 10 times more than the Bray extractable P. The difference could be attributed to the types of soils used. De Jager and Claassens (2005) used incubated soils for 5 months at ± 28 ^{0}C. In this study the last time the soils received any P was in 1985 for NPK and 1990 for MNK, which means the soils were incubated on average for nearly 20 years. In addition to this, 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 in this study.



Series 1: Data (MNPK)

Series 2: Two component first order model (MNPK) $R^2 = 0.993$

Series 3: Data (MNK)

Series 4: Two component first order model (MNK) $R^2 = 0.918$

Series 5: Data (NPK)

Series 6: Two component first order model (NPK) $R^2 = 0.947$

Figure 3.1. Cumulative desorption data of the different P treated soils fitted to a two component first order model

The rate of desorption has not reached maximum, indicating that desorption will continue for longer period than 56 days. The experimental data were fitted with a two-component first order model. The correlation coefficients were 0.947, 0.918 and 0.993 for NPK, MNK and MNPK treated soils respectively as shown in Figure 3.1. The control is not considered here, as the amount of P extracted was negligibly small albeit the rate of desorption followed the same trend like the P treated plots. The rate constants (0.0003-0.0043 h^{-1}) of P release from all treatments were lower than the rate constant of P transport through the DMT (0.09 h^{-1}) reported by Freese et al. (1995), indicating that it is the P release from the soil solid phase and not P diffusion through the DMT that was the rate limiting step. This result concurs positively with the results of De Jager and Claassens (2005) (0.0046-0.0064 h^{-1}).

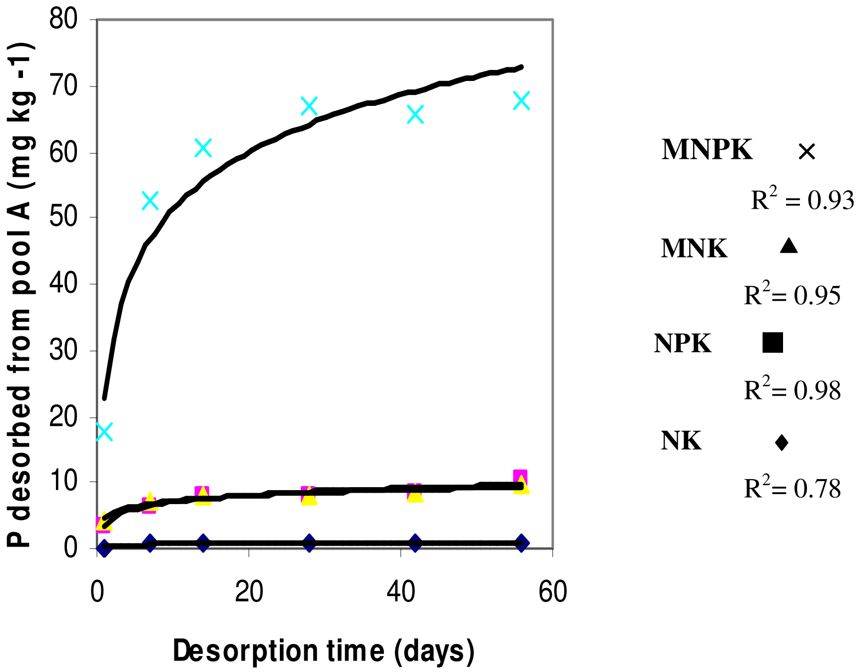


Figure 3.2. Simulated P desorption from pool A (SP_A) for the different treatments over 56 days

In Figures 3.2 and 3.3, the simulated P release from respective SP_A and SP_B pools were plotted against time to show the different release kinetics of each pool over 56 days. The contributions of SP_A and SP_B to $P_{R\,(t)}$ can therefore be calculated from the following equations (De Jager and Claassens, 2005).

$$SP_A\,(t) = \alpha_A\, P_{R(t)} \qquad \text{and} \qquad (7)$$

$$SP_B\,(t) = \alpha_B\, P_{R(t)} \qquad (8)$$

Where $\alpha_A = (1\text{-}e^{-KA\,t})\, SP_{A0}/\, P_{R(t)}$ and $\alpha_B = (1\text{-}e^{-KB\,t})\, SP_{B\,0}/\, P_{R(t)}$

The release kinetics of SP_A was faster in the first 14 days but declined with increasing time, where as the contribution made by SP_B increased with time, the increment being dominant especially with increasing time of extraction. The contributions of both SP_A and SP_B to the total P extracted varied among treatments following the order: MNPK>>MNK≈NPK>>NK. This is in accordance with the total P content of the plots (Table 3.1). The higher the P status of the soil, the greater was the contribution made by both SP_A and SP_B. This could be attributed to higher degree of P saturation of the adsorption sites with increasing P status of the soil (De Jager and Claassens, 2005). Toor and Bahl (1999) also reported the higher P desorption rate in fertilizer and manure treated soils. In their investigation, manure appeared to play significant role in enhancing the P desorption possibly due to complexation of Fe and Al ions.

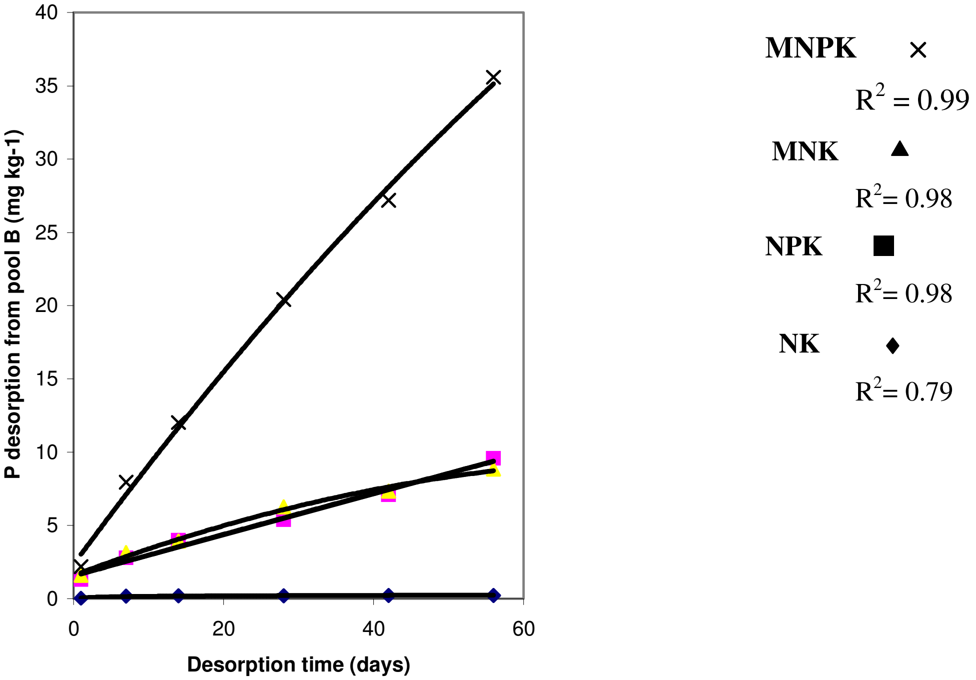


Figure 3.3. Simulated P desorption from pool B (SP_B) for the different treatments over 56 days

Figure 3.4 indicates the desorption rate of the differentially P treated soils. The rate at which P desorbed from MNPK dropped faster up until 28 days and started to change slowly with progressive desorption time. The same trend was also observed for NPK and MNK treated soils although the rate of desorption declined faster up until 14 days and varied slowly afterwards. Moreover, the degree of variation was much less pronounced as compared to MNPK. The reason for such variation could be attributed to the difference in the amount of desorbable P, which is much greater for MNPK than either NPK or MNK treated plots. The control, however, showed almost negligible variation with time. These results are consonant with the reports made by De Jager and Claassens (2005). The reason for this could be ascribed to the very low P contents of the treatments that received no P and faster release kinetics are usually associated with desorption of adsorbed P directly in contact with the soil solution (Lookman et al. 1995).

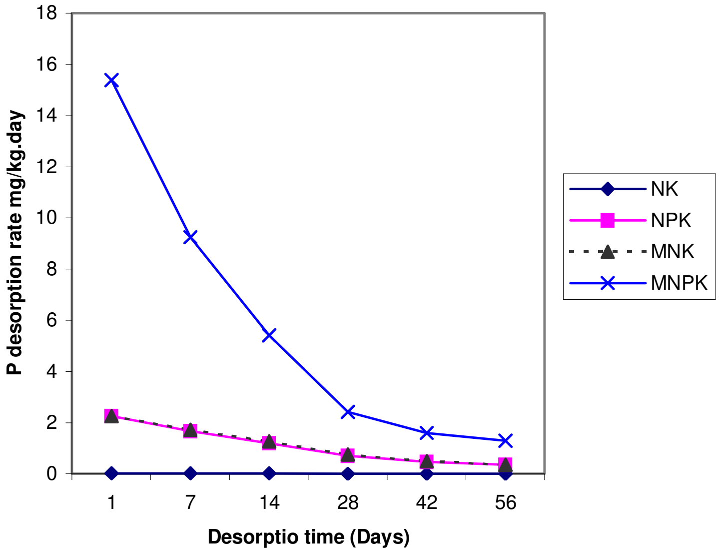


Figure 3.4. Desorption rates for the different P treatments over 56 days

2.3.2 Plant growth as related to phosphorus desorption kinetics

In this work, correlations between the rate coefficients k_A and k_B (day $^{-1}$) with maize grain yield (t ha $^{-1}$) were made as illustrated in Table 3.4. The grain yield considered for this comparison was the yield (four replications) obtained in the year of sampling. However, the yield obtained on the subsequent year was also comparable revealing the consistency of the data considered. A significant correlation was obtained between the labile pool rate coefficient k_A and maize grain yield ($r = 0.93^{**}$). This pool represents the P pool with fast release kinetics that comprises presumably primarily P bound to the reactive surfaces that is in direct contact with the aqueous phase. This pool is presumed to be easily available to plants in a reasonably short period of time (Lookman et al., 1995). The rate coefficient k_B also showed a significant but moderate correlation ($r = 0.78^{*}$) with maize grain yield. This pool represents the P pool with slow release kinetics that results from slow dissolution kinetics or from slow diffusion from the matrix of sesquioxide aggregates (Koopmans et al., 2004). This pool will be available only over a long period of time and that is probably why the correlation was not so strong.

Table 3.4. Correlation between maize grain yield (t ha^{-1}) and kinetic parameter k (day $^{-1}$)(Rate coefficient) of the DMT-HFO method

	Yield	k_a	k_b	k_a+k_b
Yield	1.00			
k_A	0.93^{**}	1.00		
k_B	0.78^{*}	0.52	1.00	
k_A+k_B	0.97^{**}	0.99^{**}	0.62	1.000

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

Although the P pools are theoretically grouped in to these two discrete pools for the sake of convenience, the fact that both pools involve simultaneously in the uptake process indicates that one should take into account the effect of both when such a correlation is made. Thus, the sum of the rate constants (k_A+k_B) showed a significant correlation ($r = 0.97^{**}$) with maize grain yield, which is even stronger than the correlation with which each showed with maize grain yield. The rate coefficient for the labile fraction, $k_{A,}$ strongly correlated ($r = 0.99^{**}$) with the sum of k_A and k_B (k_A+k_B) unlike the less labile fraction, k_B ($r = 0.62$) revealing the predominant contribution of the labile P fraction in replenishing the soil solution P than the less labile form for the extraction period considered in this study.

Table 3.5.Correlation between the cumulative amounts of P (mg kg^{-1}) extracted by the DMT-HFO and Bray 1 P (mg kg $^{-1}$) with maize grain yield (t ha $^{-1}$)

P (mg kg $^{-1}$)	Maize grain yield (t ha^{-1})	Bray 1P (mg kg $^{-1}$)
DMT-HFO-P_i	0.58	0.92^{**}
Bray-1P	0.84^{*}	-

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

The cumulative amount of P extracted by the DMT-HFO over a 56-day period was also correlated with yield and Bray 1P as depicted in Table 3.5. According to Sharpley (1996), Bray 1 provides the best indication of labile P in slightly to highly weathered soils. This extraction method is currently widely used as a soil P test method. Correlation between the cumulative P extracted and maize yield was not significant. However, the correlation between Bray 1P and maize grain yield was highly significant ($r = 0.84^{*}$). Unlike the correlation between DMT-HFO-P_i and yield, the correlation of the former with Bray-1P was found to be highly significant (r =

0.92**). This observation probably indicates the ability of these extractants to extract the labile P. Although the correlation between DMT-HFO-P_i and Bray-1P was found to be highly significant, the correlation each showed with maize yield was apparently opposite, the former resulted in weak correlation, while the latter resulted in moderately strong and significant correlation with the yield parameter. A possible explanation for the observed difference between the two extractants could be obtained by comparing the amount of P extracted by both extractants as depicted in Table 3.1. NK and MNPK treated soils released roughly similar amount of P by both extractants where as NPK and MNK desorbed a DMT-HFO-P_i extract, which was nearly half extracted by Bray-1P. The relatively lower amount of P desorbed by these treatments could be a possible reason for the poor correlation observed between DMT-HFO-P_i and maize grain yield.

Judging from the r-values, the rate coefficient showed a better correlation with maize grain yield than the cumulative amount of P desorbed. The rate coefficient, therefore, appeared to be a good index to assess the P supplying capacity of the soils studied. The relatively weaker correlation of the amount of P released with maize grain yield is an indication of the need for further fine-tuning (optimization) of the existing approach. Moreover, correlation with other plant indices such as plant P uptake and relative plant response should also be included in the further assessment of this methodology.

3.4 CONCLUSIONS

According to this study, cumulative P released with time followed the same pattern for all P treated soils, 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. No desorption plateau was reached during the entire period of extraction time, indicating that desorption can continue for a longer period than 56 days. P desorption kinetics were described relatively well by a two-component first-order model (R^2 = 0.947, 0.918, & 0.993 for NPK, MNK, & MNPK respectively). The contributions of both SP_A and SP_B to the total P extracted varied among treatments following the order: MNPK>>MNK≈NPK>>NK. The contribution made by SP_A was found to be higher than SP_B in the 56 days of extraction. However, the degree of increment with time showed that it is the less exchangeable pool (SP_B) that will control the release kinetics of the soil in the long term.

In this study the rate coefficient showed a better correlation with maize grain yield than the cumulative amount of P desorbed. The rate coefficient, therefore, appeared to be a good index of plant availability. However, in this research correlation with other plant yield parameters such as P uptake and relative plant response was not conducted due to lack of relevant data. More work relating these plant indices with desorption indices is therefore required. Data from a wider range of soils is also needed to evaluate the universality of this method. Besides, this method employed 100% exploitation of soil volume, which is in contrast to plants where the root exploitation is much less than this. Recent works related to the percent root exploitation of the soil volume revealed that 3-4% of the top soil volume was exploited at full maturity of a maize crop. This value was 1% during the first two weeks, when most P uptake was anticipated to occur (Kamper & Claassens, 2005). Therefore, exploiting the whole volume of the soil by continuous shaking, as has been done in this technique, may not well simulate the plant mode of action.

CHAPTER 4*

Effect of long-term phosphorus desorption using dialysis membrane tubes filled with hydrous iron oxide on phosphorus fractions

4.1 INTRODUCTION

The application of phosphorus (P) as either fertilizers or manures in excess of plant requirements causes a build up of P in the soil. However, some of this accumulated P may not be readily plant available (Myres, et al., 2005).

Presently conventional soil P tests, which consist of single extraction procedure, are used to estimate fertilizer requirements and represent an index of plant available P. Since 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 (Solomon et al., 2002). This can be achieved by characterizing both labile and less labile inorganic and organic P pools.

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, however, takes many years. For example, Johnston and Poulton (1976) showed that a Batcombe clay loam soil (Typic Hapludalf), which had received no manure since 1901 took in excess of 50 yr to deplete Olsen P concentrations via crop removal from approximately 65 mg kg^{-1} to the minimum

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concentration required for optimal crop growth. McCollum (1991) estimated that with out further P fertilizer additions, it would take about 14 yr of maize (Zea mays. L) or soybean (Glycine max (L.) Merr.) production to deplete Melich-3 extractable P concentration of a Portsmouth fine sandy loam (mixed thermic Typic Umbraquult) from 100 mg kg^{-1} to an agronomic threshold of 20 mg kg^{-1}. As estimating residual P by this approach is time consuming and very expensive, 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.

Several methods can be used to estimate plant available P in soils called ion sink tests that employ a P adsorbing surface. Some of these P sink methods such as anion exchange resin /FeO-coated paper strips are used for a short term desorption studies while others such as dialysis membrane tubes filled with hydrous ferric oxide solution (DMT-HFO) can be used for long-term desorption studies (Freese et al., 1995; Lookman et al., 1995). These P testing methods have an advantage over conventional chemical extractants such as Bray-1P, Olsen-P and Melich-3P because the ion sink methods function similarly to a plant-root surface adsorbing available P ions from the *in situ* labile P pool in soil (Menon et al., 1989). In contrast, the use of single extractants in chemical tests for soil P may solubilize non-labile P more tightly bound to Al, Fe, and Ca complexes, which may not be plant available. When this occurs, accurate interpretation of test results becomes more difficult (Myres et al., 2005).

Ion sinks have advantages over typical chemical extractants, as they do not react with the soil, but only sorb the chemical entering the soil solution. Hence, they can be favorably employed to estimate plant-available P for soils with large variations in physical and chemical properties (Sarkar and O'Connor, 2001). Furthermore, as extraction with these P-sink methods is a mild process conserving the chemical structure of soil, it has been possible, by this way, to make a series of extractions from one soil sample (Indiati and Sharpley, 1996). Depletion of soil P artificially using these methods therefore could simulate the action of P removal by crops in successive cropping experiment, which would normally take many years to realize. Consecutive extraction procedures carried out by these ion sink methods (McKean and Warner, 1996) combined with subsequent fractionation procedure (Tiessen and Moir, 1993) may, therefore, constitute a convenient laboratory method to characterize the P supplying capacity of a soil and to understand which P pools are involved in replenishing the soil solution 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) investigated the desorption kinetics of residual and applied phosphate to red sandy clay soils. 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 in two soils [Alfisols], a red sandy clay soil [Haplo-Palcustafs] and a red sandy loam soil [Pale-Xerults] after P application and incubation. 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. However, information regarding the effectiveness of this modified method on soils which have a long term fertilization history is limited. On top of this, there is still 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 successive P desorption by DMT-HFO followed by a subsequent fractionation method and 2) to investigate wich P pools contribute to the P requirements of maize for some soils with a long term fertilization history.

4.2 MATERIALS AND METHODS

4.2.1 Fertilization history and soil analyses

The sampling procedure and experimental site history of the soil samples used in this experiment are detailed in Sections 3.2.1 and 3.2.2. Table 3.1 shows some selected physical and chemical properties of the different treatments.

4.2.2 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). Detail of this particular step was also documented in Section 3.2.3.

4.2.3 Fractionation procedure

Soil samples were sequentially extracted for P using Tiessen and Moir (1993) method with a slight modification made on the first 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 P fractionation procedure used consists of the following steps: 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 periods (1, 7, 14, 21, 28, 42 & 56 days). This was followed by sequential extractions in the order: (i) 0.5M $NaHCO_3$ at pH 8.5, extracts weakly adsorbed P_i (Hedley et al., 1982) and easily hydrolysable organic P (P_o)-compounds like ribonucleic acids and glycerophosphate (Bowman and Cole, 1978) (labile-P_i and P_o), (ii) 0.1M NaOH, extracts P_i associated with amorphous and crystalline Al and Fe (oxy) hydroxides and clay minerals and P_o associated with organic compounds (fulvic and humic acids). This is designated as slow labile Pi and P_o, (iii) 1.0M HCl extracts P_i associated with apatite or octacalcium P (Frossard et al., 1995) and this represents slow-labile P_i, (iv) Hot concentrated HCl extracts P_i and P_o from more stable pools. Organic P extracted by concentrated HCl may also come from particulate organic matter (Tiessen and Moir, 1993). This represents occluded /recalcitrant/lattice fixed P_i and P_o, (v) 5ml of concentrated H_2SO_4 (approx. 18M) and 2-3 ml of H_2O_2 represents the very recalcitrant P_i and P_o which is considered as a residual P. A separate soil sample (0.5 g) was analyzed for total P content by means of concentrated H_2SO_4 digestion to verify the total soil P determined by summation of all fractions as described by Schmidt et al., (1997). The extracted P was determined in solution according to the colorimetric method described by Murphy and Riley (1962).

4.2.4 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 grain yield (12% moisture content) grown in the same and subsequent years as the soil analysis.

4.2.5 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).

4.3 RESULTS AND DISCUSSION

4.3.1 P recovery and distribution

The total P ($\sum P_i + \sum P_o$) extracted using this fractionation method was compared with the total P obtained by direct method. In the first case average of the total P obtained for day1 and 56 days of extraction was considered. The results showed that treatments

NK, NPK, MNK and MNPK extracted about 102.55, 107.31, 104.32 and 104.51 percent of the total P determined by direct method (Table 4.1).

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). Accordingly, the percentage contributions of labile, less labile and stable fractions varied between 3.02-25.11, 13.58-39.45 and 39.42-82.61 of the total soil 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) for Avalon and Clovelly soils and Ochwoh et al. (2005) for a red sandy clay soil [Haplo-Palcustafs] and a red sandy loam soil [Pale-Xerults] of South Africa. According to Table 4.1, the relative proportion of the stable fraction was largest in the control (NK) and least in the high P treated soil (MNPK). This indicated that over 65 years of continuous cropping resulted in the depletion of the more labile pools in the control (NK) and what is left is predominantly stable in nature. The fact that there was a small decline of P in the stable P pool after the 56-day extraction period indicated that the stable P pool must have contributed to the extracted P. Long-term application of P in the form of fertilizer or manure therefore changed the distribution of P in the P treated soils compared to the control. Hence, the labile and less labile fractions increased relative to the stable form. However, the stable P pool also increased indicating that some of the excess applied P was transformed to the stable P pool.

Comparison of the gain/loss (difference) between day1 and 56 days of extraction for each fraction and all treatments are presented in Table 4.1. The gain/loss was calculated by subtracting the value of day 1 from day 56 for each fraction. The fact that there was a small difference in the total P extracted on day 1 and 56 can be attribute to experimental error. On average, however, more than 96% of the variation could be resulted from P redistribution due to continuous P extraction by DMT-HFO.

4.3.2 Effect of P application level and extraction time on the labile P (DMT-HFO-P_i +HCO_3- P_i+P_o) fraction

4.3.2.1 DMT-HFO-extractable P_i

The amount of P_i extracted by DMT-HFO was significantly influenced ($P \leq 0.05$) both by the levels of P applied and extraction time (Table 4.2). Temporal change of this fraction, however, was not significant for the control. The cumulative P desorbed was higher in the MNPK treated soil (19.83-103.46 mg kg $^{-1}$) and lower in the control NK (0.08-1.13 mg kg $^{-1}$) at all levels of extraction time (1 –56 days). In this study, NPK (4.87 – 19.34 mg kg $^{-1}$) and MNK (5.85 – 18.76 mg kg $^{-1}$) treated soils resulted in a comparable amount of extracted P at all levels of extraction time despite the different source of applied P. This is possibly because P might have been accumulated in inorganic forms in preference to organic forms (Sharpley, et al., 1993; Koopmans et al., 2003). This is evidenced by having nearly similar amount of Bray 1P and total P for both NPK and MNK treated soils (Table 3.1). The P source therefore seemed not to influence the amount of P extracted from both types of treatments. 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 as depicted in

Figure 4.1a though the degree of increment was more pronounced for the high P treatments than the others. 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). 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).

The percentage distribution of DMT-HFO-P_i fraction ranged from 0.02 –0.30, 0.77 – 3.18, 0.89 – 3.07 and 2.24 – 11.84 for NK, NPK, MNK and MNPK treated soils respectively from day 1 to 56 days of extraction time (calculated from Table 4.1). The percent P extracted in all cases was very low as compared to the total P. Similar results have also been reported by other researchers where the proportion of P extracted by DMT-HFO method was low compared to the large amounts of P initially present in the soils investigated (Koopmans et al., 2001; De Jager and Claassens, 2005; Ochwoh et al., 2005).

Table 4.1. Phosphorus content (mg kg^{-1}) in different inorganic (P$_i$) and organic (Po) fractions for the differentially P treated soils

	NK[‡]			NPK			MNK			MNPK		
P fractions	Day 1	Day 56	Difference[§]	Day 1	Day 56	Difference	Day 1	Day 56	Difference	Day 1	Day 56	Difference
DMT-HFO	0.08[†]	1.13	1.05	4.87	19.34	14.47	5.85	18.76	12.91	19.83	103.46	83.63
HCO$_3$Pi	6.33	0.9	-5.43	77	52.72	-24.28	66.48	46.78	-19.7	108.5	70.17	-38.33
HCO$_3$Po	8.04	9.23	1.19	21.11	15.49	-5.62	23.19	36.77	13.58	54.87	45.65	-9.22
Labile	14.45	11.26		102.98	87.59		95.52	102.31		183.2	219.28	
%Labile	**3.80**	**3.02**		**15.87**	**13.71**		**14.53**	**15.83**		**20.73**	**25.11**	
OH-Pi	17.75	11.09	-6.66	116.03	110.67	-5.35	122.82	100.28	-22.54	167.83	145.14	-22.62
OH-Po	28.32	47.36	19.04	51.14	77.31	26.17	74.07	96.17	22.1	80	119.76	39.76
1M HCl-Pi	5.65	2.29	-3.36	41.75	25.67	-16.08	29.99	23.33	-6.66	100.72	44.58	-56.14
Less-labile	51.68	60.67		208.92	213.84		226.88	219.78		348.55	309.55	
%Less-labile	**13.58**	**16.28**		**32.20**	**33.42**		**34.51**	**34.00**		**39.45**	**35.45**	
C/HCl-Pi	52.03	42.63	-9.4	110.9	67.69	-33.76	96.9	61.16	-35.74	106.7	72.75	-27.25
C/HCl-Po	25.22	22.98	-2.24	36	46.22	-9.15	38.31	46.22	7.61	69.32	53.22	-16.1
C/H$_2$SO$_4$-P	237	235	-2	190	223.65	43.65	196.7	216.93	17.42	201.11	215.64	14.53
Stable	314.25	300.61		336.9	337.56		331.91	324.31		377.13	341.61	
%Stable	**82.61**	**80.68**		**51.92**	**52.83**		**50.95**	**50.17**		**41.49**	**39.24**	
$\sum$P$_i$+Po	380.42	372.61	-7.81	648.8	638.85	9.95	654.31	646.4	-7.91	908.88	870.44	-38.48
♦P$_{total}$	367.16			600			623.43			851.22		
($\sum$P$_i$+Po)/ P$_{total}$ (%)	103.61	101.48	-2.13	108.13	106.48	-1.65	104.95	103.68	-1.26	106.77	102.25	-4.52
Average	102.55			107.31			104.32			104.51		

[§] 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}) and 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

Table 4.2 Effect of P levels and extraction time on soil P desorption

P fractions (mg kg-1)	Treatments	Extraction time (days)				
		1	7	14	28	56
HFO-P_i	NK	[‡]**x** 0.08[†]**a**[§]	x1.05a	x1.07a	x1.08a	x1.13a
	NPK	x4.87a	y9.29a	y11.84ab	y13.5ab	y19.34b
	MNK	x5.85a	y10.46ab	y11.91ab	y12.91ab	y18.76b
	MNPK	y19.83a	z60.72b	z73.33c	z87.62d	Z103.47e
HCO3-P_i	NK	x6.33a	x4.11a	x5.46a	x2.29a	x0.88a
	NPK	z77.00a	z69.4ab	y65.92b	y55.73c	y52.72c
	MNK	y66.53a	y55.86b	y58.46ab	y51.51bc	y46.78c
	MNPK	w108.5a	w97.84b	z91.25b	z85.9c	z70.17d
HCO_3-Po	NK	x8.04a	x8.66a	x8.99a	x8.55a	x9.23a
	NPK	y21.11b	y31.09a	y12.32c	y16.75bc	Y15.49bc
	MNK	y23.19b	y23.87a	y18.89b	z35.88a	z36.77a
	MNPK	z54.87a	z49.90ab	z45.35b	w44.88b	W45.65b
NaOH-P_i	NK	x17.75a	x16.58a	x15.68a	x14.47a	x11.09a
	NPK	y116.03a	y117.03a	y105.88a	xy114.17a	Y110.67a
	MNK	y122.82a	y121.80a	y116.59ab	y106.6b	Y100.28b
	MNPK	z167.83a	z160.03a	z154.60b	z150.80b	Z145.14b
NaOH-Po	NK	x28.37a	x25.71a	x26.26a	x31.06a	x47.98a
	NPK	xy51.14ab	y44.4b	y55.79ab	y82.73a	y77.31a
	MNK	yz74.1b	y61.62b	y71.41ab	y76.32ab	yz96.17a
	MNPK	z80.42bc	y57.97c	y69.46bc	y86.58b	Z119.76a
1M HCl-P_i	NK	x5.65a	x6.33a	x3.64a	x3.64a	x2.29a
	NPK	z41.75a	z37.97a	y32.86ab	y26.67b	y25.67b
	MNK	y29.99a	y26.23a	y25.76a	y24.69a	y23.33a
	MNPK	w100.72a	w81.62b	z73.33bc	z65.65c	z44.58d
C/HCl-P_i	NK	x52.3a	x49.78a	x50.31a	x44.48a	x42.63a
	NPK	z110.9a	z93.7b	yz78.90c	yz73.07c	yz67.78c
	MNK	y96.9a	y75.19b	y68.84b	y62.48bc	y61.6c
	MNPK	yz106.7a	z95.31ab	z85.87b	z75.72bc	z72.54c
C/HCl-Po	NK	x25.22a	x17.75a	x11.66a	x18.89a	x22.98a
	NPK	xy36.31a	x16.55c	x21.51b	y33.31bc	y46.22a
	MNK	y38.31ab	y34.39bc	x25.52c	y33.31bc	y46.22a
	MNPK	z69.32a	z47.77b	x32.54c	y35.54c	y53.23b
Residual P	NK	x237.1a	x232.93a	x225a	x239.03a	x235a
	NPK	x190.02a	x211.1a	x227.96a	x217.78a	x223.65a
	MNK	x196a	x203.53a	x203.53a	x241.18a	x216.93a
	MNPK	x200.73a	x202.68a	x202.69a	x222.14a	x215.64a

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

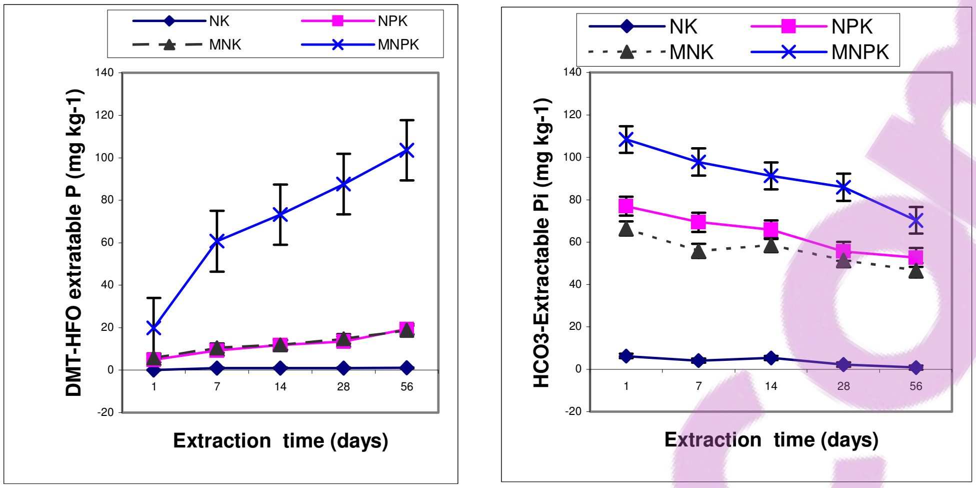
[†] Mean values of three replicates

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

4.3.2.2 0.5M $NaHCO_3$- Extractable P_i

The temporal change of the 0.5M $NaHCO_3$ extractable P_i due to continuous DMT-HFO extraction was significant ($P \leq 0.05$) for all P treated soils as shown in Table 4.2. The amount of this fraction (Table 4.2) ranged from 6.33 –0.88, 77.0 –52.72, 66.53 - 46.71 and 118.44 - 72.33 mg kg^{-1} respectively between 1 and 56 days of extraction for NK, NPK, MNK and MNPK treatments respectively. In general the HCO_3-P_i decreased in the order MNPK>NPK>MNK>NK. The bicarbonate extractable P_i decreased with increasing time of extraction revealing the contribution of this fraction to the solution P depleted by DMT-HFO as shown in Figure 4.1b. Ochwoh (2002) and De Jager and Claassens (2002) also reported similar results for some South African soils, which have been incubated for 6 months and subjected to the same successive extraction by DMT-HFO from 1 to 56 days. However, the amounts extracted are relatively low in both cases as compared to our results possibly due to the low amount of total P in their soils (≅ 400mg/kg) compared to this study (≅ 800 mg/kg).

The percentage distribution of this fraction was 1.68 – 0.23, 12.26 – 8.67, 10.12 – 7.03, and 12.27 – 8.06 for treatments NK, NPK, MNK, and MNPK respectively. As an average of all extraction time, the P treated soils extracted 11(NPK), 9(MNK) and 10.7(MNPK) times as much phosphate extracted from the control. The application of P in the form of fertilizer or manure, therefore, increased the $NaHCO_3$-P_i.



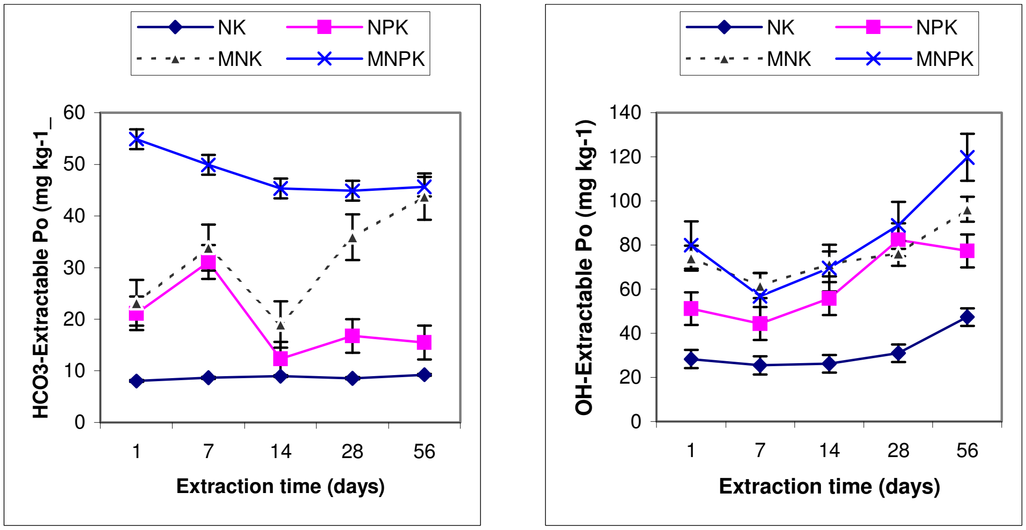
a b

Figure 4.1(a-b) Changes in the cumulative DMT-HFO-P_i (a) and HCO_3-extractable P_i (b) fractions over time. The values in the figures are means of three replicates. Vertical bars represent the standard error

4.3.2.3 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 P treated soils ($P \leq 0.05$) (Table 4.2). The amount of HCO_3-P_o extracted for the respective treatments was NK (8.04-9.23 mg kg^{-1}), NPK (21.11-15.49 mg k^{-1}), MNK (23.19-36.77 mg kg^{-1}), and MNPK (54.57-45.39 mg kg^{-1}) after 1 day and 56 days of extraction. In general the amount of this fraction followed the order MNPK>MNK>NPK>NK. The change in 0.5MHCO_3-extractable Po with time showed a different pattern for the different treatments (Figure 4.2a). The control showed little variation with time. This clearly indicated that the organic material content of the soil was very low and probably no Po was extracted during extraction with DMT-HFO. For the NPK treatment the Po extracted was relatively lower than MNK treatments. This is because this treatment received only inorganic P and the DMT-HFO extraction did not influence the extractable Po significantly especially after 14 days of extraction. The Po extracted from the MNK treatment was higher than the NPK treatment obviously due to the long history of applied Po in the form of manure (Table 3.1). The reason for the increased amount of this fraction with time could be due to microbial immobilization of P (Stewart and Tiessen, 1987). MNPK treated plots showed a reduction in 0.5MHCO_3-extractable Po until the 14th day and remained constant afterwards. The observed general decline in 0.5MHCO_3-extractable Po for soils with MNPK treatment might be due to the relatively high amount of P extracted by the DMT-HFO compared to others. In all other cases, the amount of P extracted by DMT-HFO was less than the Bray-1P except the MNPK treated plots. The involvement of 0.5MHCO_3-extractable Po for MNPK treated soils, therefore, could be to replenish at least in part the P removed by the DMT-HFO (Tables 4.2 and 3.1).

The percentage distribution of HCO_3-extractable P_o was 2.13-2.45, 3.36-2.55, 3.53-5.69 and 6.17- 5.23 for NK, NPK, MNK and MNPK respectively between 1 day and 56 days of extraction. The addition of fertilization in the form of fertilizer or manure therefore increased the 0.5M $NaHCO_3$-Po compared with the unfertilized control (NK). As an average of all extraction time and P levels, the percent 0.5M $NaHCO_3$-extractable P_o was about 3.89. 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).



a b

Figure 4.2(a-b) Changes in the HCO_3-extractable Po(a) and NaOH-extractable P_o(b) fractions over time. The values in the figures are means of three replicates. Vertical bars represent the standard error

4.3.3 Effect of P level and extraction time on the less labile P (0.1MNaOH-P_i +0.1M NaOH-Po+1M HCl-P_i) fraction

4.3.3.1 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 (Table 4.2). The decline of this fraction with time (Figure 4.3a) indicated that this fraction contributed to the soil solution P depleted as the result of extraction by DMT- HFO. The extractable P_i for the NPK and MNK treatments was nearly similar and significantly better than the control. The highest P_i was extracted from the MNPK treatment. The P_i extracted from the control did not significantly alter due to the extraction time with DMT-HFO, indicating that very little DMT-HFO extractable P was available in this fraction. In soil from the MNPK treatment there was a steady decline in the NaOH-P_i indicating that some of this fraction was extracted with DMT-HFO over the 56-day period. The same tendency was observed for the MNK treatment but to a lesser extent. 0.1M NaOH extractable P_i ranged from 13.94- 11.09, 116.03-110.00, 122.82-100.28 and 167.83- 145.21 mg kg^{-1} for NK, NPK, MNK and MNPK respectively between 1 day and 56 days of extraction. This fraction is therefore the second largest fraction of all the P fractions.

As an average of all extraction time, the percent NaOH-Pi contributed 3.33, 18.85, 17.10 and 17.81 for NK, NPK, MNK, and MNPK treated soils respectively. The result of this study was comparable with the results from previous reports especially for the P treated soils. 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 treated soils after 1 day and 56 days of extraction by DMT-HFO. In a similar work done by Ochwoh (2002), 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 of the control in this study could be the inherently lower inorganic fractions due to P depletion over time.

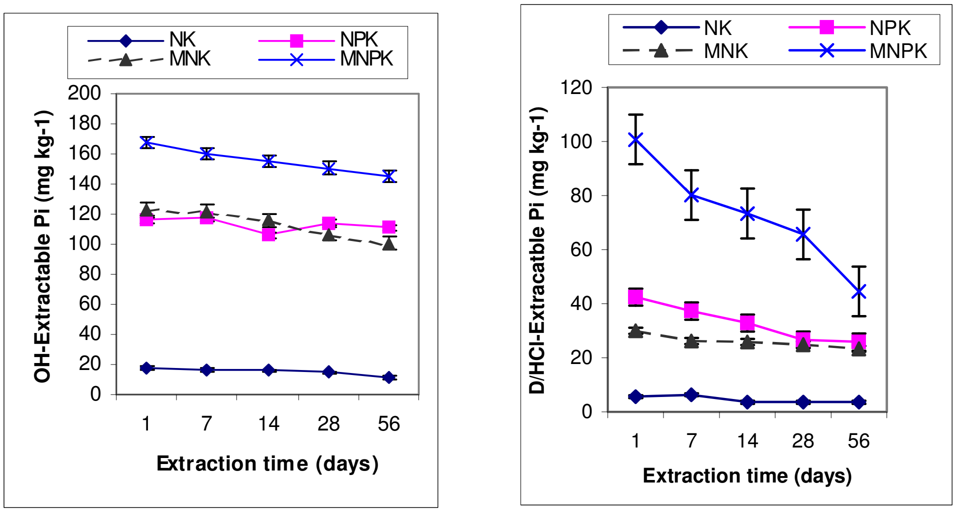
As compared to the control, on average, about 5.5 times more phosphate was extracted from P treated soils. The addition of fertilization in the form of fertilizer or manure, therefore, increased the 0.1M NaOH extractable P_i on the P treated plots.

4.3.3.2 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 4.2). The amount of this fraction ranged from 28.32- 47.36, 51.14-77.31, 74.07-96.17, and 80.42-119.76 mg kg^{-1} for NK, NPK, MNK, and MNPK respectively after 1 day and 56 days of extraction by DMT-HFO. There were significant increases in extractable NaOH Po due to increasing of P application compared to the control. The increased Po extracted from the NPK treatment that did not received any organic P could be due to the higher yields obtained from this treatment compared to the control and the subsequent higher additions of organic material including P from the crops roots. The amount of extractable OH-P_o followed the order NK<NPK<MNK<MNPK. In all treatments the OH-P_o extracted increased with time of extraction (Figure 4.2b). The reason for the increased amount of this fraction could be due to microbial immobilization of P (Stewart and Tiessen, 1987) or possibly due to the removal of NaOH-P_i and the subsequent dissolution of Po that could be extracted with NaOH.

Soil P_o has been recognized as a significant source of available P particularly for grassland and forest soils (Gracia-Mounteil et al., 2000) where as for soil with a long-term fertilization history the contribution of P_o to the crop-available P pool seems rather limited. Examining the ratio of $NaHCO_3$-P_o to NaOH-P_o served as a means to determine whether the P_o can be a source for available P (Kuo et al, 2005). Where P_o was an important source of available P for crops (Hedley et al., 1982; Tiessen et al., 1984; Zhang and Mackenzie 1997b), the ratio of $NaHCO_3$-P_o to NaOH-P_o was high

(25.23%). Where the ratio is <10%, the contribution of P_o to plant available P could presumably be less important (Schmidt et al. 1997; Kuo et al. 2005). Based on this, the ratio was found to be >30% for this study and the contribution of P_o to plant available P could, therefore, be important especially in the long-term when the current inorganic P gets exhausted to induce Po mineralization.



a

b

Figure 4.3(a-b) Changes in the 0.1M NaOH-extractable Pi and D/HCl-extractable P_i fractions over time. The values in the figures are means of three replicates. Vertical bars represent the standard error

As an average of all extraction time, the percent NaOH-Po contributed 10.03, 10.43, 13.06 and 11.44 of the total P for NK, NPK, MNK, and MNPK treated soils respectively. The percentage distribution of OH-P_o therefore followed the order: MNK>MNPK>NPK>NK. However, there seemed to be no big difference on the percent recovery of this fraction from P treated soils as compared to the control.

4.3.3.3 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 4.2). However extraction time did not influence the extractable Pi for the NK and MNK treatments significantly. In both these treatments is was obvious that it did not contribute to the Pi extracted with DMT-HFO. The NPK and MNPK treatments contributed significantly to the DMT-HFO extractable P. 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 much more pronounced on the P received plots than the control as depicted in Figure 4.3b. The amount of extracted 1M HCl-P_i was in the range from 5.79-2.29, 41.75-25.67, 29.99-23.33, and 100.71-44.58 mg kg^{-1} for the plots treated in NK, NPK, MNK, and MNPK respectively after 1 day and 56 days of extraction.

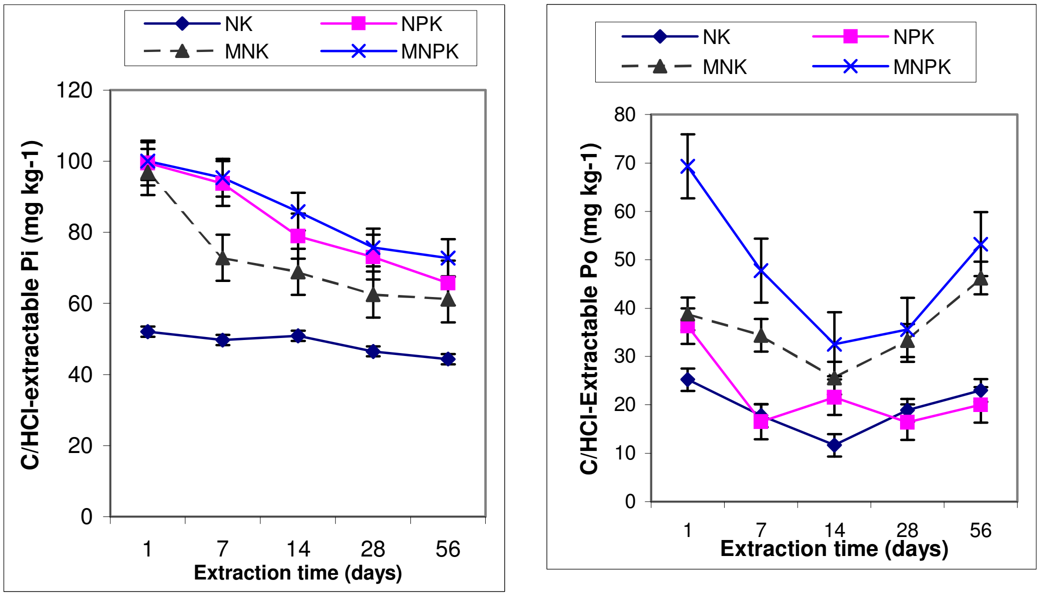
The P_i extracted by this extractant from P received plots is 5.08, 3.82, and 7.71 times as much compared to the control for NPK, MNK and MNPK respectively. The addition of fertilization in the form of commercial fertilizer or manure therefore significantly increased this fraction as compared with the unfertilized control. The contribution of this fraction was on average 6% for all P treated soils. Du Preez and Claassens (1999) reported <1% of contribution to the total P of this fraction for the Clovelly soil. While other similar studies revealed 5-8% contribution of this fraction to the total P (Hedley et al., 1982; Sattel and Morris, 1992; Ochwoh et al., 2005).

4.3.4 Effect of P level and extraction time on the stable P (C/HCl-P_i +C/HCl Po+C/H_2SO_4 + H_2O_2 -P) fraction

4.3.4.1 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 4.2). The amount extracted by this extractant (mg kg^{-1}) varied from 52.30-42.62, 110.90-67.78, 96.90-61.60 and 106.69-72.54 for treatments NK, NPK, MNK and MNPK respectively after day 1 and 56 days of extraction. This fraction is the third largest fraction of all. Besides, the decrease in this fraction (Figure 4.4a) with increased time of extraction by DMT-HFO was the largest of all both for the control and the P treated soils. This clearly indicated that this fraction contributed significantly to the P extracted by DMT-HFO. This also suggests that this fraction may be a buffer to more labile P fractions in the long-term.

Averaged over all extraction time, the percent C/HCl-P_i constituted 12.63, 14.40, 12.12 and 10.19 for NK, NPK, MNK, and MNPK treated soils respectively. The contribution of this fraction is on average 12.33% for all soils. Ochwoh (2002) reported between 15-25% contribution of this fraction to the total P for Loskop and Rustenburg soils of South Africa.



a b

Figure 4.4 (a-b) Changes in the C/HCl-Extractable P_i and C/HCl-Extractable P_o fractions over time. The values in the figures are means of three replicates. Vertical bars represent the standard error

4.3.4.2 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 4.2). The amount extracted by this extractant (mg kg^{-1}) varied from 25.22-22.98, 36.31-20.03, 38.31-46.22, and 69.32- 53.23 for treatments NK, NPK, MNK and MNPK respectively after 1 day and 56 days of extraction. The C/HCl-P_o showed a decreasing tendency until 14 days of extraction followed by slight increment as the days of extraction increased as shown in Figure 4.4b. The reason for a slight increment of C/HCl-P_o at the later days of extraction could be attributed to microbial immobilization of P (Stewart and Tiessen, 1987).

As an average of all extraction time, the percent of this fraction constituted 6.41, 4.45, 6.49 and 4.58 for NK, NPK, MNK, and MNPK treated soils respectively. Averaged over all extraction time and treatments, the contribution of this fraction to the total P was 4.23%, which is nearly comparable to the reports made by other researchers. Ochwoh (2002) reported 2-4% contribution of this fraction to the total P. Du Preez and Claassens (1999) reported 6.4-8.5% and 1.6-3.4% contribution to the total P for Avelon and Clovelly soils respectively. The C/HCl-P_o extracted by Hedley et al. (1982) was also found to be 3%. Bashour et al., (1985) however reported that the contribution made by this fraction ranged from 0-26.7%.

4.3.4.3 C/H_2SO_4 + H_2O_2 extractable P

This fraction showed no statistically significant differences for both extraction time and P level (Table 4.2). The fact that no significant decrease in extractable P took place after extraction with DMT-HFO over the 56-day period indicated that it contributed very little to the available 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 Santos et al., (2006) on the study made on Cerrado soils. They observed that on average terms, the residual fraction corresponded to half of the recovered total P in all soils regardless of the source of applied P and method of applications.

Averaged over all extraction time, the percentage distribution of this fraction was 66.34, 33.40, 31.68 and 23 53 for NK, NPK, MNK and MNPK respectively. The P treated soils therefore showed less proportion (20-30%) of this fraction than the control where more than 60% of the pool was C/H_2SO_4 + H_2O_2 extractable P. In this regard, the result of this study concurs with the result of Ocwoh et al. (2005), as the contribution of this fraction ranged between 20-25% to the total P pool for the P treated soils they investigated.

4.3.5 Plant growth as related to phosphorus fractions

The amount of P extracted by the different extractants (including total P) was also correlated with yield as illustrated in Table 4.3. This comparison was made between the cumulative DMT-HFO extractable P, the subsequent fractions and maize yield. The same kind of comparison was also made between Bray1P and maize yield. Significant correlations were observed between maize grain yield and all the P pools and the total P except DMT-HFO-P_i (r=0.58) and HCO_3-P_o (r=0.77). A significant correlation was also observed between maize grain yield and Bray1P (r = 0.84^{*}). Cajuste et al., (1994) reported highly significant correlations between Bray-1P and the different P fractions for oxisol and alfisol soils they studied under laboratory conditions. Unlike the correlation between DMT-HFO-P_i and yield, the correlation of the former with Bray-1P was found to be highly significant (r = 0.95^{**}). This observation probably indicates the ability of these extractants to extract the labile P. A possible explanation for the observed difference between extractants DMT-HFO and Bray-1P could be obtained by comparing the amount of P extracted by both extractants as depicted in Table 3.1. NK and MNPK treated soils released roughly similar amount of P by both extractants where as NPK and MNK desorbed a DMT-HFO-P_i extract, which was nearly half extracted by Bray-1P. The relatively lower amount of P desorbed by these treatments could be a possible reason for the poor correlation observed between DMT-HFO-P_i and maize grain yield.

Table 4.3. Correlations among the cumulative P desorbed over 56 day period, the subsequent fractions, Bray 1P(mg kg $^{-1}$) and maize grain yield (t ha^{-1}), N=4

P extracts (mg kg^{-1})	Yield (t ha^{-1})	Bray 1P (mg kg^{-1})	Change in P fraction(mg kg $^{-1}$)	Yield (t ha^{-1})
HFO-P_i	0.58	0.92^{**}	ΔHFO-Pi	0.59
HCO_3-P_i	0.96^{**}	0.95^{**}	ΔHCO_3-P_i	-0.85^{**}
HCO_3-P_o	0.76^{*}	0.83^{**}	ΔHCO_3-P_o	-0.08
OH-P_i	0.96^{**}	0.95^{**}	ΔOH-P_i	-0.62
OH-P_o	0.88^{**}	0.95^{**}	ΔOH-P_o	0.62
D/HCl-P_i	0.88^{**}	0.99^{**}	ΔD/HCl-P_i	-0.54
C/HCl-P_i	0.95^{**}	0.93^{**}	ΔC/HCl-P_i	-0.92^{**}
C/HCl-Po	0.98^{**}	0.92^{**}	ΔC/HCl-Po	-0.21
Total P	0.88^{**}	0.99^{**}		
Bray-1P	0.84^{*}	-		

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

Correlation between P fraction decrease and maize grain yield was also done. The change in P fraction can be calculated as the difference between day1 and 56 days of DMT- HFO-P extraction (Table 4.1). The correlation between the change in P of each fraction and dry maize grain yield was also made as illustrated in Table 4.3. The only two fractions that showed strong and highly significant correlation with grain yield were HCO_3-P_i (r = -0.85**) and C/HCl-P_i (r = -0.92**). All the other fractions were not significant. Changes in the inorganic fractions with time revealed the decreasing tendency of these fractions with time as depicted in Figures 4.1b – 4.3b and 4.4a although the degree of contribution differed from one fraction to the other. The values in Table 4.3 indicate the importance of the inorganic fractions especially $NaHCO_3$-$P_{i,}$ NaOH-P_i and C/HCl-P_i in replenishing the soil solution P than the organic fractions. From the inorganic fractions, C/HCl-P_i was the fraction that decreased most suggesting that this fraction may be the major P source to buffer the 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 and Claassens, 2005; De Jager and Claassens, 2005). The present investigation 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.

4.4 CONCLUSIONS

In this 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 soil solution P at this stage. They could act as a source of P only in the very long term when the inorganic P becomes too low to induce Po mineralization. The amount of P extracted by the different fractions in general followed the order MNPK>NPK>NPK>NK for inorganic fractions whereas for the organic fraction the order appeared to be MNPK>MNK>NPK>NK. Highly significant correlations were observed between maize grain yield and the different P fractions including total P. The correlation between the change in P of each fraction and maize grain yield was highly significant for the fractions HCO_3-P_i ($r = -0.85^{**}$) and C/HCl-P_i ($r = -0.92^{**}$). From the inorganic fractions, C/HCl-P_i was the fraction that decreased most suggesting the importance of this fraction in replenishing the labile P fractions.

CHAPTER 5 [*]

Effect of shaking time on long-term phosphorus desorption using dialysis membrane tubes filled with hydrous iron oxide

5.1 INTRODUCTION

Several investigators showed that continuous application of phosphorus (P) either in the form of fertilizer or manure over a long-term can accumulate large amounts of residual P. This is principally due to the low amount of P removed from a field by crops, which in general varies from 3-33% of applied P fertilizer (Bolland & Gilkes, 1998; Csatho et al., 2002; Aulakh et al., 2003; Kamper & Claassens, 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). As depletion of the soil can take many years to study in the field or green house studies, which makes it very expensive and time consuming, more rapid soil extractions methods are required to assess the effect of P addition on the rate of P decrease in available soil P. One promising method uses ion sinks such as Fe-oxide impregnated filter paper strips that can act as infinite sinks for soil P release (Sharpley, 1996; McDowell and Sharpley, 2002). The Fe-oxide strips have a better theoretical basis for estimating plant available P in different soil types than chemical extractants (Sarkar and O'Connor, 2001; Hosseinpur and Ghanee, 2006). This method however has two major drawbacks making it unsuitable for studying long-term P desorption from soils. First,

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the paper strips are mechanically unstable during longer desorption times (weeks), leading to relatively large losses of the P sink to the soil sample. Second, fine P rich particle adhere to the filter paper during every desorption step resulting in an overestimation of the amount of P desorbed, since any P associated with these particles is accounted for as desorbed after analyzing the filter paper (Freese et al., 1995; Lookman et al., 1995).

The use of dialysis membrane tubes filled with hydrous ferric oxide has recently been reported as an effective way to characterize long-term P desorption (Freese et.al., 1995). This method is similar to Fe-oxide impregnated filter paper strips but in this case the HFO is placed in a dialysis membrane tube instead of being impregnated in the filter paper. This has the advantage of not allowing strong chemicals to come in to contact with the soil. This system is mechanically stable and capable of maintaining low P activity in solution for longer period of time ,and, therefore, P release over long periods of time can be measured in a more natural environment than a routine soil tests (Freese et al., 1995; Lookman et al., 1995).

In studies to relate extraction methods with plant availability, the study of root systems especially in connection with the percent exploitation of the soil volume is important. Recent works related to the percent root exploitation of the soil volume revealed that 3-4% of the top soil volume was exploited at full maturity of a maize crop. The value was as low as 1% during the first two weeks, when most P uptake was anticipated to occur (Smethurst, 2000; Kamper & Claassens, 2005). However, the DMT-HFO method, similar to other soil tests, exploits 100 percent of the sample volume that is much more than the percent root exploitation of plants. Therefore, exploiting the whole volume of the soil by continuous shaking, as has been done in this technique, may not simulate the plant mode of action very well. One possible solution to simulate the root P uptake could be by modifying the shaking procedure using different shaking periods. The objectives of this paper were to investigate the influence of shaking time variation on the P desorbed by DMT-HFO and to relate the desorption indices generated with maize yield.

5.2 MATERIALS AND METHODS

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

5.2.1 Long-term Phosphate desorption experiment

The procedure in this section is also detailed in Section 3.2.3.

5.2.2 Modification of the shaking time

The shaking period was adjusted to investigate the influence of the different shaking periods on the amount of P extracted and to identify which shaking option better mimics the plant mode of action.This was done by comparing the conventional approach which served as a control with the modified approaches. Continous shaking for 1, 7, 14, 28, and 56 days, which is the usual approach, was assumed to be a conventional approach. The modification was then carried out by reducing the length of shaking time by certain percentages such as 25%, 50% and 75% of the control. For example, if the shaking period is shortened by 25%, then the shaking procedure will assume a different pattern. So instead of shaking for 1, 7, 14, 28 and 56 days continously it will be shaken for ¾, 5¼, 10½, 21and 42 days continously. This is equivalent to 75% of the respective shaking times of the control. The following shaking options were considered. Option 1 was the conventional approach which served as a control. Options 2, 3 and 4 are the modified approaches continously shaken for 75%, 50% and 25% of the control respectively. Table 5.1 indicates the different possibilties one can obtain by considering the different shaking options.

Table 5.1 The different shaking patterns according to the conventional and modified approaches

Shaking time (days) §Conventional approach	Shaking time (days) ‡Modified approach		
Option1 Control	Option2 75%	Option3 50%	Option4 25%
1	0.75	0.5	0.25
7	5.25	3.5	1.75
14	10.5	7	3.5
28	21	14	7
56	42	28	14

§Conventional approach is the continuous shaking time for 1, 7, 14, 28, & 56 days; control

‡Modified approach is a continuous shaking for 75%, 50%, & 25% of the conventional approach for the shaking options 2, 3, and 4 respectively.

5.2.3 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 grain yield (12% moisture content).

5.2.4 Data Analysis

The data obtained were analyzed by using Statistical Analysis System Program (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 rate constants k_A and k_B values were determined from equation [6] described in section 2.1.1 by splitting the solid phase P in to two pools: labile pool, Pool A and the less labile pool, Pool B as described by Lookman et al. (1995). Correlation with the plant yield parameter was done using Pearson linear correlation, PROC CORR (SAS Institute 2004).

5.3 RESULTS AND DISCUSSION

5.3.1 DMT-HFO-P_i

The effect of varying shaking options on the extractable DMT-HFO-P_i for each treatment is illustrated on Table 5.2. No statistically significant differences were observed for all treatments amongst the different shaking options except treatment MNPK. These treatments received high P and resulted in relatively large amount of P at all extraction periods. The relatively high releasing capacity of these soils might have contributed to the difference shown on these treatments. The amount of P extracted was found to be consistent with the time of extraction for all treatments in all four shaking options. Thus, in general the pattern of release followed the order: option1>option 2> option3>option 4, consistent with the general expectation that the amount of P extracted by a given extractant increases with increasing time of extraction (Damodar et al., 1999; Pasricha et al., 2002).

The cumulative amount of P (mg kg^{-1}) desorbed over a 56-day period of extraction ranged from 1.74-1.57(NK), 23.61- 15.69 (NPK), 21.48- 15.7 (MNK) and 132.81-103.97 (MNPK) for shaking options 1 to 4 (Table 5.2). Cumulative P released with time followed, in general, the same pattern for all shaking options and in all P treatments, with an initial rapid release of P that continued up until 14 days of extraction (Option 1-3) and 7 days of extraction (option 4) followed by a slower release that was still continuing after the respective 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).

Table 5.2. The effect of different shaking options on the extractable DMT-HFO-Pi for different P levels

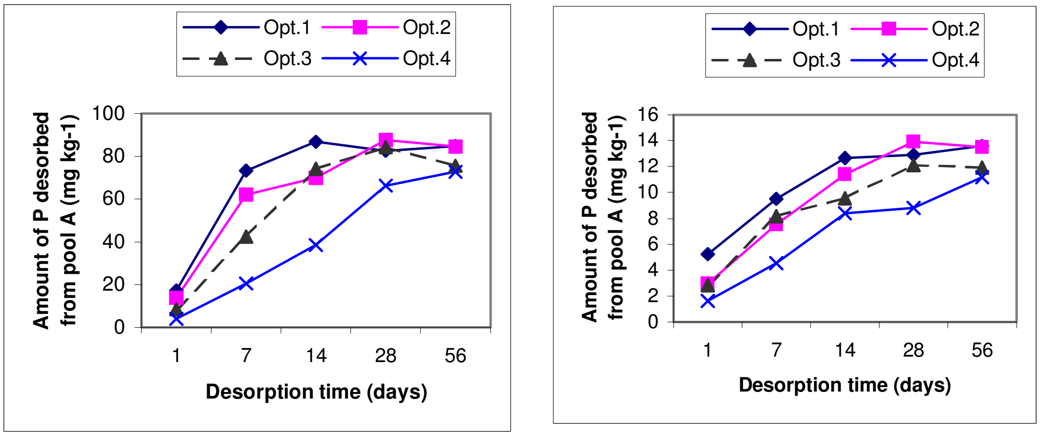
		Percentages of continuous shaking			
	Conventional approach	100% (Control)	75%	50%	25%
Treatment	Ext time	Opt1	Opt2	Opt3	Opt4
NK	1	[§]x1.47[†]a[‡]	x1.41a	x1.36a	x1.28a
	7	x1.54a	x1.56a	x1.54a	x1.52a
	14	x1.57a	x1.57a	x1.54a	x1.54a
	28	x1.62a	x1.6a	x1.57a	x1.54a
	56	x1.74a	x1.69a	x1.62a	x1.57a
NPK	1	x4.59a	x3.03a	x2.19a	x1.8a
	7	x11.31a	x10.56a	x7.99a	x5.3a
	14	x15.69b	x11.73b	x11.31b	x7.99a
	28	x19.44b	x17.46bc	x15.69bc	x11.31b
	56	x23.61b	x21.37c	x21.37c	x15.69b
MNK	1	x6.15a	x3.18a	x3.18a	x1.98a
	7	x11.6a	x8.27ab	x9.46ab	x5.58a
	14	x15.76b	x13.39bc	x11.6bc	x9.47ab
	28	x17.55bc	x16.85c	x15.76bc	x11.6b
	56	x21.48c	x18.56c	x17.55c	x15.76b
MNPK	1	z18.87a	yz14.56a	xy8.97a	x4.52a
	7	w 85.8b	z67.21b	y46.01b	x24.03b
	14	z103.97c	y78.14c	y85.8c	x46.01c
	28	y108.85c	y105.17d	y103.97d	x85.80d
	56	z132.81d	y115.63e	x108.85d	x103.97e

[§] 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, c, d, and e are significantly different ($\alpha = 0.05$).

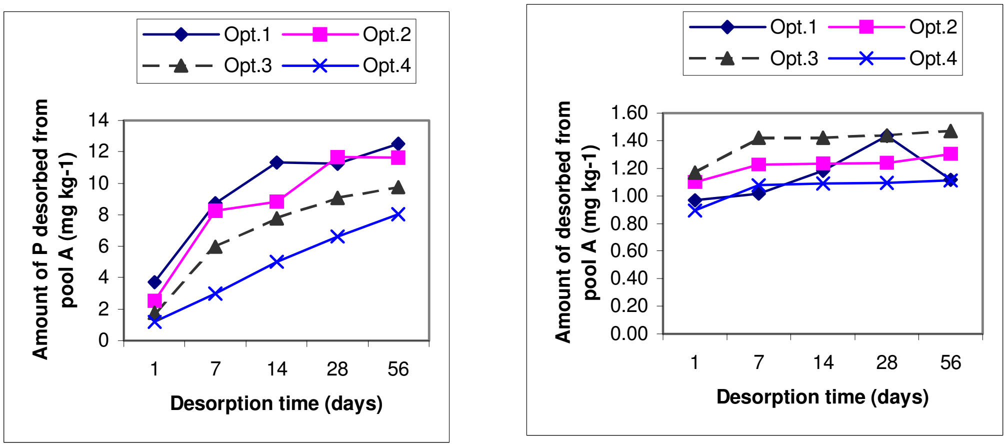
[†] Mean values of three replicates

The contributions of both SP_A and SP_B to the total P extracted varied among treatments and shaking options following the order: MNPK>>NPK≈MNK>>NK (Figure 5.1a-d for SP_A and Figure 5.2a-d for SP_B). This is in accordance with the total P content of the treatments (Table 3.1). The higher the P status of the soil, the greater was the contribution made by both SP_A and SP_B. This could be attributed to higher degree of P saturation of the adsorption sites with increasing P status of the soil (De Jager and Claassens, 2005). Toor et al., (1999) also reported the higher P desorption rate in fertilizer and manure treated soils. In their investigation, manure appeared to play significant role in enhancing the P desorption possibly due to complexation of Fe and Al ions. The change of these pools with time in general varied in the same way. The contribution of SP_A increased with time for all P levels and shaking options as well. The only exception noted was for MNPK treated soils where by the contribution of SP_A consistently increased with time only for option 4 (note that the maximum period of extraction according to this option is only 14 days!) but started to decline for options 1-3 (Figure 5.1a-d). This indicates that the contribution of this pool is more pronounced only to short desorption period. On the other hand, the contribution made by the slowly released pool, SP_B, increased with time, the degree of increment being higher at the latter extraction time, revealing the predominant role played by this fraction in replenishing the soil solution P in long-term desorption studies (Figure 5.2a-d). The control resulted in negligible variation in this respect too for the reason reported previously.



a) MNPK

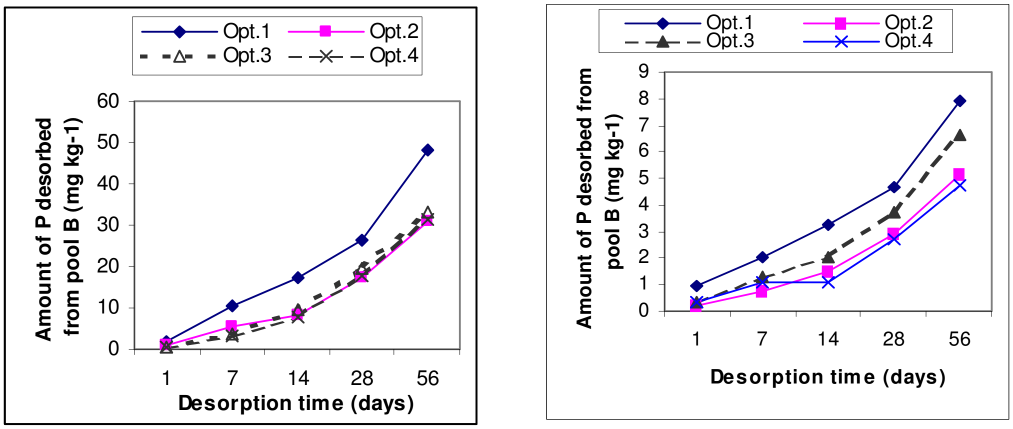
b) MNK



c) NPK

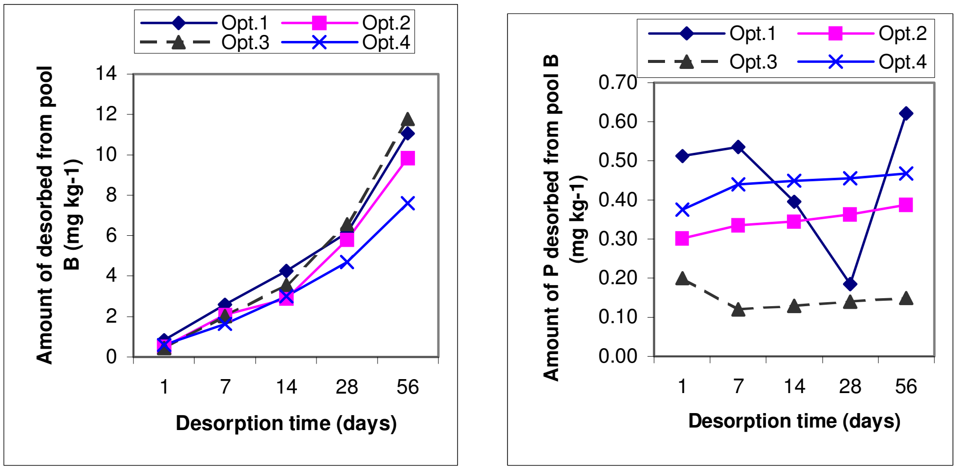
d) NK

Figure 5.1(a-d). Simulated P desorption from pool A (SP_A) of the different P treatments and shaking options



a) MNPK

b) MNK



c) NPK

d) NK

Figure 5.2 (a-d). Simulated P desorption from pool B (SP$_B$) of the different P treatments and shaking options

5.3.2 Plant growth as related to phosphorus desorption kinetics

Correlations between the rate coefficients k_A and k_B (day $^{-1}$) with maize grain yield (t ha $^{-1}$) for the different shaking options were made and the results are presented in Table 5.3. The rate coefficients for the different shaking options are labeled as illustrated on Table 5.3. Significant correlations were obtained between the labile pool rate coefficients [k_{A1} (0.92**), k_{A2} (0.99**), k_{A3} (0.92**) and k_{A4} (0.92**)] and maize grain yield. The labile pool represents the P pool with fast release kinetics that comprises presumably primarily P bound to the reactive surfaces that is in direct contact with the aqueous phase. This pool is presumed to be easily available to plants in a reasonably short period of time (Lookman et al., 1995). Comparing the values of the rate coefficients for this pool revealed that the rate coefficient for option 2 resulted in the best correlation with maize grain yield. The role of this pool in general enhanced with decreasing desorption time corroborating the pronounced contribution of this pool for short desorption studies. The only rate coefficient from the less labile pool, k_B , which showed a significant but moderate correlation ($r = 0.78^{*}$) with corn grain yield, was only k_{B1}. This pool represents the P pool with slow release kinetics that results from slow dissolution kinetics or from slow diffusion from the matrix of sesquioxide aggregates (Koopmans et al., 2004). This pool will be available only over a long period of time and that is probably why the correlation was strong only in the case of option 1 which exhibited the longest desorption period. This evidenced that the role of this pool appeared to be much less pronounced with decreasing time of desorption.

Albeit the P pools are theoretically grouped in to these two discrete pools for the sake of convenience, they are presumed to involve simultaneously in the uptake process as reported previously. It is therefore important to take in to account the sum of the rate constants when such correlations are made. The sum of the rate constants (k_A+k_B) in general showed significant correlations with maize grain yield in all shaking options considered as depicted on Table 5.3. The rate coefficient for the labile fraction, $k_{A,}$ strongly correlated with the sum of k_A and k_B (k_A+k_B) unlike the less labile fraction, $k_{B,}$ revealing the predominant contribution of the labile P fraction in replenishing the soil solution P than the less labile form in all the options considered at least for the extraction period considered in the present study.

The cumulative amount of P extracted by the DMT-HFO was also correlated with yield and Bray 1P as depicted in Table 5.4. Both the cumulative amount of P (mg kg^{-1}) extracted by DMT-HFO and the change in DMT-HFO-P_i (mg kg^{-1}) showed no statistically significant correlations with maize grain yield in all the options considered. However, option 2 seemed to correlate better in both cases as judged from their r-values. Statistically significant correlations were observed between DMT-HFO-P_i and Bray-1P in all shaking options for both cases. This observation probably indicates the ability of these extractants to extract the labile P. Bray-1P has also showed a significant correlation with maize yield. Although the correlation between DMT-HFO-P_i and Bray-1P was found to be very strong and statistically significant, the correlation each showed with maize yield was apparently opposite, the former resulted in no correlation for all the shaking procedures considered in the present study, while the latter resulted in moderately strong and significant correlation with the yield parameter.

Table 5.3 Pearson correlations between the rate coefficients k_A, k_B, and $k_A + k_B$ with dry maize grain yield for the different options, N=4

♦ Option1					Option2				
	Yield	K_{A1}	K_{B1}	$K_{A1}+K_{B1}$		Yield	K_{A2}	K_{B2}	$K_{A2}+K_{B2}$
K_{A1}	0.92^{**}	-	0.73	0.99^{**}	K_{A2}	0.99^{**}	-	0.41	0.99^{**}
K_{B1}	0.79^{*}	0.73	-	0.76	K_{B2}	0.43	0.41	-	0.44
$K_{A1}+K_{B1}$	0.93^{**}	0.99^{**}	0.76	-	$K_{A1}+K_{B2}$	0.99^{**}	0.99^{**}	0.44	-
Option3					Option4				
	Yield	K_{A3}	K_{B3}	$K_{A3}+K_{B3}$		Yield	K_{A4}	K_{B4}	$K_{A4}+K_{B4}$
K_{A3}	0.92^{**}	-	0.51	0.99^{**}	K_{A4}	0.92^{**}	-	0.40	0.99^{**}
K_{B3}	0.59	0.51	-	0.55	K_{B4}	0.60	0.40	-	0.48
$K_{A3}+K_{B3}$	0.93^{**}	0.99^{**}	0.55	-	$K_{A4}+K_{B4}$	0.94^{**}	0.99^{**}	0.48	-

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

♦ Option 1 represents the control; Options 2, 3, and 4 represent continuous shaking for 75%, 50%, & 25% of the control.

A possible explanation for the observed difference between the two extractants could be obtained by comparing the amount of P extracted by both extractants as depicted in Table 3.1. NK and MNPK treated soils released roughly similar amount of P by both extractants where as NPK and MNK desorbed a DMT-HFO-P_i extract, which was nearly half extracted by Bray-1P. The relatively lower amount of P desorbed by these treatments could be a possible reason for the poor correlation observed between DMT-HFO-P_i and maize grain yield.

Table 5.4 Pearson correlations between the cumulative DMT-HFO-P_i (mg kg^{-1}) and the change in DMT-HFO-P_i (mg kg^{-1}) with dry maize grain yield, N=4

§Cumulative DMT-HFO-P_i (mg kg^{-1})					‡ ΔDMT-HFO-P_i (P max- P min)(mg kg^{-1})			
♦Option1					Option1			
	Yield	DMT-HFO-P_i	Bray-1P			Yield	DMT-HFO-P_i	Bray-1P
DMT-HFO-P_i	0.57	-	0.92^{**}		DMT-HFO-P_i	0.56	-	0.91^{**}
Bray-1P	0.84^{*}	0.92^{**}	-		Bray-1P	0.84^{*}	0.91^{**}	-
Option 2					Option 2			
	Yield	DMT-HFO-P_i	Bray-1P			Yield	DMT-HFO-P_i	Bray-1P
DMT-HFO-P_i	0.58	-	0.92^{**}		DMT-HFO-P_i	0.57	-	0.92^{**}
Bray-1P	0.84^{*}	0.92^{**}	-		Bray-1P	0.84^{*}	0.92^{**}	-
Option 3					Option 3			
	Yield	DMT-HFO-P_i	Bray-1P			Yield	DMT-HFO-P_i	Bray-1P
DMT-HFO-P_i	0.58	-	0.92^{**}		DMT-HFO-P_i	0.54	-	0.90^{**}
Bray-1P	0.84^{*}	0.92^{**}	-		Bray-1P	0.84^{*}	0.90^{**}	-
Option 4					Option 4			
	Yield	DMT-HFO-P_i	Bray-1P			Yield	DMT-HFO-P_i	Bray-1P
DMT-HFO-P_i	0.55	-	0.90^{**}		DMT-HFO-P_i	0.55	-	0.90^{*}
Bray-1P	0.84^{*}	0.90^{**}	-		Bray-1P	0.84^{*}	0.90^{*}	-

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

♦ Option 1 represents the control; Options 2, 3, and 4 represent continuous shaking for 75%, 50%, & 25% of the control.

§ Cumulative P extracted by DMT-HFO, which is the P extracted after 56, 42, 28 and 14 days of extraction for options 1,2,3 & 4 respectively.

‡ The change in P (ΔDMT-HFO-P_i) calculated as the difference between the maximum and minimum extraction time for each options (56 vs.1, 42 vs. 0.75, 28 vs. 0.5 and 14 vs. 0.25 days).

In general, the correlation values among the rate coefficients and the cumulative P extracted by DMT-HFO with maize grain yield revealed that option 2 seemed relatively better than the other options. However, according to this study, the rate coefficients appeared to be better indices of plant availability than the amount of P extracted by DMT-HFO, as the latter showed no significant correlation with maize grain yield. In this research correlation with other plant yield parameters such us P uptake and relative plant response was not conducted due to lack of relevant data. More work relating these plant indices with desorption indices is therefore required. Data from a wider range of soils is also needed to evaluate the universality of this method.

5.4 CONCLUSIONS

The effect of varying shaking options on the extractable DMT-HFO-P_i for each treatment showed no statistically significant difference for all treatments except the MNPK treated plots. The amount of P extracted was found to be consistent with the time of extraction for all treatments in all four options: option1>option2> option3>option 4 which is consistent with the general expectation that the amount of P extracted by a given extractant increases with increasing time of extraction.

From the results observed by relating rate coefficients and the cumulative P extracted by DMT-HFO with maize grain yield, the rate coefficients appeared to be better indices of plant availability than the amount of P extracted by DMT-HFO, as the former only showed significant correlation with maize grain yield. Thus, based on the r values, option 2 seemed relatively better than the others since it showed the strongest correlation. In this research correlation with other plant yield parameters such us P uptake and relative plant response was not conducted due to lack of relevant data. More work relating these plant indices with desorption indices is therefore required. Besides, data from a wider range of soils is also needed to evaluate the universality of this method.

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.

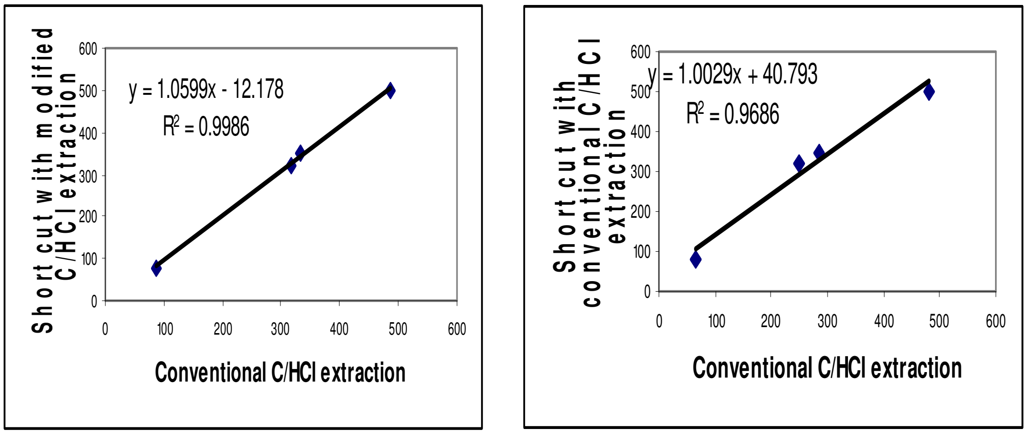
Table 6.1 Amount of C/HCl extracted P_i (mg kg^{-1}) for the different treatments according to the modified methods and the conventional approach of Tiessen and Moir (1993)

Treatment	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})
	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)

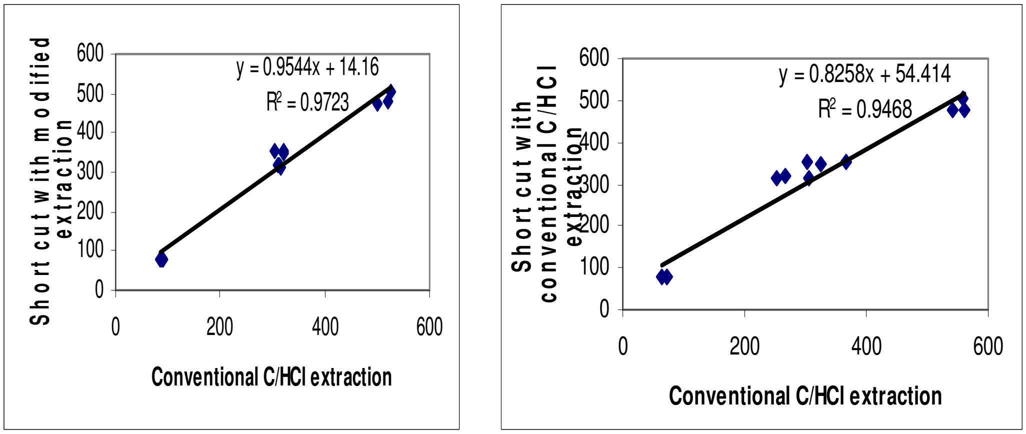
‡$\sum P_i$ refers to $\sum$DMT-HFO-P_i+$NaHCO_3$-P_i+NaOH-P_i+D/HCl-P_i+C/HCl-P_i

* 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	[§]x1.47[†]**a**[‡]	**x** 4.59**a**	**x** 6.15**a**	**y** 18.87**a**
7	x1.54**a**	**y**11.31**ab**	**y** 11.6**ab**	**z** 85.8**b**
14	x1.57**a**	**y**15.69**bc**	**y** 15.76**bc**	**z** 103.97**c**
28	x1.62**a**	**y**19.44**bc**	**y** 17.55**bc**	**z** 108.85**c**
56	x1.74**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

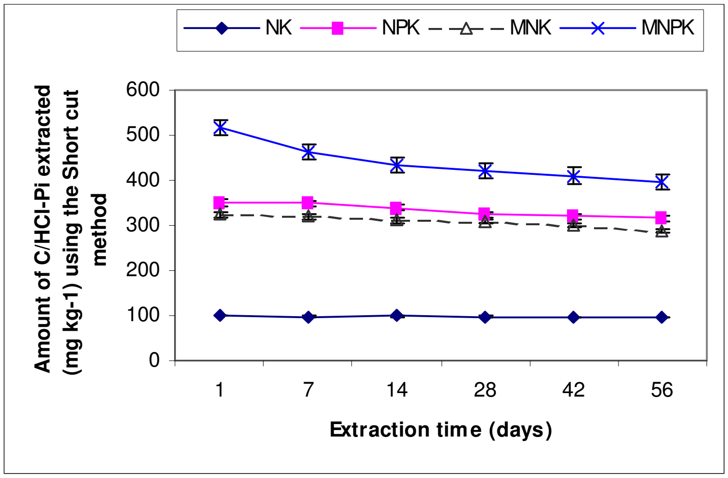


Figure 6.3 Changes in the C/HCl-extractable P_i with time. The values in the figure are means of three replicates. Vertical bars represent the standard error.

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

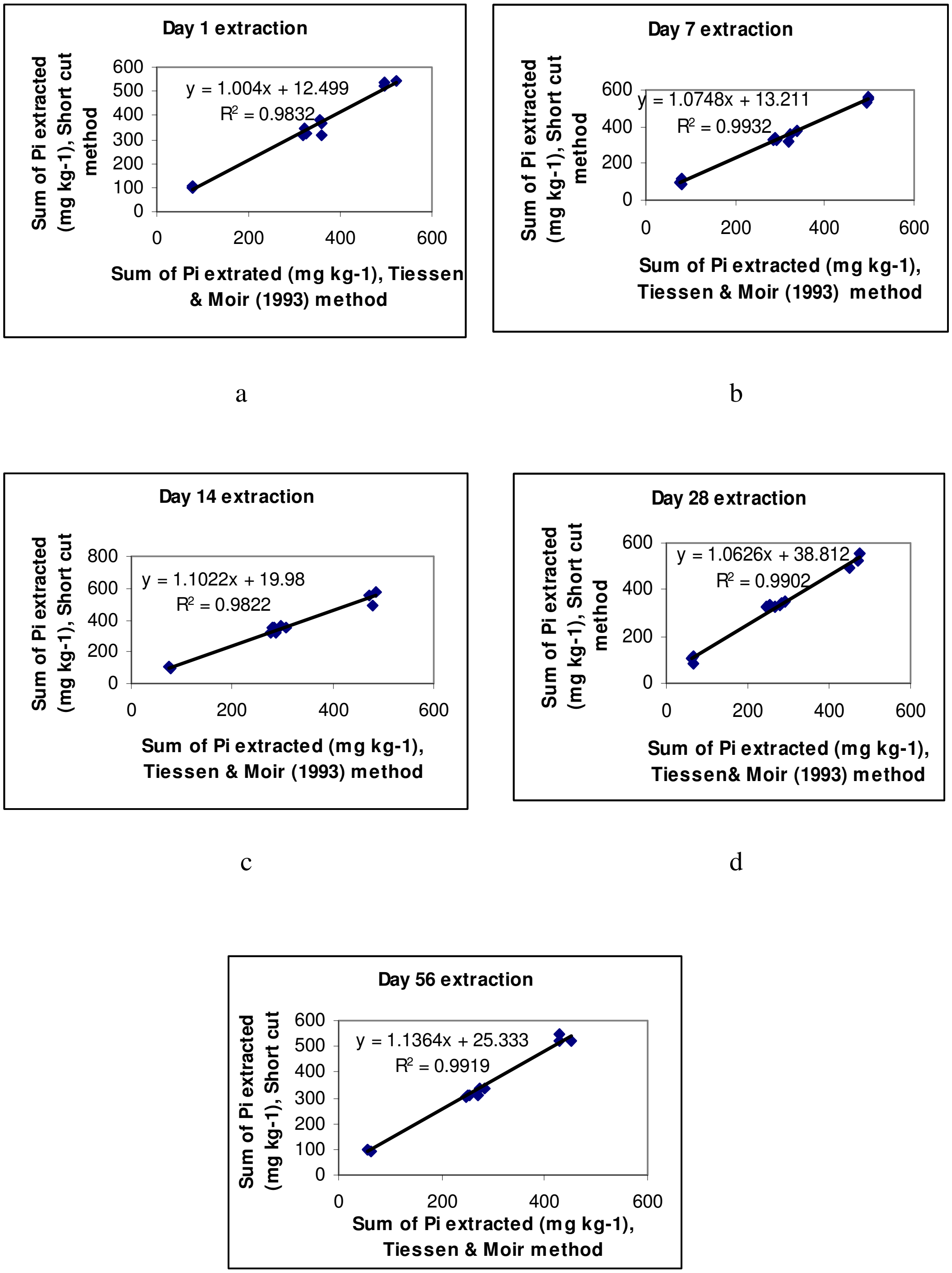
Treatment	Method	Pi fractions	Extraction time (days)				
			1	7	14	28	56
NK	Tiessen &Moir	HFO-Pi	0.08	1.05	1.07	1.08	1.13
	(1993)	HCO_3-Pi	6.33	4.11	5.46	2.29	0.88
		NaOH-Pi	17.75	16.58	15.68	14.47	11.09
		1M HCl-Pi	5.65	6.33	3.64	3.64	2.29
		C/HCl-Pi	52.3	49.78	50.31	44.48	42.63
		♦∑ Pi	**82.11**	**77.85**	**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 ($pH\geq6$)

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$_2$SO$_4$-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.

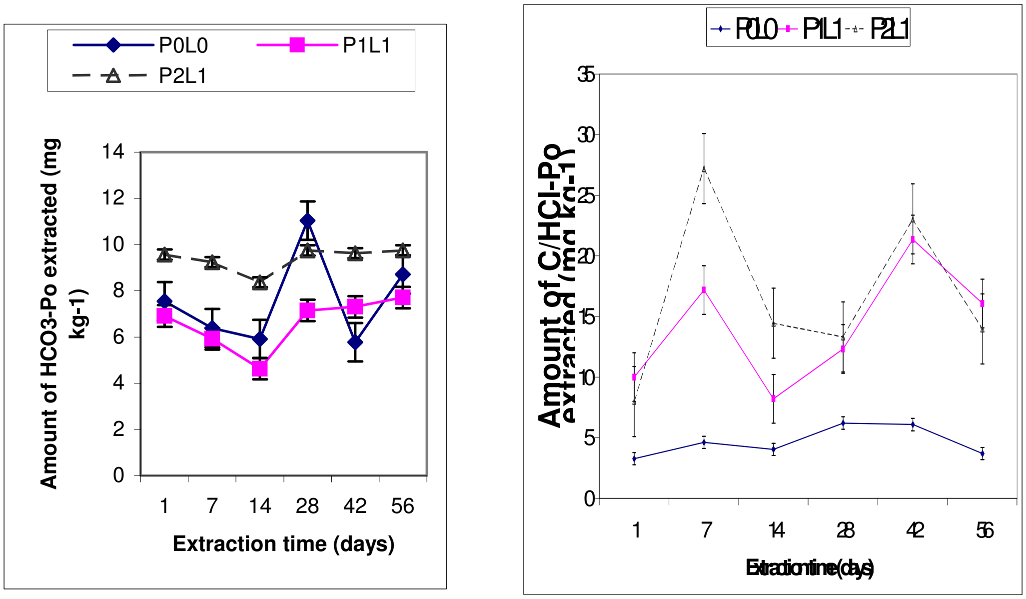
As an average of all extraction time, the percent C/HCl-P_i constituted 7.32, 8.75 and 11.31 for PoLo, P_1L_1 and P_2L_1 respectively. The contribution of this fraction is on average 9.12% for all treatments. The average percentage contribution of this fraction was reported to be about 12% for the soils 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.

CHAPTER 8 [*]

Phosphate desorption kinetics study for Avalon soils and its relationship with plant growth

8.1 INTRODUCTION

The kinetics of P desorption is a subject of importance in soil and environmental sciences primarily because P uptake by plants occurs over a span of time. Thus, kinetic information is required to properly characterize the P supplying capacity of soils, to design fertilizer-P management to optimize efficiency, to reduce environmental pollution, and to develop guidelines for the disposal of P-rich wastes onto the land (Skopp, 1986). Another reason for kinetic study is to obtain information on reaction mechanisms (Skopp, 1986).

In order to assess long-term P desorption kinetics, it is necessary to sufficiently suppress the back ward resorption reaction. Introducing effective P sink into the system can serve the purpose. 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

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chemical extractants in estimating available soil P (Sharply, 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 strips for studying long-term P dynamics has been proposed (De Jager and Claassens, 2005; Ochwoh et al. 2005).

However, relatively little information is available on the literature in relation to the use of this method. Lookman et al. (1995) studied the kinetics of P desorption using this procedure. They concluded that P desorption could be well described by a two component first order model. They also reported that no desorption maximum was reached in the entire period of desorption (1600hrs). Research was also done which linked short-term soil P tests to long-term soil P kinetics (Koopmans et al., 2001; Maguire et al., 2001). Recently, studies were also made on some South African soils using DMT-HFO method as a phosphate sink. De Jager and Claassens (2005) investigated the desorption kinetics of residual and applied P to an acid sandy clay soils from Mpumalanga, South Africa. They reported that no desorption maximum was reached after 56 days of shaking. However, there is still a paucity of information on the relationship between kinetics of phosphorus release using this new method and plant yield parameter. The objective of this research was to relate the kinetic data generated using the DMT-HFO method to maize yield at the green house level.

8.2 MATERIALS AND METHODS

The sampling procedure and experimental site history of the soil samples used in this experiment are detailed in Section 7.2. Table 7.2 shows some selected physical and chemical properties of the different treatments.

8.2.1 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. We followed the same procedure for these samples too.

8.2.2 Greenhouse experiment

The detail of this particular experiment is as detailed in Section 7.2.4.

8.2.3 Data analysis

The data obtained were analyzed 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 $\alpha = 0.05$. Correlation of the rate parameters and the cumulative amount of P released with plant yield parameter was done using Pearson linear correlation, PROC CORR (SAS Institute 2004).

8.3 RESULTS AND DISCUSSION

8.3.1 Long-term desorption of P

The amount of P_i extracted by DMT-HFO was significantly influenced ($P \leq 0.05$) both by the P content and extraction time although the difference of this fraction was not significant between P_1L_1 and the control (Table 7.3). 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 (Figure 8.1).

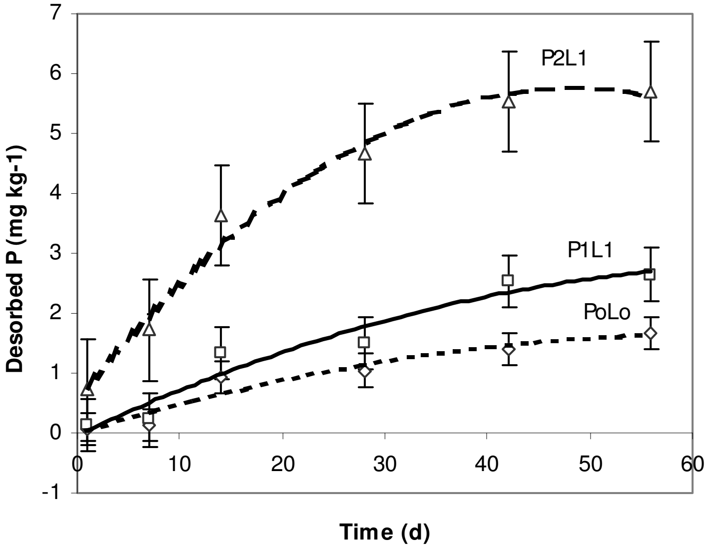

Figure 8.1. Cumulative P desorbed over time, extracted using DMT-HFO for the different treatments; error bars represent standard errors of the mean.

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). This can be explained by P desorbing quickly from 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 analogous to the result documented in section 2.3.1 for the long-term fertilized soils collected from the University of Pretoria. 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).

The percent P extracted in all cases was very low as compared to the total P. 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 was 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 labile P would be lower.

8.3.2 Plant growth as related to phosphorus desorption kinetics

Correlations between the rate coefficients k_A and k_B (day^{-1}) with yield parameters such as shoot dry matter yield and plant P uptake were made as illustrated in Table 8.1. The correlation between the rate coefficients and plant yield was negative in all cases because of the decreased rate of P release with increased cumulative P content of the soils considered. The rate of P release followed the order $P_oL_o > P_1L_1 > P_2L_1$ whereas the cumulative P released followed the reverse order. The plant yield obtained was also in accordance with the total P content of the treatments. The labile pool desorption rate coefficient k_A showed a highly significant correlation with both shoot dry matter yield (r = -0.994**) and P uptake (r = -0.982**). This pool represents the P pool with fast release kinetics that comprises presumably primarily P bound to the reactive surfaces that is in direct contact with the aqueous phase. This pool is presumed to be easily available to plants in a reasonably short period of time (Lookman et al., 1995). The less labile rate coefficient k_B also showed a significant correlation with both shoot

Table 8.1 Correlation between the kinetic parameter k (day $^{-1}$)(Rate coefficient) shoot dry matter yield and P uptake; N=3

	Dry matter Yield	P-uptake
	mg kg^{-1}	
k_A	-0.994**	-0.982**
k_B	-0.856*	-0.893*
k_A+k_B	-0.999**	-0.994**

**Significant at 0.01 probability level, *Significant at 0.05 probability level dry matter yield (r = -0.856*) and P uptake (r = -0.893**). This pool represents the P pool with slow release kinetics that results from slow dissolution kinetics or from slow diffusion from the matrix of sesquioxide aggregates (Koopmans et al., 2004). This pool will be available only over a long period of time and that is probably why the correlation was lower. Although the P pools are theoretically grouped in to these two discrete pools for the sake of convenience, the fact that both pools involve simultaneously in the uptake process indicates that one should take in to account the effect of both when such correlations are made. Thus, the sum of the rate constants (k_A+k_B) showed a highly significant correlation with both shoots dry matter yield (r = -0.999**) and P uptake (r = -0.994**).

Table 8.2. Correlations between the cumulative P desorbed over a 56-day period, shoot dry matter yield, P uptake and Bray 1P; N=3

P extracted	Dry matter yield	P uptake	Bray 1P
		mg kg^{-1}	
HFO-Pi	0.997**	0.999**	0.982**
Bray 1P	0.965**	0.982**	-

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

The cumulative amount of P extracted by the DMT-HFO over a 56-day period was also correlated with yield and Bray 1P as shown in Table 8.2. The correlation between the cumulative P extracted and maize yield was highly significant. The correlation between Bray 1P and maize yield was also highly significant. Based on the r-values, both the kinetic parameters and the cumulative amount of P desorbed could serve as reliable indices of plant available P. This is contrary to the results obtained for the other long-term soil samples carried out at the field level as documented in Section 3.3.2. In the later case it was only the rate coefficient parameter that showed a significant correlation with the yield. The reason for this disparity could be that experiments conducted at the field level are difficult to control and plant response could be influenced by several interacting soil, plant and climatic factors besides P content.

8.4 CONCLUSIONS

According to this study, cumulative P released with time followed the same pattern for all P treated soils, 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. Desorption maximum was not reached during the entire period of extraction time, indicating that desorption can continue for a longer period than 56 days. Both the kinetic parameters and the amount of P extracted showed highly significant correlations with yield parameters and hence could be reliable indices of plant available P. However, data from a wider range of soils is also necessary to evaluate the universality of this method.

CHAPTER 9

General conclusions and recommendations

9.1 Kinetics of phosphorus desorption and its relationship with plant growth

The kinetics of phosphate desorption was done using DMT-HFO method. The cumulative P released with time followed the same pattern for both soils from UP (University of Pretoria) and Ermelo, Mpumalanga; with an initial rapid release of P followed by a slower release that was still continuing after 56 days of extraction. No desorption plateau was reached on both cases during the entire period of extraction time, indicating that desorption can continue for a longer period than 56 days. The rate coefficients were in the range of 0.0059 - 0.104 day $^{-1}$ for the UP soils and 0.2294 - 0.1313 day^{-1} for Ermelo soils. The P desorption rate was higher for Ermelo soils than the UP soils. These variations could be ascribed to the differences in the physical properties of the two soils. The clay content of the UP soils was about three times higher than the Ermelo soils and hence the lower rate of release. Nonetheless, the cumulative desorbed P was consistent with the total P content of the soils (UP>>Ermelo). The other notable difference observed on these two long-term trials was that in the case of UP soils the rate coefficient increased with increased P content of the different treatments hence the control being the least in its P content, resulted in the lowest rate of P release. However, the rate of P release declined with increased P content for Ermelo soils. The contribution made by SP_A was found to be higher than SP_B in the 56 days of extraction. However, the degree of increment with time showed that it is the less exchangeable pool (SP_B) that will control the release kinetics of the soil in the long term.

The rate coefficients showed significant correlations with plant yield parameters for both cases. The rate coefficient, therefore, appeared to be a good index of plant availability. The correlations between the cumulative amount of P desorbed and plant yield parameters however were not similar. Correlation between the cumulative P extracted and maize yield was not significant for soils collected from UP where as significant difference was observed for soils collected from Ermelo. The reason for this contrasting result could be attributed to the difference in the actual approach in making such a comparison. In the case of UP soils, the treatments were from a field trial where as in the case of Ermelo soils it was done in a pot trial. Experiments carried out at the field level are in general less controlled compared to what it would be when the same experiments were to be done at the green house level. The result from UP soils indicates that the plant response is probably influenced by several interacting soil, plant, and climatic factors besides P deficiency. However, in this research correlation with other plant yield parameters such us P uptake and relative plant response was not conducted due to lack of relevant data. More work relating these plant indices with desorption indices is therefore required to validate the above results especially under field condition. Besides, data from a wider range of soils is also needed to evaluate the universality of this method.

9.2 The dynamics of phosphorus and the relationship between different pools and plant growth

The use of successive desorption of P by DMT-HFO followed by subsequent fractionation as described by Hedley et al., (1982) or Tiessen and Moir (1993) was employed to study the P dynamics of long-term fertilized soils from South Africa. The effect of P levels and extraction time was found to be statistically significant for all P fractions except the residual P pool in both soils. Almost all the inorganic P fractions decreased with increased time of extraction by DMT-HFO. The only inorganic fraction that showed an increasing trend was the OH-P_i fraction from Ermelo. All the organic fractions in general increased with increased time of extraction for both cases that is attributed to the microbial immobilization of P (Stewart and Tiessen, 1987). Therefore, the contribution of the labile and non-labile Pi fractions in replenishing the solution Pi was significant where as the contribution by organic fractions was limited in replenishing the soil solution P for the desorption periods considered in this study.

The amount of P extracted by the different extractants (including total P) was correlated with dry matter yield and plant P uptake. This comparison was made between the different P extracts after 56 days of extraction by DMT-HFO and maize grain yield for UP soils and plant yield parameters such as shoot dry matter yield and P uptake for Ermelo Soils. Significant correlations were observed between maize grain yield and all the P pools and the total P except DMT-HFO-P_i and HCO_3-P_o for the UP soils where as in the case of Ermelo soils, significant correlations were observed between the different P pools and plant yield except HCO_3-P_o, C/HCl-P_o and P_t.

The decreasing trend in the inorganic fractions with time revealed their different contributions to the soil solution P extracted with DMT-HFO. In the case of UP soils, $NaHCO_3$-Pi, NaOH-P_i and C/HCl-Pi were the most important fractions that contributed in replenishing the soil solution P. Among the inorganic fractions, C/HCl-P_i was the major contributor. This suggests that this fraction may be a buffer to the more labile P fractions. The C/HCl-P_i was also the fraction that decreased most for the Ermelo soil especially for the high P treatments indicating the importance of this fraction in replenishing the more labile P fractions.

9.3 Effect of varying shaking time on phosphorus desorption

The DMT-HFO method, similar to other soil P tests, exploits 100 percent of the soil sample volume, which is, much more than the percent root exploitation of plants. Therefore, exploiting the whole volume of the soil by continuous shaking may not represent the plant mode of action very well. One possible solution to simulate the root P uptake could be by modifying the shaking procedure using different shaking periods. In this case an attempt to investigate the effect of varying the shaking periods was done. Four shaking options were considred. Continous shaking for 1, 7, 14 ,28, and 56 days, which is the usual approach, was assumed to be a conventional approach (option 1). Option 2 referred to a continous shaking for 75% of option 1. Option 3 referred to a continous shaking for 50 % of option 1 and option 4 referred to a continous shaking equivalent to 25% of option 1. The effect of varying shaking options on the extractable DMT-HFO-Pi for the different P treatments showed significant difference only for treatment that received the highest P (MNPK). Significant correlations were obtained between the labile pool rate coefficients [k_{A1} (0.92^{**}), k_{A2} (0.99**), k_{A3} (0.92^{**}) and k_{A4} (0.92^{**})] and maize grain yield for shaking options 1, 2, 3, and 4 respectively. The only rate coefficient from the less labile pool, k_B , which showed a significant but moderate correlation ($r = 0.78^{*}$) with maize grain yield, was $k_{B1.}$ This pool will be available only over a long period of time and that is probably why the correlation was strong only in the case of option 1 which exhibited the longest desorption period. This evidenced that the role of this pool appeared to be much less pronounced with decreasing time of desorption. However, the higher correlations observed between the sum of the rate coefficients and plant parameters as illustrated in the previous chapters indicates that this pool could contribute in replenishing the solution P over long periods and it appears that this approach to simulate the plants mode of action is not ideal.

The cumulative amount of P (mg kg^{-1}) extracted by DMT-HFO showed no statistically significant correlations with maize grain yield in all the options considered. Judging from the r-values, the rate coefficients appeared to be better indices of plant availability than the amount of P extracted by DMT-HFO. Option 2 seemed relatively better than the others since it showed the strongest correlation. So for soils with high releasing kinetics and high total P content, provided that the P release from the soil is the rate-limiting step, reducing the length of shaking time could shorten the duration one needs to complete the experiment with out influencing the predicting capacity of the methodology.

9.4 Short cut to the combined method

We employed a short cut combined method characterize the P supplying capacity of a soil and to understand the dynamics of soil P. The procedure used consecutive extraction of P from a soil sample, firstly by dialysis membrane tubes filled with hydrous ferric oxide (DMT-HFO) followed by subsequent P fractionation. However, this procedure is lengthy and time consuming and an approach to develop a way to shorten these P desorption studies in soils was important. The objective of chapter 6 was to propose a short cut method as an alternative to the conventional step-by-step method in understanding 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. The C/HCl-P_i was identified as a major source in replenishing the labile P pool from chapter 4. 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 the 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 the extraction periods, resulted to very strong and significant correlations. Correlations between C/HCl-P_i and maize grain yield were 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.

9.5 General remarks

Consecutive extraction procedures using dialysis membrane tubes filled with hydrous ferric oxide (DMT-HFO) followed by subsequent P fractionation procedure as described by De Jager and Claassens (2005) and Ochwoh et al., (2005) constitute a convenient laboratory method to investigate the kinetics of residual P release and to understand the dynamics of soil P. From the kinetic study, one can estimate the time frame of P release and the distribution of P between the labile and less labile forms. However, categorizing the P pools as labile and less labile P pools in the above way could be crude in the sense that it is not possible to identify which particular P pool involves in the replenishment of the P taken by the plants, which in this case was approximated by using the DMT-HFO step. The importance of the subsequent fractionation would be to identify the principal less labile P fraction that acts as a major contributor to the more labile P pool. This combined method can approximate successive cropping experiments carried out either in the green house or field condition. The work done in this study focused on relating the P extracted by this method especially the DMT-HFO-P_i with plant yield parameter, to identify the P pools that served as a buffer in replenishing the labile P fractions, and to shorten the time required to carry out this kind of experiment. In general characterization of P using the combined method could be a more practical and environmentally responsible approach to P fertilizer recommendations. The DMT-HFO step has been regarded as a mild and nondestructive extractant influenced less by the soil's physicochemical properties making it suitable to study the fate of residual P. However, data from a wider range of soils is needed to evaluate the applicability of this method in this context.

9.6 Research needs

Use of successive extraction procedure employing hydrous ferric oxide in dialysis membrane tubes (DMT-HFO) as a phosphate sink is one of the promising methods to evaluate the fate of residual P especially for long-term fertilized soils. Only a limited research has been done since it was introduced for the first time about 12 years ago. One particular problem associated with this method is that it is lengthy and time consuming despite the attempt made in this study to shorten it to a certain extent. Besides, this methodology has not been done for soils of wider physical properties and documents relating the desorption indices of this method with plant yield parameters are also scarce. More research in this regard is important to further the progress made so far. Little is also documented on the influences of soil type, pH, ionic strength and temperature on the labile and less labile rate constants and hence research related to these is also required.

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