Chapter 2

2) Materials and Methods

2.1) The soil used in the study

The soil used was a red sandy clay soil that came from Piet Retief, Mpumalanga. Selected chemical and physical properties in of the studied soil are given in **Table 2.1** and **2.2**. According to Turner & Laker, (1999), clay and sandy clay Hutton soil forms from this region originated from the Piet Retief biotite granite. This particular soil was chosen because it is known that red clay and red sandy clay soil have a high phosphate sorption capacity (Bainbridge *et al,* 1995). The high sorption capacity of the studied soil is also illustrated in **Table 2.3**. The high phosphate sorption capacity can mainly be contributed to two factors; firstly, the dominant clay mineral in these soils is kaolinite, which has a pH dependable charge on the edges of the 1:1 clay mineral; and secondly, the precipitation of Fe(III) oxy-hydroxide on the planar surfaces of these clay mineral increases the pH dependable charge of the clay mineral and greatly enhances the phosphate adsorption capacity.

Table 2.1. Selected chemical properties of the soil used in the study.

pH (H_20)	Bray 1 P (mg kg^{-1})	Total[a] P (mg kg^{-1})	CEC ($cmol_c$ kg^{-1})	C content (%)	Oxalate extractable Fe (mg kg^{-1})	Oxalate extractable Al (mg kg^{-1})
4.1	1.16	161.09	7.2	0.87	1516	1300

a) H_2SO_4 digestion

Table 2.2. Texture of soil used in the study

Sand (%)	Clay (%)	Silt (%)
59	39	2

Table 2.3. Phosphate sorption capacity of the studied soil

Phosphate added ($\mu g.g^{-1}$)	Phosphate in solution ($\mu g.g^{-1}$) after 72 hour shaking with 0.02M KCl	Phosphate sorbed ($\mu g.g^{-1}$)
0	1.92	0
250	7.46	242.54.
10000	8370.17	1629.83
1000 (and incubated for a month)	2.49	997.51

2.2) The different soil treatments

Two 250g soil samples were wetted to field capacity with a KH_2PO_4 solution with phosphate concentration equivalent to 75 and 150mg P $.kg^{-1}$ soil respectively. The soil samples were then thoroughly mixed and incubated for 5 months at ± 21 °C, while the soils were kept at field capacity. Along with the two samples that were incubated with 75mg.kg^{-1} (R75) and 150mg kg^{-1} (R150) phosphate, a third samples was also wetted to field capacity with deionised water and incubated for 5 months as the control (R).

2.3) The preparation of the DMT-HFO.

Reagents

200 g $Fe(NO_3)_3$,

4 *M* NaOH

1 *M* HCl

Preparation

HFO was prepared by a precipitation reaction. The increase in pH of a acidic ferric (III) solution causes the precipitation of a red brown gelatinous polymer $Fe_2O_3.xH_2O$ (usually written as $Fe(OH)_3$).

$$Fe^{3+} + 3OH^- \leftrightarrows Fe(OH)_3(s)\ (amorph) \qquad \textbf{(2.1)}$$

Amorphous ferri oxy-hyroxide ($Fe(OH)_{3(am)}$) is sparingly soluble with a solubility product (K_{sp}) of 2.6×10^{-39} (McMurry & Fay, 1995, Shriver &Atkins, 1999).

Two hundred grams (0.83 moles) of ferric nitrate nonahydrate was dissolved in 2 ℓ of deionised water, and 4 *M* NaOH was added drop wise to the solution until a pH of 7 - 8 was obtained, because maximum precipitation occurs at this pH according to **Figure 2.1**. The suspension was then decanted in centrifuge tubes and centrifuged to separate the $Fe(OH)_{3(am)}$ from the Na^+ and NO_3^- containing solution. After the solution was decanted, the remaining $Fe(OH)_{3(am)}$ in the centrifuge tubes was re-suspended in deionised water and centrifuged again. This procedure was repeated three times to thoroughly clean the $Fe(OH)_{3(s)(am)}$. Finally, the pH was adjusted to more or less the pH of the soil with 1*M* HCl. The $Fe(OH)_{3(am)}$ was re-suspended in deionised water to obtain a volume of 4 ℓ with a total ferric iron concentration of $10^{-0.68}$ *M*. **Figure 2.1** shows that when pH $\leq$ 2, all the ferric iron (or $10^{-0.68}$ *M*) will be in solution, and when the pH is increased to 4.1 the total Fe^{3+}concentration in solution drops to $10^{-4.77}$ *M* which means that, at this pH, 99.99% of the ferric iron will precipitated out.

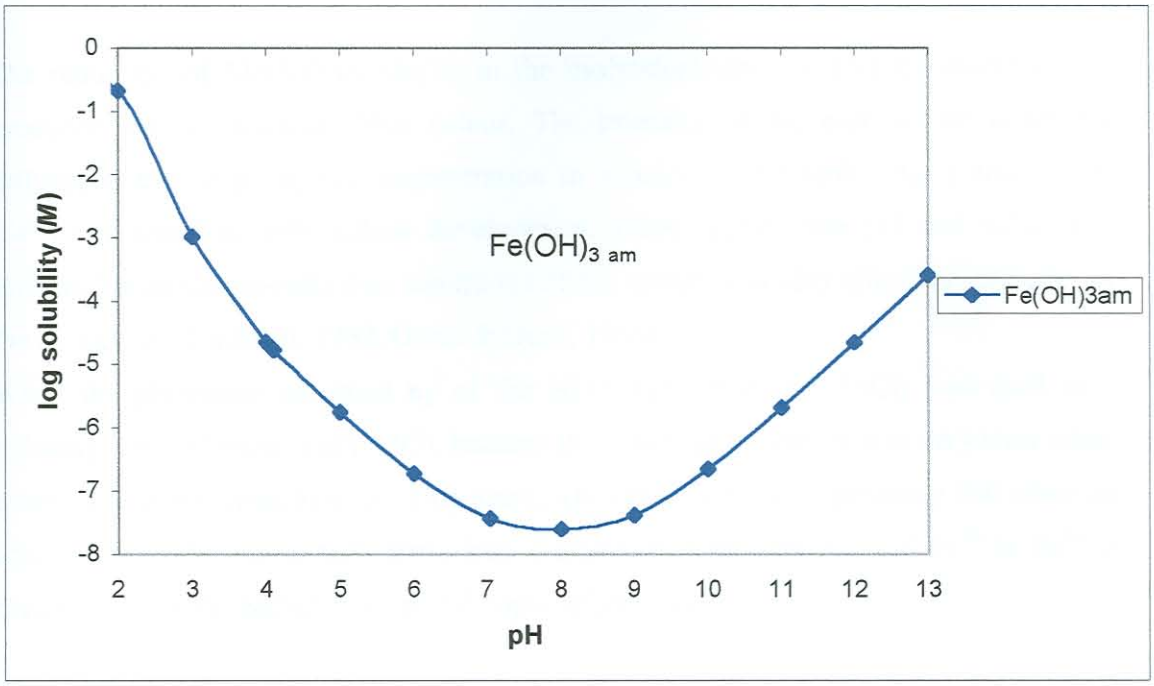

Figure 2.1. Solubility of amorphous $Fe(OH)_3$ calculated with the geochemical transport model, Phreeqc (Parkhurst & Appelo, 2001).

Fifteen centimetre length DMT (diameter of 14.3 mm, approximate pore size 2.5 – 5 nm; membrane thickness 3 μm obtained from Medicell International Ltd) were boiled twice for 5 minutes each in deionised water and thoroughly rinsed. Each dialysis tube was filled with 10 ml HFO, containing ± 2.07 *mmol* $Fe(OH)_{3(am)}$. The ends of each DMT were folded tightly and closed with household plastic clips to ensure that no HFO would leak into the soil solution during the period of shaking.

2.4) Determination of phosphate in the extracts

Total phosphate and inorganic phosphate were determined colourimetrically with the method of Murphy & Riley (1962). This method is based on the principle that a heteropoly molybdophosphoric acid complex forms when phosphate is added to an acid molybdate solution:

$$H_3PO_4 + 12\ H_2MoO_4 \leftrightarrows H_3(PO_4MoO_{10})_4 + 12H_2O \qquad \textbf{(2.2)}$$

The reduction of Mo(VI) to Mo(V) in the molybdophosphoric acid by ascorbic acid produces the characteristic blue colour. The intensity of the blue colour is mainly influenced by the phosphate concentration in solution, but arsenic (As^{5+}) and silicon (Si^{4+}) can interfere with colour development. Other factors like pH and substances influencing oxidation-reduction conditions of the system can also affect the intensity of the blue colour (Jackson, 1962, Olsen & Dean, 1965).

When the phosphate adsorbed by of the HFO was determine, $SnCl_2$ was used as a reducing agent (Freese *et al* (1995), because the colour development was very slow when ascorbic acid was used as a reducing agent. The reason for this is probably that when an acid solution also has a high ferric iron concentration the reduction of Fe^{3+} to Fe^{2+} is favoured above the reduction of molybdophosphoric acid.

Reagents

0.25 *M* H_2SO_4

4 *M* NaOH

Paranitrophenol (indicator)

The following reagens were the **colour developing reagents**

0.032 *M* $(NH_4)_6\ Mo_7O_{24}.4H_2O$ (ammonium molybdate)

0.3 *M* $C_6H_8O_6$ (ascorbic acid) solution.

0.01 *M* $K(SbO)C_4H_4O_6$. 0.5 H_2O (potassium antimonyl tartrate hemihydrate)

$SnCl_2$ (reducing agent used for determination of phosphate adsorbed by of the HFO)

Method

A suitable aliquot was pipetted into a 50 cm^3 volumetric flask, and a drop of the indicator, paranitrophenol was added. The pH of the aliquot was adjusted to ± 5.6, first by adding 4 *M* NaOH until the indicator turned yellow and then 0.25 *M* H_2SO_4 until the indicator just turned clear again. Alkaline extractions were only acidified untill the indicator turned clear, 8 ml of the colour developing solution was added, made up to volume, shaken and the colour intensity was read after 10 minutes on a spectrophotometer at 712 nm.

2.5) The sequence of the phosphate fractionations

2.5.1 DMT-HFO extraction

The sequence of the fractionation is illustrated in **Figure 2.2.** After the DMT-HFO was prepared, it was immersed in glass flasks containing 40 cm^3 of deionised water and 1g of soil. The flasks were placed in a constant temperature room on a horizontal shaker and shaken for 56 days. On days 1, 7, 14, 21, 28, 35,42 and 56, the DMT-HFO's were replaced with new DMT-HFO. With each replacement four of the DMT's were removed from the flasks, cut open and the HFO was washed out of the tubes into a glass beaker. The HFO suspension was then dissolved with 5 cm^3 5.4 *M* H_2SO_4 and the phosphate concentration was determined colorimetrically with the molybdophosphoric blue method using $SnCl_2$ as reductant. A standard series was prepared with the same background Fe and H_2SO_4 concentrations. After the DMT-HFO was removed the soil suspensions in the glass flasks were transferred to centrifuge tubes and centrifuged to separate the soil and the solution. The supernatant solution was discarded and the soil was sequentially extracted for phosphate according to the flow chart in **Figure 2.2.**

2.5.1) $NaHCO_3$ extraction

The $NaHCO_3$ - extraction is based on the method of Olsen & Dean (1965). The $NaHCO_3$ -extraction is used on a wide range of acid and calcareous soils. In calcareous soils the addition of $NaHCO_3$ changes the equilibrium between $CaCO_3$ and calcium phosphate. The increased CO_3 concentration in solution will favour the dissolution of calcium phosphate due to the precipitation of $CaCO_3$. In acid soils the increase in pH increases the solubility of ferric-and aluminium phosphates as illustrated in **Figure 1.5**. As explained earlier the excess of CO_3 in solution favours the precipitation of $CaCO_3$ and prevents the precipitation of calcium phosphate. Therefore the phosphate that goes into solution because of the dissolution of ferric and aluminium phosphates will not re-precipitate as calcium phosphate (Olsen & Dean, 1965, Hesse, 1971, Lindsay, 1979, Tiessen & Moir, 1993).

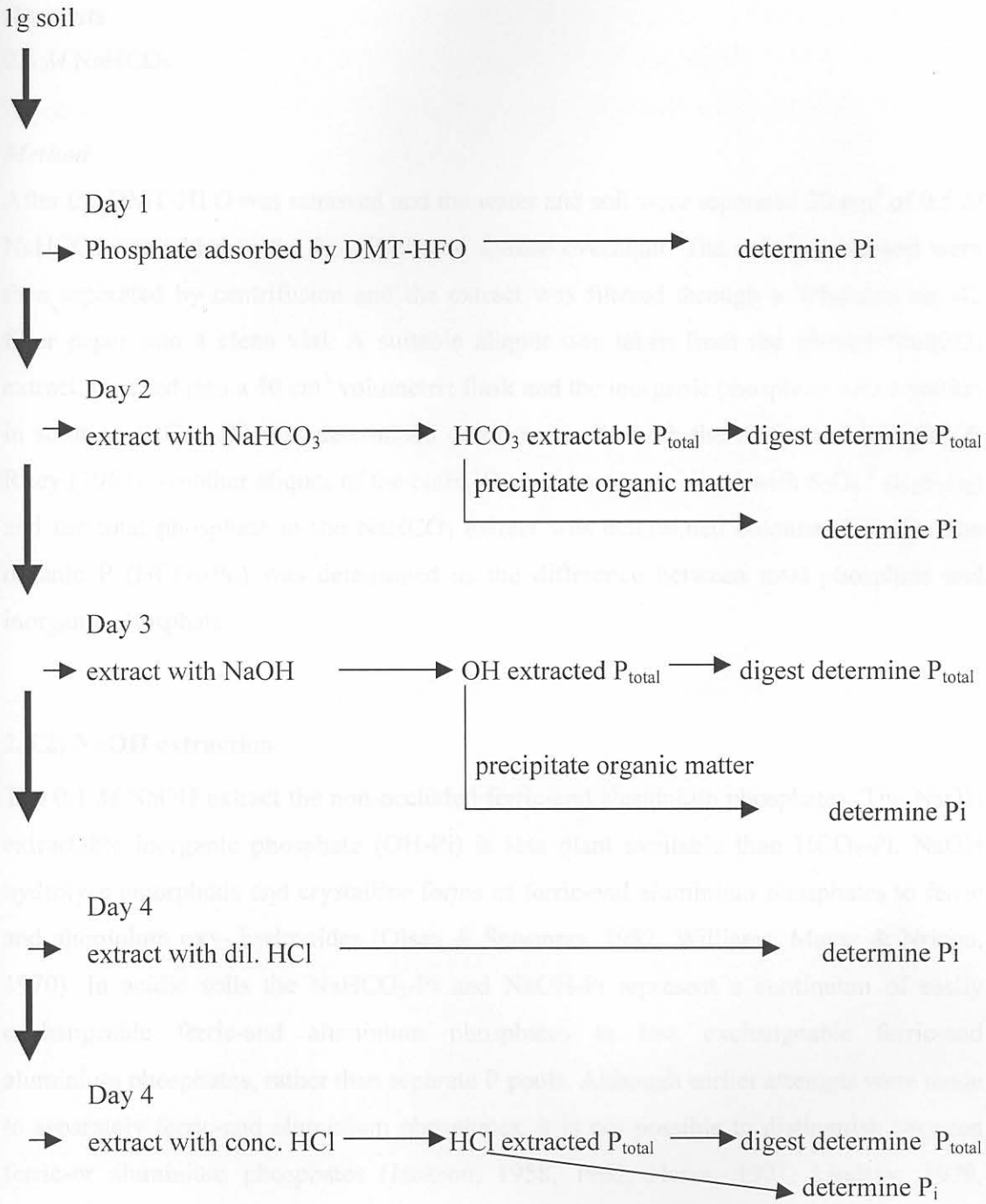

Figure 2.2. Flow chart of the sequential P extraction method

Reagents

0.5 *M* $NaHCO_3$

Method

After the DMT-HFO was removed and the water and soil were separated 30 cm^3 of 0.5 *M* $NaHCO_3$ was added to the 1g of soil and shaken overnight. The solution and soil were then separated by centrifusion and the extract was filtered through a Whatman no. 42 filter paper into a clean vial. A suitable aliquot was taken from the filtered $NaHCO_3$ extract, pipetted into a 50 cm^3 volumetric flask and the inorganic phosphate concentration in solution (HCO_3-Pi) was determined colorimetrically with the method of Murphy & Riley (1962). Another aliquot of the $NaHCO_3$ extract was oxidised with $S_2O_8^{-2}$ ($K_2S_2O_8$) and the total phosphate in the $NaHCO_3$ extract was determined colourimetrically. The organic P (HCO_3-Po) was determined as the difference between total phosphate and inorganic phosphate.

2.5.2) NaOH extraction

The 0.1 *M* NaOH extract the non-occluded ferric-and aluminium phosphates. The NaOH extractable inorganic phosphate (OH-Pi) is less plant available than HCO_3-Pi. NaOH hydrolyse amorphous and crystalline forms of ferric-and aluminium phosphates to ferric and aluminium oxy- hydroxides (Olsen & Summers, 1982, Williams, Mayer & Nriagu, 1970). In acidic soils the $NaHCO_3$-Pi and NaOH-Pi represent a continuum of easily exchangeable ferric-and aluminium phosphates to less exchangeable ferric-and aluminium phosphates, rather than separate P pools. Although earlier attempts were made to separately ferric-and aluminium phosphates, it is not possible to distinguish between ferric-or aluminium phosphates (Jackson, 1958, 1962, Hesse, 1971, Lindsay, 1979, Tiessen & Moir, 1993).

According to Anderson (1975), NaOH is also the most effective extractant for soil organic phosphate. NaOH hydrolyses the organic P esters in the soil, releasing it in solution. Because the nature of a large part of the organic phosphate compounds in the

soil are still unknown, it is not yet possible to determine exactly which organic phosphate was extracted (Anderson, 1975). According to Tiessen & Moir (1993), the organic phosphate extracted with $NaHCO_3$ ($NaHCO_3$-Po) and NaOH (NaOH-Po) probably also represents the same organic phosphate pool.

Reagents

0.1 *M* NaOH

Method

30 cm^3 of 0.1 *M* NaOH was added to the 1g of soil that was brought over from the $NaHCO_3$ extraction and shaken overnight. The solution and soil were then separated by centrifusion and the extract was filtered through a Whatman no. 42 filter paper into a clean vial. A suitable aliquot was taken from the filtered NaOH extract, pipetted into a 50 cm^3 volumetric flask and the inorganic phosphate concentration (OH-Pi) in solution was determined colorimetrically with the method of Murphy & Riley, (1962). Another aliquot of the NaOH extract was oxidised with $S_2O_8^{-2}$ ($K_2S_2O_8$) and the total phosphate in the NaOH extract was determined colorimetrically. The organic phosphate (OH-Po) was determined as the difference between total phosphate and inorganic phosphate.

2.5.3) 1 *M* HCl extraction

1 *M* HCl is very effective in extracting phosphate associated with calcium phosphates, from dicalcium phosphate ($CaHPO_4$) dicalcium phosphate dihydrate ($CaHPO_4.2H_2O$), octacalcium phosphate [$Ca_8H(PO_4)_6.5H_2O$] to the least soluble calcium phosphate, namely fluorapatite [$Ca_5(PO_4)_3F$] (Lindsay, 1979, Williams, Syers, Harris & Armstrong, 1970). Organic phosphate is seldom present in this fraction (Tiessen & Moir, 1993). When the pH of this soil is considered it is unlikely, that calcium phosphate would have precipitated in this soil. If calcium phosphate was present in the soil it was most probably trace amounts of residual phosphate fertilizer. In this study it is more likely that the 1 *M* HCl extracted aluminium- and ferric phosphates not extracted with 0.1 *M* NaOH.

Reagents

1 *M* HCl

Method

30 cm^3 of 1 *M* HCl was added to the 1g of soil that was brought over from the NaOH extraction and shaken overnight. The solution and soil were then separated by centrifusion and the extract was filtered through a Whatman no. 42 filter paper into a clean vial. A suitable aliquot was taken from the filtered 1 *M* HCl extract, pipetted into a 50 cm^3 volumetric flask and the inorganic phosphate (1*M* HCl-Pi) concentration in solution was determined colorimetrically with the method of Murphy & Riley (1962).

2.5.4) Hot concentrated HCl extraction

Hot concentrated HCl extract most of the residual organic and inorganic P left in the soil and represents the very stable organic and inorganic P pools. The organic P extracted with concentrated HCl could also represent plant available P that is not alkali soluble (Tiessen & Moir, 1993).

Reagents

Concentrated HCl (11.3 *M*)

Method

10 cm^3 concentrated HCl was added to the 1 g soil brought over from the 1 *M* HCl extraction and heated on a water bath at 80°C for 10 minutes. After 10 minutes the samples were removed, 5 ml concentrated HCl was added and left at room temperature for one hour. The extract was then centrifuged and decanted into a clean vial. The soil residue was washed twice with deionised water, centrifuged and the extract was filtered through a Whatman no. 42 filter paper into a clean vial. A suitable aliquot was taken from the HCl extract, pipetted into a 50 cm^3 volumetric flask and inorganic phosphate (HCl-Pi) concentration in solution was determined colorimetrically with the method of Murphy & Riley,(1962). Another aliquot of the concentrated HCl extract was oxidised with $S_2O_8^{-2}$ ($K_2S_2O_8$) and the total P in the concentrated HCl extract was also determined

colorimetrically. The organic P (HCl-Po) was determined as the difference between total phosphate and inorganic phosphate.

2.6 Statistical analysis

An analysis of variance (Anova) of the data was done on the statistical program SAS. The Tukey test was used to determine significant differences at $\alpha = 0.05$.