5-1996

The solubilization of phosphates in the presence of organic acids

William Lawrence Holden

To the Graduate Council:

I am submitting herewith a thesis written by William Lawrence Holden entitled "The solubilization of phosphates in the presence of organic acids." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Plant, Soil and Environmental Sciences.

Michael E. Essington, Major Professor

We have read this thesis and recommend its acceptance:

Donald D. Howard, Dave L. Coffey
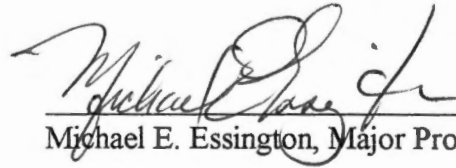
Accepted for the Council:
Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)
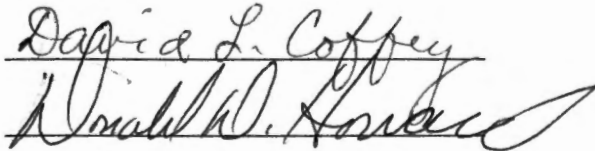
To the Graduate Council:

I am submitting herewith a thesis written by W.L. Holden entitled "The Solubilization of Phosphates in the Presence of Organic Acids". I have examined the final copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Plant and Soil Science.

Michael E. Essington, Major Professor

We have read this thesis
and recommend its acceptance:

Accepted for the Council:

Associate Vice Chancellor and
Dean of The Graduate School

The Solubilization of Phosphates in

the Presence of Organic Acids

A Thesis

Presented for the

Master of Science

Degree

The University of Tennessee, Knoxville

W. L Holden

May 1996

ACKNOWLEDGMENTS

ABSTRACT

Loess based soils of West Tennessee are insensitive to the Mehlich I soil P test. A more accurate assessment of plant available P may be obtained using organic acids produced in the rhizosphere. Two such organic acids, citric acid and 2-ketogluconic acid, have been shown to effectively solubilize soil P. The objectives of this study were to: 1) develop extraction procedures that utilize citric and 2-ketogluconic acid and that maximize P solubilization; 2) compare extraction capabilities of the organic acid extraction procedures to those of the Mehlich III and Olsen bicarbonate methods; 3) correlate organic acid extractable P to P solid phase speciation; 4) correlate corn (*Zea mays* L.) yield and ear leaf P concentration to organic acid extractable P, Mehlich III-P, Olsen-P, and to the inorganic P fractions of the soil solids; and 5) investigate the mechanism of soil P solubilization by 2-ketogluconic acid. Two extraction procedures were found to maximize P solubility from a loess based soil: 1) 10 g soil sample extracted with 25 ml of a 10 mM citric acid, pH 3.5 solution for 3 hrs (OE1), and 2) 5 g soil sample extracted with 25 ml of a 5 mM citric acid + 5 mM 2-ketogluconic acid, pH 3.5 solution for 6 hrs (OE2). Soil samples were obtained from a study that investigated the use of swine manure as a P fertilizer source (at Ames Plantation Experiment Station) and from a study that investigated the long-term influence of tillage practice and fertilizer P rates on corn production (at Milan Experiment Station). Both Experiment Stations are located in West Tennessee. In the Ames soil, OE1 was significantly correlated with Mehlich III-P ($r = 0.84^{***}$), Olsen-P ($r = 0.50^{*}$), and Al-P ($r = 0.85^{***}$), but OE2 was only correlated with Mehlich III-P ($r = 0.74^{***}$) and Al-P ($r = 0.74^{***}$). In the Milan soil, OE1 and OE2 were

iii

significantly correlated with Mehlich III-P, Olsen-P, Al-P, and Fe-P. None of the available

P indices or inorganic P fractions were significantly correlated with plant P from the Ames

or Milan studies. In the Milan soil, corn yield was significantly correlated with OE1-P (r =

0.68[**]), OE2-P (r = 0.68[**]), Mehlich III-P (r = 0.70[**]), Olsen-P (0.68[*]), Al-P (0.67[*]), and

Fe-P (r = 0.64[*]). All of the P indices were poorly correlated with yield in the Ames soil,

perhaps due to the relative abundance of P in the organic P phase. Therefore, all four

extractants inadequately assessed plant available P in the Ames and Milan soils. The

ability of 2-ketogluconic acid to solubilize soil P may be attributed to the formation of

soluble Al^{3+} and Fe^{3+} complexes and to competition with phosphates for adsorption sites.

The former mechanism was investigated by examining the equilibrium solubility of gibbsite

as a function of 2-ketogluconic acid concentration, pH, and ionic strength. The solubility

results suggest the formation of $[Al(C_6H_9O_7)^{2+}]$, $[Al(OH)(C_6H_9O_7)^+]$,

$[Al(OH)_2(C_6H_9O_7)^0]$, and $[Al(OH)_3(C_6H_9O_7)^-]$, with log K values of 3.0, -1.1, -5.2, and -

10.6, respectively. The formation of these soluble complexes would promote the

dissolution of aluminum phosphates (and possible iron phosphates), thereby releasing the

soil P into the extracting solution.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF TABLES (continued)

LIST OF FIGURES

Chapter I

Introduction

Phosphorus (P) is an essential element for plant growth. Compared to other macroelements, the total concentration of P in soil is relatively small. Mean total P concentrations vary widely: rock masses-1240 mg kg^{-1}, humid soils-490 mg kg^{-1}, arid soils-510 mg kg^{-1}, and loess-790 mg kg^{-1}. In soil, total P is found predominantly in the solid phase, with only 0.1 to 1 mg L^{-1} existing as plant available P in solution. Total P can be divided into two broad categories: organic P and inorganic P. The percentage of P in these two categories varies widely. Estimates in current literature show organic P in soils to range from 3% to 75% of the total soil P. The total P present in soil can also be divided into three pools: (1) soil solution P, which is bioavailable and includes organic and inorganic forms, (2) labile P, which is also bioavailable and includes sparingly soluble mineral and organic P phases that are in equilibrium with solution P, and (3) non-labile P, which is unavailable for plant uptake. The non-labile P is present in three forms: (a) adsorbed to the surface of the soil particles, (b) fixed in the lattices of clays and other silicate minerals, and (c) a constituent of organic compounds. Soil P fixation, which is the conversion of solution P and labile P to non-labile P, occurs as a result of sorption. Sorption collectively describes: (1) precipitation, which is the formation of a new three dimensional solid, and (2) adsorption, which is the concentration of an adsorbate at the soil surface (two-dimensional). These mechanisms result in a reduction of available P and thereby influence and limit the concentration of plant available P present in the soil.

1

The accessibility of phosphate for plant uptake is directly affected by the metabolic production of phosphatase, an enzyme that hydrolyzes a broad spectrum of organic phosphates, by the plant root. The synthesis of these enzymes is often greatest under conditions of low orthophosphate (inorganic phosphate) availability. Plants are also able to readily utilize orthophosphates by excreting organic acids to solubilize sorbed inorganic P. These organic acids enhance the dissolution of solid phase mineral P and displace adsorbed P from soil surfaces. The assessment of soil P with commonly used soil tests, such as Bray I & II, Mehlich I & III, and Olsen, in loess based soils has proven inadequate. In recent studies, these tests produced poor correlations between extractable soil P and plant P and underestimated the quantity of plant available soil P present.

Clearly, soil tests that more accurately assess plant available P are needed. Further, the developement of such soil tests should be based on rhizosphere chemistry. Two organic acids present in the rhizosphere and known to increase P solubility are citric acid ($C_6H_8O_7$) and 2-ketogluconic ($C_6H_{10}O_7$) acid. This research project was conducted to investigate the potential for these organic acids to enhance P solubilization and predict plant available P. Citric acid is known to be a strong complexing agent, with a high affinity for Al, Fe, and Ca. Although work investigating the mechanism by which citric acid increases P solubilization has been substantial, 2-ketogluconic acid's mechanism for P solubilization has not been well characterized. Therefore, the potential for 2-ketogluconic acid to enhance P solubilization through complexation was investigated. The objectives of this research are to: 1) develop an easily applied extraction procedure for orthophosphates using citric acid and 2-ketogluconic acid, 2) compare to other soil P tests and to chemical

2

pools in soil, and 3) quantify the aqueous speciation for 2-ketogluconic acid with aqueous Al^{3+} species.

Chapter II

Literature Review

1. Soil Chemistry of Phosphorus

Aqueous Chemistry

The concentration of inorganic phosphate, orthophosphate, in solution ranges from 0.1 to 1 mg L^{-1} and constitutes a very small portion of the total P in soil. The soluble P concentration is affected by the properties of both the solid and solution phases and also varies with the ratio between solid and solution. The soluble P concentration is dependent upon such factors as pH, redox, salt content, and mineralogy. The forms in which phosphates exists in solution are governed by acid-base and ion pair formation reactions. Protonation reactions distribute P between phosphoric acid, $H_3PO_4^0$ and its dissociation products: $H_2PO_4^-$, HPO_4^{2-}, PO_4^{3-} (Larsen, 1967) (Figure 2.1). At pH 7.2 there are approximately equal amounts of $H_2PO_4^-$ and HPO_4^{2-} in solution. Below this pH, $H_2PO_4^-$ is the major form in soil solution, whereas HPO_4^{2-} is the predominant form above pH 7.2.

The dissociation products of phosphoric acid are ligands (L^{l-}) (anions or neutral molecules that reside in the coordination sphere of a central metal ion (M^{m+})) that form soluble complexes with an array of metal ions, such as Al, Fe, Ca, and Mg (Larsen, 1967; Smith and Martell, 1976)(Table 2.1). Two types of complexes can form: inner sphere and outer sphere. The essential difference between these two types of soluble complexes is the location of the water molecules.

$$[(M^{m+})(L^{l-})H_2O]^{m-l} \text{ - inner sphere complex}$$

$$[(M^{m-})H_2O(L^{l-})]^{m-l} \text{ - outer sphere complex}$$

4
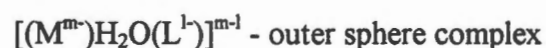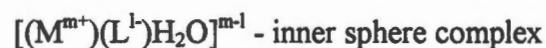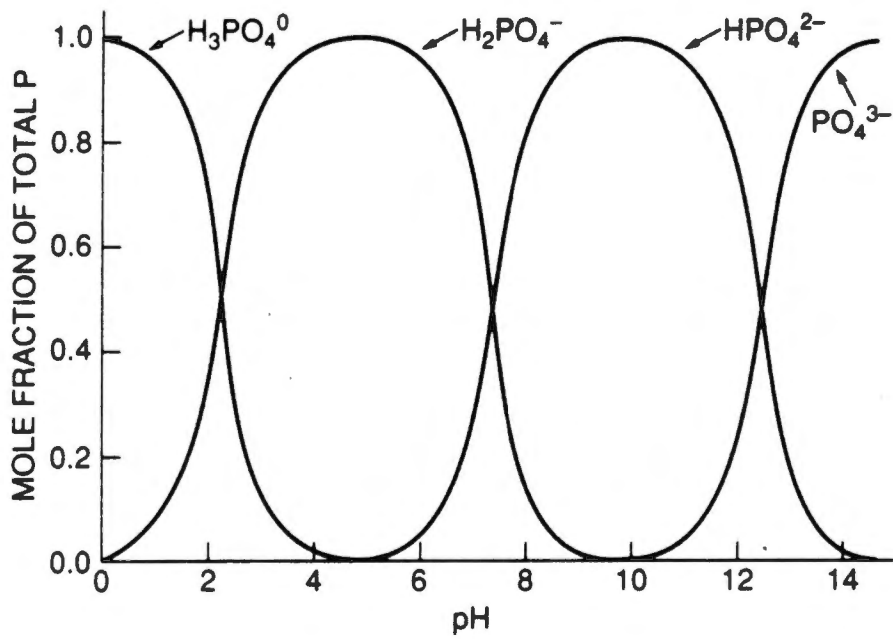
Figure 2.1. Influence of pH on the distribution of orthophosphate species in solution (Tisdale et al., 1993).

Table 2.1. Stability constants for metal-phosphate complexation reactions at 25° C and 0 M ionic strength (Smith and Martell, 1976; Larsen, 1967).

Reaction	log K
$Na^+ + HPO_4^{2-} = NaHPO_4^-$	0.60[a]
$K^+ + HPO_4^{2-} = KHPO_4^-$	0.49[a]
$Mg^{2+} + HPO_4^{2-} = MgHPO_4^0$	2.91
$Ca^{2+} + PO_4^{3-} = CaPO_4^-$	6.46
$Ca^{2+} + HPO_4^{2-} = CaHPO_4^0$	2.74
$Ca^{2+} + H_2PO_4^- = CaH_2PO_4^+$	1.4
$Al^{3+} + H_2PO_4^- = AlH_2PO_4^{2+}$	3.00[b]
$Al^{3+} + 2H_2PO_4^- = Al(H_2PO_4)_2^+$	5.30[b]
$Al^{3+} + 3H_2PO_4^- = Al(H_2PO_4)_3^0$	7.60[b]
$Fe^{2+} + HPO_4^{2-} = FeHPO_4^0$	3.6
$Fe^{2+} + H_2PO_4^- = FeH_2PO_4^+$	2.7
$Fe^{3+} + HPO_4^{2-} = FeHPO_4^+$	8.3[c]
$Fe^{3+} + H_2PO_4^- = FeH_2PO_4^{2+}$	3.47[c]

a - 0.2 M ionic strength.

b - 0.1 M ionic strength, 18° C.

c - 0.5 M ionic strength.

The inner sphere complex is much more stable than the outer sphere complex, because the latter does not involve covalent bonding (Sposito, 1989). Phosphorus speciation in soil solutions has been illustrated by Lindsay (1979). At pH 7, with $Ca^{2+} = 10^{-2.5}$ M and $Mg^{2+} = 10^{-3}$ M, the $H_2PO_4^-$ and $CaHPO_4^0$ species each account for approximately 30% of total P. Approximately 20% of total P is predicted to occur as HPO_4^{2-}, 15% as $MgHPO_4^0$, 3% as $CaH_2PO_4^+$, and < 1% as $CaHPO_4^{2-}$. At pH 5, 90% of total P is predicted to occur as $H_2PO_4^-$, with $CaH_2PO_4^0$ accounting for 8% of the total. The $CaHPO_4^0$ and HPO_4^{2-} species each account for approximately 1% of the total P content of pH 5 soil solutions. Clearly, a thorough understanding of phosphate chemistry in soil solutions requires a knowledge of complex ion formation, in addition to acid-base chemistry.

Solid-Phase Chemistry

Precipitation is an accumulation of a substance to form a bulk solid phase resulting in a three dimensional array of atoms in the soil environment (Sposito, 1984). The precipitation of P-bearing solids is favored when the activity of PO_4^{3-} and associated cations in the soil solution exceed the solubility product (Ksp) of a mineral. Each P mineral will support specific PO_4^{3-} activities, depending on the solubility product of the mineral and soil solution characteristics. For example, $FePO_4 \cdot 2H_2O$ may dissolve accordingly,

$$FePO_4 \cdot 2H_2O + H_2O = H_2PO_4^- + H^+ + Fe^{3+}$$
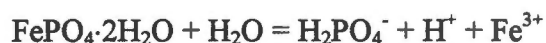
As illustrated in this reaction the activity of solution P supported by a mineral phase will be highly dependent on solution pH, as well as the activity of the associated metal ion. Phosphorus forms sparingly soluble precipitates with many metal ions, but predominantly

7

with Ca, Fe, and Al (Figure 2.2). Phosphorus mineralogy in low pH soils, ~ 4.5, which maintain relatively high soluble Al and Fe activities, is predicted to be dominated by the Al and Fe phosphates, $AlPO_4 \cdot 2H_2O$ and $FePO_4 \cdot 2H_2O$ (Sample et al., 1980)(Figure 2.3). As pH increases to values above 6.0, $H_2PO_4^-$ activities decrease due to the abundance of soluble Ca^{2+} and the precipitation of Ca-phosphates.

Adsorption Chemistry

Adsorption is the net accumulation of matter at the interface between a solid phase and an aqueous solution phase that forms a two dimensional structure (Sposito, 1989). Surface functional groups are the molecular units that protrude from a solid surface into soil solution. An important surface functional group in P adsorption is the hydroxyl group (OH⁻). This group is found on metal oxides, oxyhydroxides, hydroxides, clay minerals, and on amorphous silicate minerals. There are three distinct types of surface hydroxyl groups: (1) a Type A hydroxyl is coordinated to a single cation in the mineral structure, (2) a Type C hydroxyl is coordinated to two cations, and (3) a Type B hydroxyl is coordinated to three cations (Sposito, 1989). Clay minerals expose singly coordinated OH groups at the edge of the octahedral sheet with Al and at the edge of tetrahedral sheets with Si cations. Because of the high valence of Si the OH groups tend only to dissociate protons, whereas the OH groups coordinated to Al protonate and dissociate (Sposito, 1989):
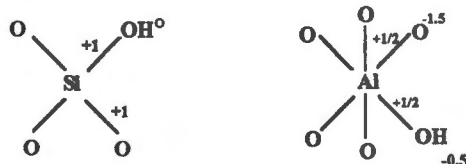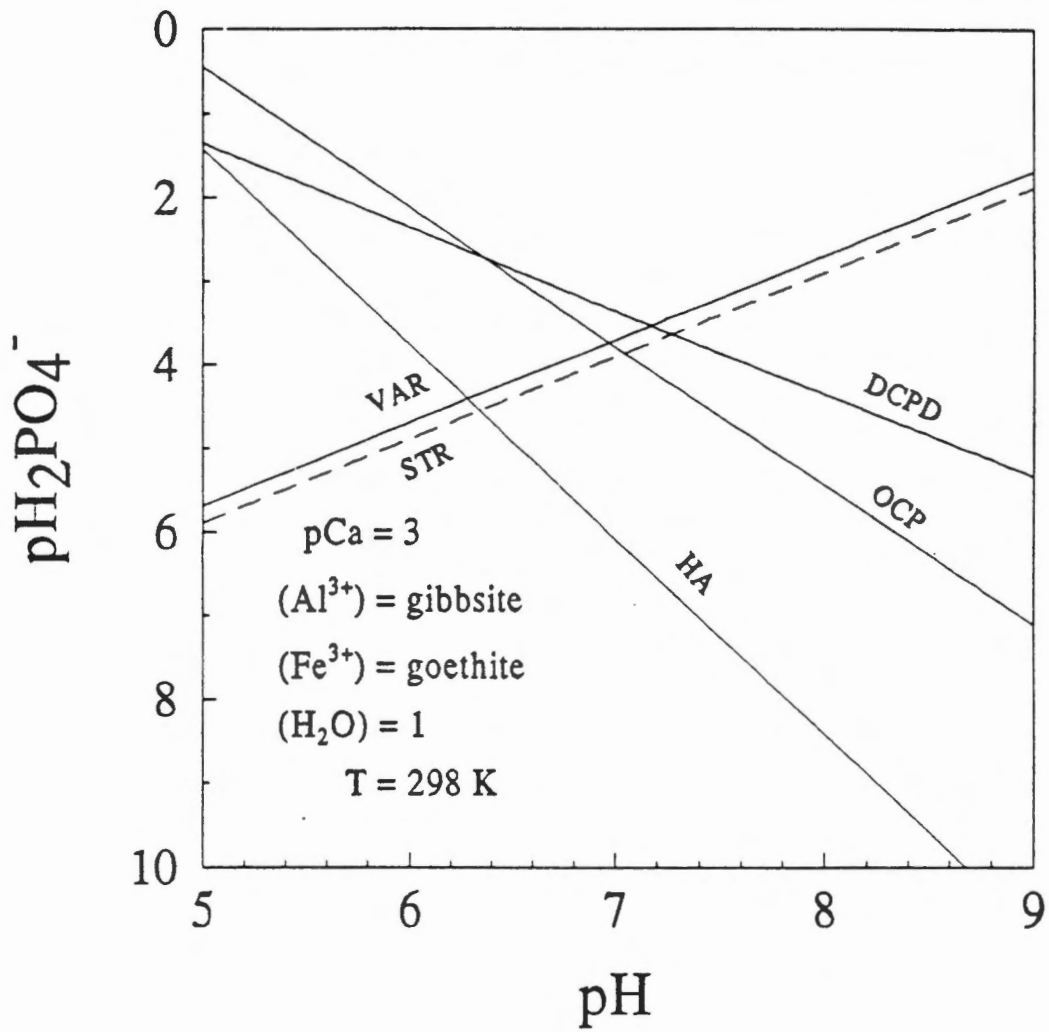
Figure 2.2. Solubility of variscite (VAR), strengite (STR), hydroxyapatite (HA), octacalcium phosphate (OCP), and dicalcium phosphate dihydrate (DCPD) as a function of pH.

9
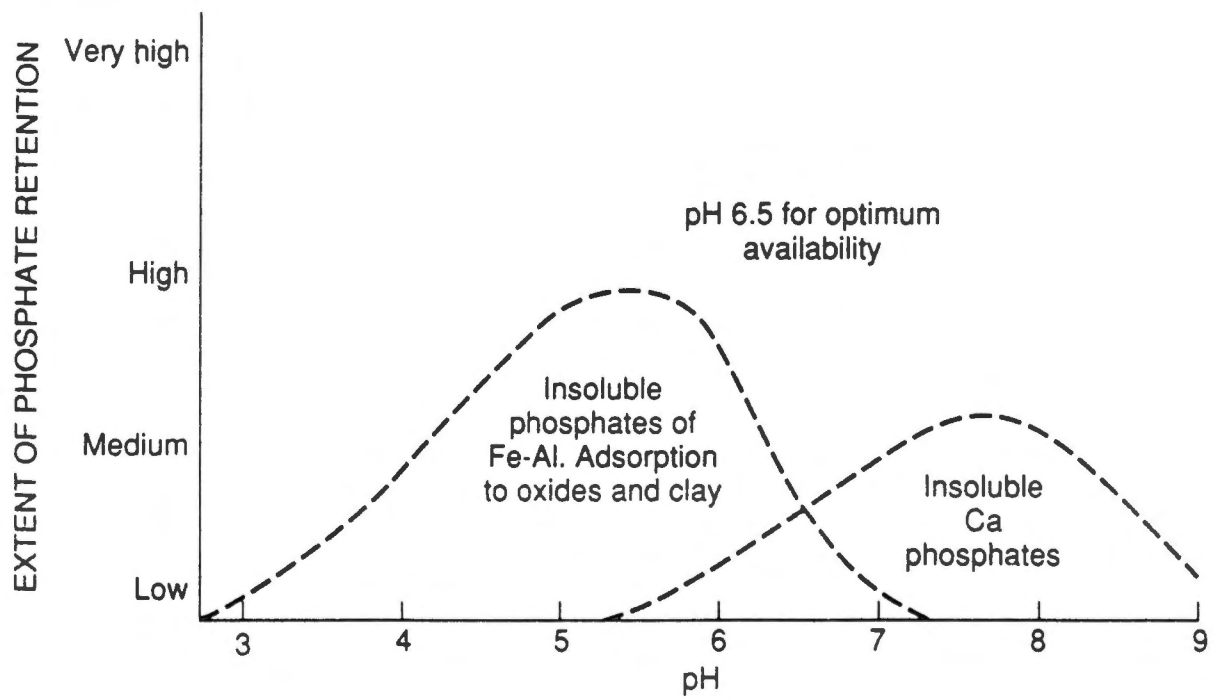
Figure 2.3. The influence of soil pH on P adsorption and precipitation (Stevenson, 1986).

Surface functional groups form complex species with ions or molecules dissolved in solution to form more stable units. Two types of complexes can form; inner sphere surface complexes and outer sphere surface complexes, mechanistically similar to aqueous complexation reactions as previously described. Phosphate is adsorbed primarily through an inner-sphere surface complexation reaction, which almost always proceeding through a ligand exchange process. Ligand exchange refers to a direct bond formation between a ligand and either Al or Fe^{3+} in a soil mineral bearing hydroxyl groups. Ligand exchange occurs because the water molecule, formed at the protonated Lewis acid site, is unstable relative to the phosphate molecule and is readily exchangeable with the phosphate ion in the soil solution (Sposito, 1989). The ligand exchange process for phosphate ions is pH dependent. The influence of pH on phosphate adsorption is the result of changes in net proton charge on soil particles and is illustrated by the following reactions and in Figure 2.4:

$$AlOH(s) + H^{+}(aq) = AlOH_2^{+}(s)$$

$$AlOH_2^{+}(s) + H_2PO_4^{-}(aq) = AlH_2PO_4(s) + H_2O(l)$$
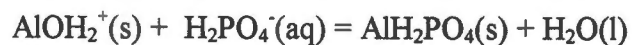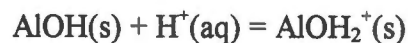
2. Plant Chemistry of Phosphorus

The readily available source of P for plant uptake is the small amount of orthophosphate in the soil solution. As orthophosphate is absorbed by the plant, the equilibrium between solution and labile P is disturbed. This results in the replenishment of soil solution P by the labile fraction. Nonlabile P has very little influence on soil solution P during a single growing season, as the rate of release is too slow (Larsen, 1967). The availability of P to the plant then depends upon the P in solution and the factors that
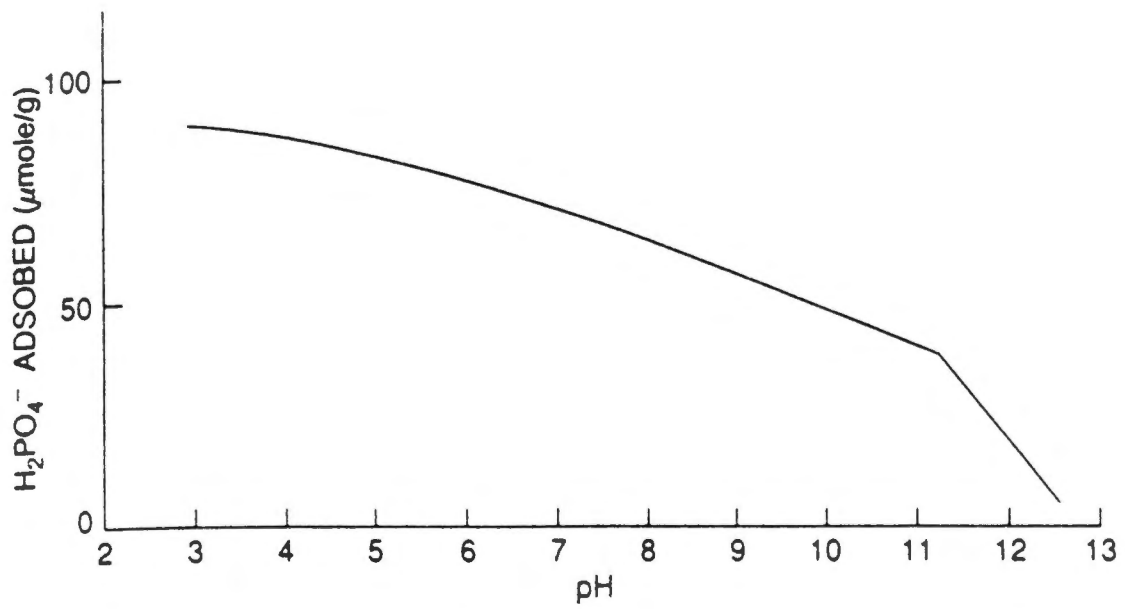
Figure 2.4. The adsorption of P on geothite (FeOOH) as influenced by soil pH (Hingston et al., 1972).

influence the re-supply of solution P from the labile pool.

The important factors within the soil which affect P availability to the plant are the intensity, kinetic, capacity, and diffusion factors (Larsen, 1967). The intensity factor is the measure of P concentration in solution. The kinetic factor describes the rate at which the solution P is replenished from the labile P pool. The capacity factor is the amount of solution P capable of being replenished by the labile P pool. The diffusion factor is the rate at which the rhizosphere P is replenished from the adjacent soil solution.

The concentration of orthophosphate in a normal soil solution ranges from 0.1 to 1 mg L^{-1}. The solution P concentration required by most plants varies from 0.003 to 0.3 mg L^{-1} and depends on the crop species and level of production. To a limit, the P absorption and the growth of plants increase with the concentration of P maintained in the soil solution. Using the analysis of twenty-one soils from the southeastern and midwestern U.S., Pierre and Parker (1927) illustrated that the soil solution contains only 0.4% of the total P required by the plant and with complete removal and renewal in each cycle the non-labile P would be required to replenish the soil solution P 249 times during the growth of the crop. Therefore, a highly efficient mechanism of absorption is required by the cells of the roots to maintain adequate P levels in the plant.

In plants, phosphates are essential for cell division, growth, and development. Plants absorb and transport phosphates primarily as orthophosphate. In young plants, the phosphate tends to concentrate in the shoots, root tips, vascular bundles, and all meristematic regions. The process of entry into the root is affected by temperature, pH, oxygen tension, and the presence of other essential nutrients, e.g. calcium and boron

13

(Lougham, 1981). Phosphate uptake by plants begins in seedlings and continues as the plant matures. After the initial process of phosphorylative absorption by the cytoplasm of the cells of the root epidermis and cortex, the inorganic phosphate is then released into the xylem and transported to the leaves. The phosphate is redistributed before flowering from the vegetative to the reproductive organs (Lawton, 1961).

Once in the plant, phosphates, are essential compounds for storage and structure, primarily organic in form. These storage compounds include phytin, phospholipids, and nucleoproteins available at different developmental stages (Black, 1957). Phosphorus in seeds is stored as phytin. Phytin is hydrolyzed during germination and is used by the developing seedling. Phospholipids are esters of glycerol or inositol with phosphoric acid, fatty acids, and other substances. Nucleoproteins are conjugated phosphorus compounds that contain a protein and nucleic acid.

There are a number of structural P compounds that occur in certain enzymes and metabolites (Black, 1957). The P in the enzymes is present as co-enzymes. In the metabolites P appears to play a vital role in energy transport. The capacity of P to carry energy is made possible by the hydrolysis of organic linkages which result in a high yield of energy. Therefore, the effectiveness by which the plant can utilize available P is very essential to the plant's survival and yield produced.

3. Assessment of Plant-Available Phosphorus

Several procedures have been developed to evaluate plant available P in soils. The commonly used extractants employ complexometric agents and have pH values ranging from 2.5 to 8.5. The extractants generate a measurable amount of extractant P that can be

14

correlated to plant uptake and yield. Extractants that have been used to assess the availability of soil P include water, and solutions of dilute salts, strong acids and complexing ions, and weak acids. In addition, exchange resins and isolated isotopic dilution (^{32}P) techniques have been employed. The available P soil tests can be grouped in two categories: (1) intensive measurements, and (2) quantitative measurements. The intensive measurements include both soil solution P and labile P, which are the forms of P most readily available for plant uptake. The quantitative measurements include plant P along with the chemical extractants used to evaluate soil P availability.

Soluble P

Soil solution P is the amount of P in solution readily available for plant uptake. The concentration of P in solution is often very low and therefore difficult to accurately quantify. Factors such as soil solution pH, composition, temperature, and duration of contact with added fertilizer P, influence the amount of P released into solution (Lopez-Hernandez et al., 1986; Barrow, 1983; Ryden et al., 1977). Procedures developed to assess the quantity of soluble P often consume large amounts of time.

One extractant commonly used to quantify soluble P is distilled water. Sharpley et al. (1988) found that the major portion of H_2O-extractable P (H_2O-P) is derived from calcium phosphate (Ca-P) phases. They attributed H_2O-P to metastable fertilizer reaction products, such as dicalcium phosphate, and reported that only a small fraction of the total Ca-P was removed during the extraction process (Sharpley et al., 1988). Pratt and Garber (1964) illustrated that H_2O-P was positively correlated with NH_4Cl-soluble P, and negatively correlated with clay content. Therefore, this procedure is of limited usefulness

15

in clay-rich soils. Although used in many experiments for correlation purposes, H_2O-P does not accurately measure the quantity of soluble P in soil.

Another commonly used method for solution P determination is the acidified molybdate and isobutanol method. This method was originally developed by Pons and Guthrie (1946) to remove the interference of organic P in the assessment of inorganic P. In the extraction process, molybdate complexes with inorganic P to form phosphomolybdate. This complex is then extracted into isobutanol, leaving organic P in the aqueous phase (Jayachandran et al., 1992). Using the acidified molybdate and isobutanol method, Jayachandran et al. (1992) tested the efficiency of separation between the organic and inorganic P. They found the method to be very effective in recovering soluble inorganic P (Jayachandran et al., 1992). Although this method has proven effective in removing interferences of organic P forms, Watanabe and Olsen (1962) illustrated that dissolved organic matter in the aqueous phase alters the assessment of soluble P. The efficiency in correlating plant P to soluble P in soils with high organic matter is thereby limited.

Labile P

Labile phosphate in soil, commonly measured by isotopic dilution of ^{32}P, is usually regarded as the "pool" of inorganic phosphate from which plant available phosphate is withdrawn (Schofield, 1955). The size of the labile pool is often quantified by isotopic dilution of ^{32}P. Labile P is in isotopic equilibrium with the soil solution over short periods of time (Tinker, 1975). This determination is based on the exchange reaction,

$$S^{31}PO_4 + {}^{32}PO_4 = S^{32}PO_4 + {}^{31}PO_4$$

16
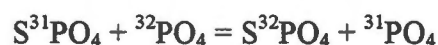
which occurs when the radioactive isotope ^{32}P is added to the soil solution (Russell et al., 1954). The ^{31}P-surface represents the portion of soil P which is readily exchangeable with ^{32}P. The exchangeable isotopes "E values" have been widely used to estimate labile P, which is the sum of the P in soil solution and exchangeable P at the soil surface (Tran et al., 1988).

Laboratory estimations of isotopically exchangeable phosphate are poorly correlated with plant uptake, particularly for relatively short growth intervals, and are highly dependent on soil type (Williams and Knight, 1963). The isotopic dilution ^{32}P method does not adequately assess labile P in soils with high P-fixation capacity and low solution P levels (Hardin et al., 1989). The addition of ^{32}P to soils which have a high P-fixing capacity results in the irreversible complexation of the ^{32}P which prevents the isotopic exchange with ^{31}P on the surface (Amer et al., 1969; Dahal and Hallsworth, 1977). Due to the lack of isotopic exchange on the surface, equilibrium can not be achieved and the calculated labile P value would overestimate the amount of exchangeable P on the surface (Wolf et al., 1985). In soils where the P concentrations are low, the assessment of labile P is hampered because of the decreased accuracy in solution P measurements (Russell et al., 1954).

A second method widely used for the estimation of labile P involves the use of anion exchange resin. Anion exchange resin represents an *in situ* sink for soil P and is non-destructive to the soil. Amer et al. (1955) suggested the anion exchange resin procedure to be more analogous to P withdrawal by plant roots than the process of isotopic exchange. Van Raij et al. (1986) found resin extractable P to be significantly

17

correlated with cotton response to added P in a large number of field studies. Of the two types of resins widely used: chloride- and bicarbonate-saturated resins, Sibbesen (1978) found the bicarbonate-saturated resins to be more effective in extracting P. He reported that the bicarbonate from the resin equilibrates with soluble carbonates resulting in a decrease or an increase in pH depending on the soil type. This indicated that the amount of P extracted and the suspension pH were independent of the soil-water ratio. This occurs because the overall process of P transport from the soil phase through the water phase to the resin phase is regulated by the desorption of phosphates. The bicarbonate ions released from the resin displace adsorbed phosphate ions and enhance resin effectiveness.

Although the resin extraction procedure has proven effective in assessing labile P, with a high correlation to plant available P, the method is difficult to use in routine soil testing. The resin method is often very time consuming and difficult to carry out on a large scale. Furthermore, no standard procedure for the method has been officially adopted and the effect of varying experimental conditions on the method is not well understood.

Another test for the determination of labile P employs Fe- and Al-oxide-impregnated filter paper strips. The filter paper strips act to adsorb the P that remains in the soil suspension. This method is an *in situ* procedure and does not exert a destructive influence on the soil. The paper strip method results have been significantly correlated with plant uptake over a wide range in soil pH and P availability levels. Menon et al. (1990) also illustrated that results from the paper strip method, applied to acid and alkaline

18

(calcareous) soils, were significantly correlated with the results of standard soil test procedures (e.g. Bray and Olsen). However, difficulties with the paper strip procedure arise with variations in soil moisture levels. Under flooded conditions, the amount of P extracted by the strips increased with time whereas, under unsaturated conditions the P extracted by the paper strips was highly variable (Menon et al., 1990). Optimal conditions for the assessment of labile P require that soil moisture levels be maintained between saturation and field capacity for at least 16 hours. Finally, because of spatial variability, a large number of strips are required to obtain the average P content across a field.

Soil Test P

Many chemical extractants have been developed to obtain an analytically measurable value of solubilized P that can be correlated to a measure of plant response. Individual extractants are capable of providing a measure of plant available P in a wide range of soil types. The commonly employed methods for acid and neutral soils are: Bray I (0.03M NH_4F, 0.025M HCl); Bray II (0.03M NH_4F, 0.1M HCl); Mehlich I (0.0125M H_2SO_4, 0.05M HCl); and Mehlich III (0.2M CH_3COOH, 0.25M NH_4NO_3, 0.015M NH_4F, 0.13M HNO_3, 0.001M EDTA). The Olsen (0.5M $NaHCO_3$) procedure is commonly applied to calcareous and neutral soils.

The Bray I method (0.03M NH_4F, 0.025M HCl) was developed for the extraction of phosphates in acid soils. The Bray I method solubilizes soil P through the formation of stable coordination complexes. The F^- ion forms stable complexes with Fe^{3+}, Al^{3+}, and Ca^{2+}, with the latter potentially forming fluorite [CaF_2] (Murphy and Riley, 1962) and forcing the dissolution of $CaHPO_4$ (Thomas and Peaslee, 1973). The F^- ion, being very

effective in complexing Al^{3+} ions (log K_{11} = 7.0; log K_{12} = 12.6; log K_{13} = 16.7; log K_{14} = 19.1) forces the dissolution of aluminum phosphates and the release of P. Similarly, the formation of Fe^{3+}-fluoride complexes (log K_{11} = 6.0; log K_{12} = 9.1; log K_{13} =12.0) enhances iron phosphate dissolution. The HCl provides enough H^+ activity to dissolve Ca-P and to a lesser extent Al-P and Fe-P (Aquino and Hanson, 1984). Phosphorus extracted by the Bray I procedure is correlated with yield response on most acid soils. However, Knudsen (1980) found Bray I to be ineffective in soils containing large amounts of unreacted lime, since P may be precipitated during the extraction process as Ca-P. Also, Bray I does not produce an accurate correlation of labile P in neutral to alkaline soils (Probert and Willet, 1983).

The Bray II soil test (0.03M NH_4F, 0.1M HCl) was adopted in Missouri when rock phosphate was the major source of fertilizer P. The higher acid concentration of Bray II vs. Bray I was needed to provide a more accurate assessment of plant available P from the rock phosphate. Aquino and Hanson (1984) correlated cumulative P removal by consecutive harvest of grain sorghum with Bray II extractable P. They concluded that Bray II provided a better correlation of labile P with extractable P than did the Bray I. However, the quantity of plant available P (as measured by Bray II) will decrease in an inverse proportional relationship to the soil P buffering capacity (Aquino and Hanson, 1984).

The Mehlich I soil test (0.0125M H_2SO_4, 0.05M HCl) was developed for soils with strongly fixed P in the mountain regions of North Carolina. The HCl provides sufficient hydrogen ion activity to dissolve Ca-P, and to a lesser extent Al-P and Fe-P. The order of

solubility in acid solutions is Ca-P > Al-P > Fe-P (Thomas and Peaslee, 1973). The sulfate

in the extract contributes by complexing with cations (log $K[CaSO_4^0]$ = 2.31; log

$K[FeSO_4^+]$ = 4.14; log $K[AlSO_4^+]$ = 3.02) and saturating surface adsorption sites, thus

preventing the reprecipitation or adsorption of the phosphate dissolved by the acid

solutions (Nelson et al., 1953). Olsen and Sommers (1982) demonstrated that the mixed

acids resulted in a better extracting agent for P than did HCl alone. However, Mehlich I

results do not correlate well with P uptake in neutral to alkaline soils where the

predominant source of plant available P comes from Ca-P forms (Mehlich, 1984).

Moreover, Sharpley et al. (1984) found that the acidic solution of Mehlich I would

dissolve more non-labile Ca-P compounds, thus resulting in an overestimation of available

soil P in calcareous and slightly weathered soils.

The Mehlich III soil test (0.2M CH_3COOH, 0.25M NH_4NO_3, 0.015M NH_4F,

0.13M HNO_3, 0.001M EDTA) was developed as a multielement extractant for the

determination of bioavailable P, K, Mg, Zn, Cu, and Mn (Ibrikei et al., 1992). Mehlich

III compensates for the ineffectiveness of the Mehlich I extract in neutral to alkaline soils

through the inclusion of the strong complexing agents, F^- and EDTA. The Mehlich III

extract has been shown to be more effective than Mehlich I in assessing available P

(Mehlich, 1984).

The Olsen bicarbonate method (0.5M $NaHCO_3$) was developed for the

determination of available P in calcareous, alkaline, or neutral soils. The extraction is

effective in soils which contain high amounts of Ca-P. Soluble Ca is precipitated with the

CO_3^{2-}, thus reducing the amount of Ca in solution and thereby forcing the dissolution of

21

Ca-P. Also, the CO_3^{2-} in solution will displace PO_4 from the soil surface further improving the measure of labile P. Although the Olsen bicarbonate method has proven effective in calcareous, alkaline, or neutral soils, it has not been shown to accurately assess plant available P in acidic soils.

The assessment of plant available P on loess based soils by methods such as Mehlich I and III and Bray I and II generally predict a crop response to fertilizer P. However, several studies have shown negligible response to fertilizer P when applied to loess based soils at recommended rates. Howard et al. (1990) found soybeans to be unaffected by soil-incorporated P on several West Tennessee soils that ranged from low to high Mehlich I extractable P. Hardin et al. (1989) evaluated the ability of soil solution orthophosphate concentration and the Bray I, Bray II, Mehlich I, Mehlich III, and Mississippi (0.05 M HCl, buffered acidic AlF_3 solution at pH 4.0; Hardin, 1988) extractants to predict plant available P. For all six procedures, the extraction test underestimated the plant available P. Essington et al. (1994) assessed the effect of long-term no-till, tillage, and P fertilization on plant available P and P solid-phase speciation in West Tennessee soils. They determined Mehlich III and Olsen bicarbonate extractable P, organic P, and total P. The modified Chang and Jackson sequential fractionation procedure was also used to determine P distribution in the solid phase. The extraction results were poorly correlated with plant P. Clearly, the assessment of plant available P in loess based soils using recommended soil test procedures has proven unreliable, generally burdening producers with an unnecessary fertilizer P expense.

22

A need exists for an easily applied test which can accurately determine the quantity of plant available P and thereby produce correct fertilizer recommendations. The efficiency of a soil test should be determined by its ability to measure the total quantity of plant available residual P in the soil if recommendations are to be developed that will insure maximum economic yield. It is also clear from the West Tennessee data that plants have the ability to remove P from soils where chemical extractants inadequately assess the quantity of plant available P. Based on these findings, a more effective P extractant maybe identified through the examination of rhizosphere chemistry. Such a methodology should be based on naturally occurring constituents, such as root exudates and microbial products, in the rhizosphere which have been proven to solubilize various forms of P and adequately supply plants with P.

4. Rhizosphere Chemistry

Under natural conditions the solubilization of soil phosphate by plants depends on the phosphate uptake by roots and on the presence of both root exudates and associated microbial products in the rhizosphere. Root exudates are primarily composed of sugars, organic acids, amino acids, and phenolic compounds. These are the predominant compounds in the rhizosphere. The organic acids in root exudates are thought to be responsible for the solubilization of sparingly soluble inorganic phosphates in the soil (Moghimi et al., 1978b). Although many organic acids act as chelates, they can also be a readily available source of H^+ ions. The production of H^+ ions will thereby contribute to the dissolution of phosphate minerals such as hydroxyapatite (Moghimi and Tate, 1978).

23

The pH of the rhizosphere can be affected by the nature and quantity of the organic acids produced, as well as the balance between the H^+ and HCO_3^- released by the root (Pojasok and Kay, 1990). The balance between the H^+ and HCO_3^- released by the root is related to the balance between anions and cations absorbed by the plant (Nye, 1981). Therefore, the chelation and H^+ ion production by root exudates aid in the solubilization of soil P.

Microorganisms in the rhizosphere receive nutrition from the root exudates. The compounds utilized (primarily sugars) by the microorganisms are mostly lower molecular weight substances exuded by plant roots. In utilizing root exudates, microorganisms enhance the accessibility of P to plants. Bowen and Rovira (1966) found that the presence of bacteria increased the uptake and translocation of phosphate to plants. Sperber (1957) found that P solubilizing organisms in the rhizosphere of subterranean clover (*Trifolium subterraneum* L.), ryegrass (*Lolium perenne*), and wheat (*Triticum aetivum* L.) roots constituted 26-39% of the microbial population. Katznelson and Rose (1959) also found approximately one-third of the bacteria from the rhizoplane of wheat to show P-solubilizing abilities. They also found these organisms to be more active metabolically than other bacteria isolated from the same soil (Katznelson and Rose, 1959). Phosphate solubilizing bacteria and fungi constituted 0.5 and 0.1%, respectively, of the general soil microbial population in prairie soils, with P solubilizing bacteria outnumbering P solubilizing fungi two-fold to 150-fold (Kucey et al., 1989). Phosphate solubilizing organisms have been found to solubilize inorganic forms of P by excreting organic acids that directly dissolve P-bearing minerals (Sperber, 1958). Analysis of culture filtrates of

24

pure isolates of these microorganisms has revealed a number of organic acid products. Two such organic acids found in the rhizosphere of plants and proven to effectively solubilize soil P are citric and 2-ketogluconic acids (Inskeep and Silvertooth, 1988; Traina et al., 1986a; 1987; Moghimi and Tate, 1978; Moghimi et al., 1978a; 1978b; Halder and Chakrabartty, 1993). These organic acids may increase the available P by (1) competing for the adsorption site, (2) dissolving the P bearing minerals and absorbents, and (3) modifying the surface chemistry of P adsorbents (Traina et al., 1986b). Based on their effectiveness in solubilizing phosphates, citric acid and 2-ketogluconic acid were chosen to develop a procedure that maximizes P solubilization from loess based soils.

Citric Acid

Citric acid ($C_6H_8O_7$), a tricarboxylic acid, was hypothesized by Dyer (1894) to be a root exudate. Citric acid has proven to be important in the solubilization of phosphates in soils. The citrate ion possesses the ability to form surface complexes with Fe and Al hydroxides, thereby decreasing the number of sites available for phosphate adsorption (Struthers and Sieling, 1950). Earl et al. (1979), using a synthetic Fe and Al gel, found citrate (10^{-3} M) to reduce P sorption by 50%. Grossl and Inskeep (1989) found that citrate inhibited precipitation of phosphates by adsorbing to the surface of crystals. The organic ligand blocks the adsorption sites that act as nuclei for crystal growth. The adsorption characteristics of citric acid are related to its functional-group content (tricarboxylate) and to its size (Grossl and Inskeep, 1991). The size of the citrate ion allows it to provide optimal surface coverage with the ability to block growth sites since it is smaller than other organic acids (e.g. humic, fulvic, and tannic) and has the highest

25

COOH content per mole of C. At pH 5.7, citric acid adsorption to brushite [$CaHPO_4 \cdot 2H_2O$] is a result of surface complexation reactions with Ca. The reduction of free Ca^{2+} in solution through aqueous complexation (log K_{11} = 4.68) has also been found to inhibit the formation of Ca-phosphates, resulting in elevated levels of PO_4 in the soil solution (Inskeep and Silvertooth, 1988). Citrate also forms strong aqueous complexes with Al (log K_{11} = 9.9), which influences the crystallization of Al hydroxides. The effect of citric acid on the precipitation of Al hydroxides is dependent upon the initial citrate/Al ratio. At high citrate concentrations (citrate/Al ratio of 1.0) most Al remains in solution (Goh and Huang, 1984). The perturbation leading to the structural distortion of the Al hydroxides (Figure 2.5) is so strong that it even occurs in alkaline soils (Violante and Violante, 1978). The complexing characteristics of citric acid would thereby suggest that it is an effective extractant of P.

Soil pH and citric acid concentration have varying effects on the solubilization of soil phosphates. Traina et al. (1986b) reported a decrease in the solubility of orthophosphate in a acidic, montmorillonitic soil as the pH was increased from 4 to 7 in the absence of citric acid. Low concentrations (< 0.1 mol L^{-1}) of citric acid in the pH range 5.5 to 7.0, resulted in a sharp decrease in the solubility of orthophosphate. A recovery in orthophosphate solubility to its value in the absence of organic ligand was observed as the concentration of citric acid was increased to 0.6 mol L^{-1}. Traina et al. (1986b) postulated that the addition of citric acid at low concentrations would suppress the hydrolysis and polymerization of Al, thereby increasing the amount of exchangeable Al. The newly released Al would react with orthophosphate to form an Al-phosphate
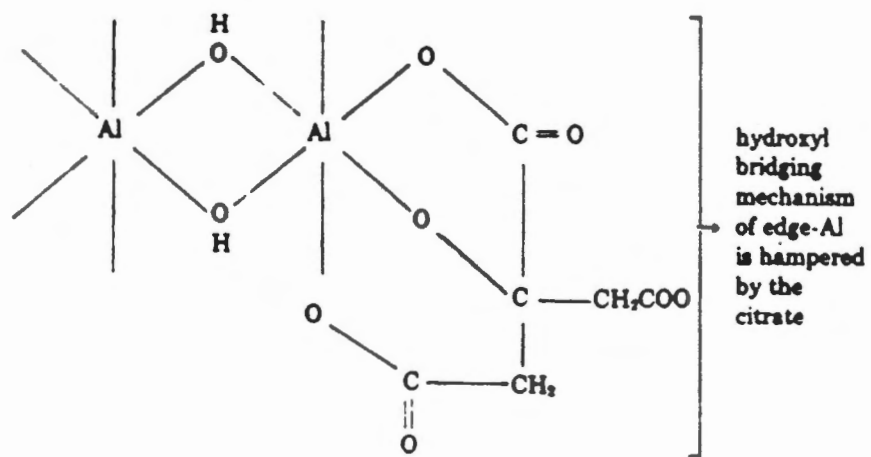
Figure 2.5. Influence of citric acid on the surface chemistry of Al-hydroxides (Kwong and Huang, 1979).

precipitate, causing a decrease in the solubility of orthophosphate (Goh and Huang, 1984). This is consistent with the retention mechanism proposed by Coleman et al. (1960) in which orthophosphate precipitates with hydrolyzed, exchangeable Al^{3+} to form $Al(OH)_2H_2PO_4(s)$. As the concentration of citric acid increases in the soil solution the ligand forms soluble complexes with the exchangeable Al^{3+} (Goh and Huang, 1984). This promotes the dissolution of Al-P, increasing orthophosphate solubility. Kafkafi et al. (1988) found citric acid to be very effective in the release of soil P when the initial acid concentration was increased to 5 mmol L^{-1}. He noted that further increases in citric acid concentrations (to 50 mmol L^{-1}) did not result in a concomitant increase in soluble P.

At pH 6.0 and EC = 1 dS/m, citrate was more effective than bicarbonate in releasing P from K-saturated kaolinite (Nagarajah et al., 1968). However, Traina et al. (1986a) illustrated that an increase in ionic strength and Ca concentration had no qualitative effect on the solubilization of PO_4 by citric acid from soil. In the absence of citrate, the orthophosphate concentrations are affected only by the amount of soluble Al^{3+} (Traina et al., 1986a).

Citrate plays an important role in the kinetics of orthophosphate solubilization. During reaction times of less than 1 hour, citrate acts to solubilize and complex with hydroxy Al species. This exposes new mineral surfaces for solute adsorption. Displacement of adsorbed orthophosphate by citrate also occurs during this time frame:

$$SH_2PO_4(s) + HCit^{2-}(aq) + H_2O(l) =$$

$$SH_2Cit(s) + H_2PO_4^-(aq) + OH^-(aq)$$
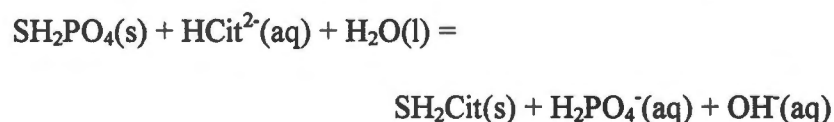
where S represents 1 mol of positive surface charge (Traina et al., 1987). Upon the initial

addition of the citric acid to the soil suspension there is an increase in solution pH due to the displacement of hydroxyl groups from mineral surfaces. During reaction times between 1 and 8 h, additional citrate (free ligand and bound with Al) is sorbed on mineral surfaces. These reactions immobilize citrate and raise the solution pH. After the initial pH increase, the pH value begins to decline because the Al^{3+}-citrate complex promotes a release of protons from the citrate carboxyl groups. The proton in the citrate reactant could be replaced by $AlOH^{2+}$. Alternatively, free Al and its hydrolysis products could be complexed by sorbed citrate.

$$Al(OH)_q X_{3-q}(s) + p\ HCit^{2-}(aq) =$$

$$Al(OH)_{q-2p}(HCit)_p X_{3-q}(s) + 2p\ OH^-(aq)$$
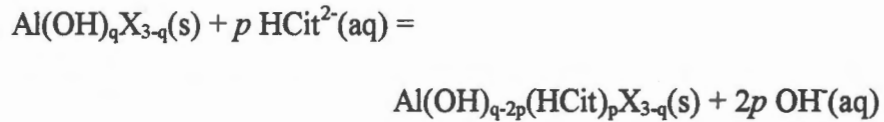
where $q \cong 2.5$ to 2.7 (Goh and Huang, 1984), X refers to amount of absorbent bearing 1 mol of negative surface charge, and p is a stoichiometric reaction coefficient (Traina et al., 1987).

Traina et al. (1987) proposed that for reaction times between 8 and 30 h, soluble orthophosphate may be reduced by the precipitation of an Al-citrate-PO_4 phase. In a NaCl background the reaction is as follows:

$$Al(OH)_{q-2p}(HCit)_p X_{3-q}(s) + (3-q)Na^+(aq) =$$

$$(3-q)NaX(s) + Al(OH)_{q-2p}(HCit)_q^{(3-q)+}(aq)$$
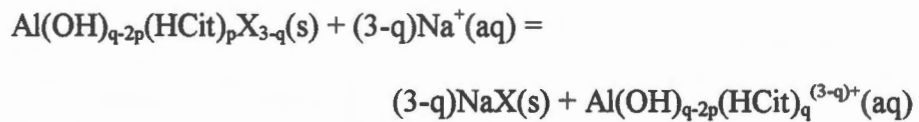
where q-2p < 2.5. This reaction is followed by the coprecipitation of an Al-citrate-phosphate.

$$Al(OH)_{q-2p}(HCit)_p^{(3-q)+}(aq) + (3-q)H_2PO_4^-(aq) =$$

$$Al(OH)_{q-2p}(HCit)_p(H_2PO_4)_{3-q}(s)$$
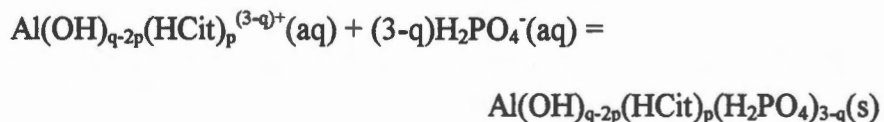
2-Ketogluconic Acid

The ability of plant roots to solubilize soil phosphates clearly depends on the presence of root exudates. Associated microbial by-products may also promote phosphate dissolution. Moghimi et al. (1978a) illustrated that a combination of root exudates and microbial by-products of wheat plants enhanced the dissolution of Ca-phosphates. The major acid component of these rhizosphere products was 2-ketogluconic acid ($C_6H_{10}O_7$).

$$HO-\underset{\underset{H}{|}}{\overset{\overset{H}{|}}{C}}-\underset{\underset{OH}{|}}{\overset{\overset{H}{|}}{C}}-\underset{\underset{H}{|}}{\overset{\overset{OH}{|}}{C}}-\underset{\underset{OH}{|}}{\overset{\overset{H}{|}}{C}}-\underset{\underset{O}{\|}}{C}-COOH$$
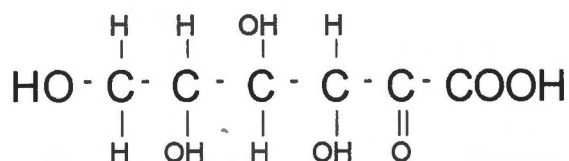
A colorimetric assay by Moghimi et al. (1978a) of the anionic fraction in the rhizosphere products of wheat found that 2-ketogluconic acid constituted approximately 20% of the total rhizosphere products. The total rhizosphere products also consisted of ~ 30% carbohydrates (principally glucose and fructose) and a negligible amount of amino acids.

Organisms that produce 2-ketogluconic acid have been isolated from the rhizosphere of plants (e.g. wheat, corn, and peas). It has been suggested that high concentrations of 2-ketogluconic acid may build up around bacteria adhering to the mineral surface (Erlich, 1981). Webley and Duff (1965) found the highest number of 2-ketogluconic acid producers in agricultural soils, particularly in soils receiving heavy and recent manure applications. They also demonstrated that these organisms were more efficient in 2-ketogluconic acid production in well drained soils as opposed to poorly drained soils.

A study by Neijssel and Tempest (1975), using *Klebsiella aerogenes*, noted a rapid rate of glucose uptake and a lower conversion of glucose to bacterial mass when phosphate was limiting. The accelerated rate of glucose metabolism resulted in the increased production of 2-ketogluconic acid and thereby an increased solubilization of phosphates. The growth of *Klebsiella aerogenes*, in a phosphate limiting environment with a dilution rate of 0.3 hr^{-1} (35°C; pH 6.8), produced the following conversion percentages of glucose carbon: 21% cells, 22% carbon dioxide, 7% acetate, 10% gluconic acid, 25% 2-ketogluconic acid, and 15% polysaccharide.

The production of 2-ketogluconic acid is a result of the oxidation of glucose by many species of *Pseudomonas*, *Bacillus*, *Streptomyces*, *Aerobacter*, *Klebsiella*, and *Acetobacter* (Sokatch, 1969; Kucey et al., 1989). Klasen et al. (1992) suggested a pathway for 2-ketogluconic acid production which stemmed from glucose oxidation. They illustrated that glucose is oxidized by *Gluconobacter oxydans* on a direct, nonphosphorylative pathway to glucono-δ-lactone and gluconic acid, respectively. This microorganism further oxidizes gluconic acid to 2-ketogluconic acid and 5-ketogluconic acid (Klasen et al., 1992). Chiyonobu et al. (1973) reported that the oxidation of glucose to 2-ketogluconic acid is catalyzed by 2-ketogluconate reductase at an optimal pH of 10.5 at 50°C.

Halder and Chakrabartty (1993) demonstrated that the production of 2-ketogluconic acid by *Rhizobium* and *Bradyrhizobium* was a major factor in inorganic phosphate solubilization. The 2-ketogluconic acid produced in the culture medium was found to solubilize hydroxyapatite *in vitro*. Further, the solubilization appeared to be

31

dependent upon the concentration of the 2-ketogluconic acid. The solubilization of phosphate increased with an increase in 2-ketogluconic acid concentration.

There has been very little investigation into the mechanism by which 2-ketogluconic acid enhances the solubility of phosphates in acid soil. Duff and Webley (1959) suggested 2-ketogluconic acid to be a strong complexing agent for Ca^{2+}, promoting the dissolution of Ca-phosphates. Webley et al. (1963) suggested that microbes may enhance the dissolution of aluminosilicates due to the production of extracellular complexing substances. Subsequently, Duff et al. (1963) using chromatography with a aniline phthalate spray showed that 2-ketogluconic acid forms soluble complexes with divalent metal cations (Ca, Mg, Mn, Zn, Sr, and Ni). Berrow et al. (1982) demonstrated that 2-ketogluconate, extracted from a batch of *Erwinium* species, was able to extract more cobalt, nickel, zinc, iron, titanium, and vanadium than ammonium acetate at the same concentration from soil. They concluded 2-ketogluconate was chelating a portion of the non-exchangeable forms of these metals in soil. Furthermore, they demonstrated that 2-ketogluconate was equal to EDTA and DTPA in extracting copper, manganese, molybdenum, nickel, and zinc at the same concentration (Berrow et al., 1982).

In contrast, Moghimi and Tate (1978) promoted 2-ketogluconic acid as readily available source of H^+ for Ca-P dissolution. 2-Ketogluconic acid is one of the stronger monobasic carboxylic acids with a pKa value of 2.8 at 25°C (Moghimi and Tate, 1978). Clearly, there is a combined effect of acid dissolution of mineral phosphates followed by metal chelation that promote P solubilized in the presence of 2-ketogluconic acid.

32

5. Objectives

The application of commonly used soil tests (e.g. Mehlich I and III) on loess-derived West Tennessee soils has proven ineffective in predicting plant response to fertilizer P. Clearly, the development of a soil test that can accurately assess available P is required. Moreover, such a soil test should be based on rhizosphere chemistry, utilizing organic acids that are responsible for P solubilization. Two organic acids that occur in the rhizosphere and have been shown to enhance P solubilization are citric acid and 2-ketogluconic acid. Citric acid possesses the capability to enhance P solubilization by forming aqueous complexes with Ca^{2+}, Al^{3+}, and Fe^{3+} and by competing with phosphates for surface sites on Fe and Al hydroxides. The aqueous association of 2-ketogluconic acid with Ca^{2+} has been suggested as a mechanism by which Ca-phosphates are dissolved in alkaline soils. However, in acidic soils containing a predominance of Fe- and Al-phosphates, the mechanisms responsible for P solubilization has not been established. It is plausible to assume, based on a comparison of 2-ketogluconic acid, EDTA, and DTPA extraction efficiencies for trace elements, that the aqueous complexation of 2-ketogluconic acid metal cations may be significant. The specific research objectives are:

1) Develop a procedure using citric acid and 2-ketogluconic acid that maximizes the extraction of P from loess based soils.

2) Compare the extraction capabilities of the developed organic acid extraction procedures to the Mehlich III and Olsen bicarbonate methods, and the modified Chang and Jackson sequential fractionation procedure.

33

3) Correlate ear leaf P concentrations and corn (*Zea mays* L.) yield to organic acid extractable P, Mehlich III and Olsen extractable P, and the P fractions extracted by the modified Chang and Jackson sequential fractionation procedure. Determine whether the organic acid procedure or the standard soil tests offer a more accurate assessment of plant-available P and identify the chemical pools accessed by the organic acids.

4) Investigate chelation as a mechanism for soil P solubilization by 2-ketogluconic acid by examining the equilibrium solubility of gibbsite and assessing aqueous aluminum speciation.

Chapter III

Materials and Methods

1. Soil P Test Development

Soil and Plant Samples

Soil samples used to evaluate P availability were obtained from on-going research projects at the Milan and Ames Plantation Experiment Stations of The University of Tennessee, Institute of Agriculture. At the Ames Plantation, a three year study was initiated in 1992 to evaluate swine manure as a source of P for crop production (O'Dell et al., 1993; O'Dell, 1995). The soil is classified as a Lexington silt loam (fine-silty, mixed, thermic, Typic Paleudalf). Manure was applied in early spring with an applicator to deliver 15, 30, and 60 kg P ha^{-1} yr^{-1} with a check plot receiving triple superphosphate (TSP) at 30 kg P ha^{-1} yr^{-1}. In 1994 (the third year of study) soil samples were collected after manure application. Approximately ten cores were removed from each plot with a 2.5 cm diameter push probe to depths of 0-10, 10-20, and 20-30 cm. At the 4 leaf, 10 leaf, and silking growth stages, four 5 cm diameter cores were collected from each plot with a tractor mounted hydraulic sampler and the soil samples were taken at depths of 0-10, 10-20, 20-30, 30-45, 45-60, 60-75, and 75-90 cm. The experimental design was arranged in a randomized complete block with treatments replicated 5 times.

The second site chosen for study was located at the Milan Experiment Station. Research was initiated in 1983 to examine the effects of tillage and fertilizer P rate on the chemical status of soil P and corn ear leaf P concentration (*Zea mays* L.) (Essington et al., 1994). The soil is classified as a Loring silt loam (fine-silty, mixed, thermic, Typic

35

Fragiudalf). Annually application rates of P as TSP were 0, 10, and 30 kg P ha^{-1}. Soil samples were obtained in 1992 from no-till (NT) and disk till (DT) plots, cropped in corn with a wheat (*Triticum aestivum* L.) cover. The depth increments were 0-4, 4-8, 8-15, 15-30, 30-45, and 45-60 cm. The plot design for the study was a split plot with four field replicates; where the main plots are tillage and subplots are the P rates.

Soil samples from both the Ames and Milan field studies were air dried and passed through a 2 mm sieve. Available P was assessed using Mehlich III (0.2M CH_3COOH, 0.25M NH_4NO_3, 0.015M NH_4F, 0.13M HNO_3, 0.001M EDTA) and Olsen (0.5M $NaHCO_3$, pH 8.5) methods. The samples were extracted using 2 g of soil with 20 ml of extracting solution (Mehlich III) and 1 g of soil with 20 ml of extracting solution (Olsen). The samples were placed in 50 ml polypropylene centrifuge tubes, shaken for 30 minutes, and centrifuged at (750) x g for 10 minutes to separate solid and solution phases. The extracted P in solution was quantified using the method described by Watanabe and Olsen (1965) which utilizes a coloring agent composed of ammonium molybdate-antimony potassium tartrate, H_2SO_4, and L-ascorbic acid. Solution P was analyzed by diluting 1 ml of the extract in 9 ml of deionized water, and 1 ml of the coloring agent. The solution was vortexed 1-2 seconds for adequate mixture and allowed to equilibrate for 10 minutes. A five point calibration curve (0, 1, 5, 10, and 15 mg P L^{-1}) was established and the absorbance of the colored solution was measured using a Milton Roy Spectronic 401 at 880 μm.

Inorganic P fractions were determined using a modified Chang and Jackson method (Peterson and Corey, 1966). The inorganic soil P was selectively dissolved in a

sequential fashion into NH$_4$Cl-extractable (soluble P), NH$_4$F-extractable (Al-P), NaOH-extractable (Fe-P), citrate-dithionite-extractable (Reductant-P), and H$_2$SO$_4$-extractable (Ca-P) P fractions. The initial step in the sequential selective dissolution procedure involved the removal of Ca and easily soluble P using 1 M NH$_4$Cl. The solid and solution phase were then separated by centrifugation. Next, Al-P was extracted using 0.5 M NH$_4$F adjusted to pH 8.2. The suspension was again centrifuged to separate the solid and solution phase, after which the soil was washed twice with saturated NaCl. Fe-P was then extracted using 0.1 M NaOH, which was followed by two additional washings with the saturated NaCl. Reductant soluble P was extracted with 0.3 M sodium citrate and 1 g of solid sodium dithionite at 80 °C. After two more washes with saturated NaCl, Ca-P was extracted using 0.25 N H$_2$SO$_4$. Phosphorus concentrations in the Al-P, Fe-P, and Ca-P solutions were measured colorimetrically, while P concentrations in the reductant soluble P solution was measured by inductively coupled plasma atomic emission spectrometry (ICP-AES). Colorimetric P concentrations were analyzed by adding a chloromolybdic boric acid solution and a chlorostannous reductant solution (Petersen and Corey, 1966) to an aliquot of extract. Absorbance was measured using the Milton Roy Spectronic 401 at 660 μm.

Plant samples for plant P analysis were collected from the Ames Plantation and Milan Experiment Station. At the Ames Plantation two varieties of corn 'DeKalb 689' and 'Pioneer 3343' were established. Whole plant samples from the Ames Plantation were harvested from a 1 m section of row at the 4 leaf and 10 leaf growth stages. Ear leaves were harvested at the silking stage from a 1 m row section. At the end of the growing

37

season all plots were harvested to measure grain yield. At the Milan Experiment Station, corn ear leaf samples were collected from each plot at silking, and plots were harvested to determine grain yield. After drying at 60°C, the plant samples from both sites were finely ground for analysis (O'Dell, 1995). A 200 mg sample of plant material was then placed in 16 x 100 mm test tube and ashed for 24 hours at 500 °C. The residue was dissolved in 10 ml of 2 N HCl and the P concentration determined by ICP-AES.

Citric and 2-Ketogluconic Acid Extraction Development

In order to maximize the extraction of P by citric acid and 2-ketogluconic acid, preliminary evaluations were performed using various organic acid concentrations, organic acid ratios, background electrolyte concentrations, pH values, extraction times, and solid-to-solution ratios. The initial citric and 2-ketogluconic acid concentrations were: 0, 2.5, 5, and 10 mM, based on the findings of Kafkafi et al. (1988). The organic acids were combined in three ratios: 100% citric; 1:1 citric:2-ketogluconic; and 100% 2-ketogluconic. Preliminary experiments using 0.1 M $CaCl_2$ as a background electrolyte produced a rapid reduction in solubilized P. This was attributed to the precipitation of calcium phosphates. The background electrolyte was then changed to 0.1 M $MgCl_2$ to minimize the reprecipitation of solubilized P. Although past studies have shown 2-ketogluconic acid to be effective in solubilizing P at a high pH range, and citric acid at a low pH range (Traina et al., 1986a, 1986b, and 1987; Neijssel and Tempest, 1975), preliminary tests were done at pH values of 3.5 and 5.5. These low pH values were preferred because the organic acid extractions were to be applied to acidic West Tennessee soils. Extraction times were chosen based on the work of Traina et al. (1986b,

1987) and on the need for rapidity in soil-P test development. Traina et al. (1986b) found

no change in the solubilization of orthophosphate by citric acid between 24 and 48 h.

They further demonstrated that a short reaction time maximized P extractability (Traina et

al., 1987). Therefore, 1 h, 3 h, and 6 h extraction times were evaluated to facilitate

handling for rapid analysis. Finally, solid-to-solution ratios were varied to examine the

influence it may have on P solubilization. Three solid to solution ratios were tested: 1g

soil per 25 ml solution; 5 g of soil per 25 ml solution; and 10 g of soil per 25 ml solution.

Preliminary extraction tests were performed using two soil samples: surface (0-15

cm) and subsurface (15-45 cm) samples from the Milan site. The organic acid extractable

P concentration in the subsurface soils was below detection limits and not reported.

Solutions for the preliminary tests were prepared with the appropriate organic acid and

ionic strength concentration at the required pH. The pH was adjusted by using

concentrated HCl or 0.1 M NaOH. The soil sample was combined with the prepared

solution in a 50 ml polypropylene centrifuge tube, shaken for the required time period and

centrifuged. Phosphate concentrations in solution were determined using the method of

Watanabe and Olsen (1965), as previously described.

The P extraction data were analyzed using the GLM procedure of the Statistical

Analysis Systems (SAS), with the mean separations conducted using Duncan's multiple

range test. The complete data set is presented in Appendix A. A complete randomized

block design was used with the following variables included in the model: organic acid

concentration, organic acid ratios, pH values, extraction time, and solid to solution ratios.

Based on the preliminary findings, two extraction procedures were chosen for further

evaluation: 1) 10 g of soil extracted with 25 ml of 10 mM citric acid at pH 3.5 for 3 hours, and 2) 5 g of soil extracted with 25 ml of 5 mM citric acid and 5 mM 2-ketogluconic acid at pH 3.5 for 6 hours.

Citric and 2-Ketogluconic Acid Extractions

The selected organic acid extraction procedures were applied to 0-10 cm and 10-20 cm depth samples from the Ames and the 0-8 cm and 8-15 cm depth samples from the Milan sites. These depth increments were chosen based on P concentrations previously determined by O'Dell (1995) and Essington et al. (1994) from these sites.

The first extraction procedure developed (OE1) to extract inorganic P had a 10 mM citric acid concentration with 0.1 M $MgCl_2$ background electrolyte. Solution pH was adjusted to 3.5 with 0.1 M NaOH. A 10 g mass of soil was combined with 25 ml of extraction solution in a 50 ml polypropylene centrifuge tube. The mixture was shaken for 3 hours at ambient temperature and centrifuged at (750) x g for 10 minutes to separate the solid and solution phases. The extract was then analyzed for P using the method of Watanabe and Olsen (1965).

The second organic acid procedure developed (OE2) to extract inorganic P was composed of 5 mM citric acid and 5 mM 2-ketogluconic acid in a 0.1 M $MgCl_2$ background electrolyte adjusted to a pH of 3.5 with 0.1 M NaOH. A 5 g mass of soil was combined with 25 ml of the extraction solution in a 50 ml polypropylene centrifuge tube. The mixture was shaken for 6 hours and centrifuged to separate the solid and solution phases. The extracted P was analyzed following a procedure by Watanabe and Olsen (1965).

40

The CORR procedure of SAS was utilized to identify significant relationships between two independent variables. Simple correlations between OE1-P, OE2-P, Mehlich III-P, Olsen-P, and the P content of the Chang-Jackson fractions were computed. Simple correlations were also conducted between plant P concentrations, yield, available P indices, and inorganic P fractions for each soil type and depth increment.

2. Influence of 2-Ketogluconic Acid on Aqueous Al^{3+} Chemistry

If 2-ketogluconate forms significant soluble complexes with Al^{3+} or its hydrolysis products, then gibbsite $[Al(OH)_3]$ solubility will be enhanced. The gibbsite used in the experiment was commercially hydrated alumina, C31, produced by the Aluminum Company of America (ALCOA). The hydrated alumina was composed of approximately: 65-67% Al_2O_3, 0.01-0.07% SiO_2, 0.002-0.006% Fe_2O_3, and 0.15-0.42% Na_2O. The gibbsite had a crystalline powder form with the following physical characteristics: specific gravity 2.4; bulk density 0.7-1.28 g/cm^3; and a pH of 8.5 in 20% solution. The gibbsite powder was analyzed by X-ray diffraction (random sample orientation, Ni-filtered Cu $K\alpha$ radiation, 45 kV and 40 mA, 2° 2θ min^{-1}) and the diffractogram was compared to Joint Committee on Powder Diffraction Standards (JCPDS) files for solid-phase identification. No impurities were detected by the X-ray diffraction analysis (Figure 3.1).

The solubility experiments were conducted in much the same manner as that used by Bloom and Weaver (1982). Samples of gibbsite (10 g) were pre-washed with 0.1 N HCl in 250 ml polypropylene bottles to remove any poorly crystalline $Al(OH)_3$ from the mineral surface. Following a 14-day cleansing period, with constant agitation, the samples were centrifuged and the clear supernatant was carefully removed. The samples were
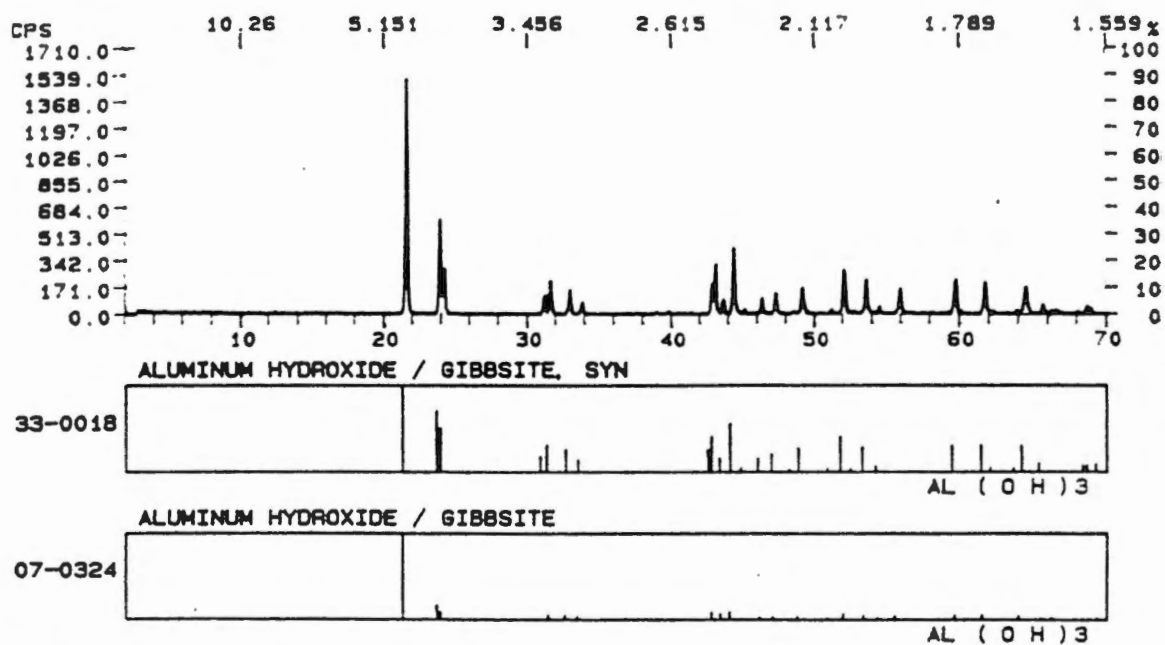
Figure 3.1. X-Ray diffractogram of ALCOA synthetic gibbsite (C31).

washed, for 30 minute intervals, three more times with the KCl background electrolyte, used in the equilibrium solubility experiments.

The equilibrium solubility experiments were conducted using a 10g:100ml solid:solution ratio, ionic strengths of 0.05 M and 0.1 M KCl, and 2-ketogluconic acid concentrations of 0 M, 0.005 M, and 0.01 M. Equilibrium was approached from both undersaturated and supersaturated initial conditions and the solubility systems were replicated 2-fold.

Pre-washed samples of gibbsite (10 g) were placed in 250 ml polypropylene bottles. For the undersaturated initial condition experiments, 100 ml of background electrolyte solution was added to each bottle. For the supersaturated initial condition experiments, 100 ml of background electrolyte containing 0.002 M $AlCl_3$ was added to each bottle. The solution pH levels (approximately 3,4,5, and 6) were adjusted with concentrated HCl or 0.1 M KOH. Solution pH was monitored three more times, within a two week period, and adjusted accordingly to ensure that the desired pH existed in solution. The sample bottles were capped and placed in a temperature-controlled Precision Low Temperature Incubator 815. The temperature was maintained at 25 ± 0.1 °C. The samples were equilibrated, with periodic shaking, for a 6 week period.

After equilibration, the solid and equilibrating solution phases were separated by centrifugation at (750) x g for 10 minutes. Aqueous-phase pH was determined with a standardized (pH 4 and 7) general purpose pH electrode and a Corning Model 255 pH/ion meter. The aqueous extract was analyzed for Al, Ca, and K by ICP-AES using a Jarrell-Ash 1100. Standard solutions were prepared and verified against EPA standard reference

43

solutions. The prepared standards, over the duration of the study, were within +2% and +4% for Ca, +1% and +5% for Al, and +0.8% and +2% for K of the standard reference solutions values. The aqueous extract was also analyzed for Cl⁻ using Dionex ion chromatography (IC). A standard calibration curve was established and verified against EPA standard reference solutions. Over the duration of the study, the prepared standards were within ± 1% of the standard reference values. Total C concentrations were determined using a Dohrmann DC80 total organic carbon analyzer. Total C values obtained were assumed to represent 2-ketogluconic acid concentrations in solution.

The determination of Al^{3+} activity, (Al^{3+}), in equilibrium with gibbsite was computed using the chemical equilibrium computer model GEOCHEM-PC (Parker et al., 1995). The program employs calculations based on chemical thermodynamics. For each component, a mole balance equation is constructed and thermodynamic equilibrium constants, corrected for ionic strength, are incorporated into various terms according to the law of mass action. The solution of the resulting set of non-linear algebraic equations by iterative approximation provides the ion speciation in solution. The aqueous species considered and the associated stability constants used by GEOCHEM-PC are listed in Table 3.1.

At any pH value, the activity of Al^{3+} is fixed by the equilibrium solubility of gibbsite. Enhanced gibbsite solubility in the presence of 2-ketogluconic acid may be attributed to the formation of aqueous Al-ligand species. In solutions containing 2-ketogluconic acid, and in equilibrium with gibbsite, the following chemical reactions may be significant:

44

Table 3.1. Ion pair formation reactions and association constants used by GEOCHEM-PC.

Reaction	log K
$Ca^{2+} + Cl^- = CaCl^+$	-1.0
$Ca^{2+} + H_2O = CaOH^+ + H^+$	-12.7
$K^+ + Cl^- = KCl^0$	-0.7
$K^+ + H_2O = KOH^0 + H^+$	-14.5
$Al^{3+} + H_2O = Al(OH)^{2+} + H^+$	-5.0
$Al^{3+} + 2H_2O = Al(OH)_2^+ + 2H^+$	-10.1
$Al^{3+} + 3H_2O = Al(OH)_3^0 + 3H^+$	-16.8
$Al^{3+} + 4H_2O = Al(OH)_4^- + 4H^+$	-22.7
$H^+ + Cl^- = HCl^0$	-8.4
$H^+ + C_6H_9O_7^- = C_6H_{10}O_7^0$	2.8

$$Al^{3+} + H_2O = Al(OH)^{2+} + H^+ \tag{1}$$

$$Al^{3+} + 2H_2O = Al(OH)_2^+ + 2H^+ \tag{2}$$

$$Al^{3+} + 3H_2O = Al(OH)_3^0 + 3H^+ \tag{3}$$

$$Al^{3+} + 4H_2O = Al(OH)_4^- + 4H^+ \tag{4}$$

$$Al^{3+} + (C_6H_9O_7^-) = Al(C_6H_9O_7)^{2+} \tag{5}$$

$$Al^{3+} + H_2O + (C_6H_9O_7^-) = Al(OH)(C_6H_9O_7)^+ + H^+ \tag{6}$$

$$Al^{3+} + 2H_2O + (C_6H_9O_7^-) = Al(OH)_2(C_6H_9O_7)^0 + 2H^+ \tag{7}$$

$$Al^{3+} + 3H_2O + (C_6H_9O_7^-) = Al(OH)_3(C_6H_9O_7)^- + 3H^+ \tag{8}$$
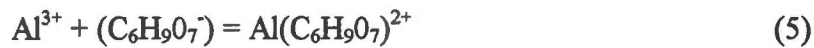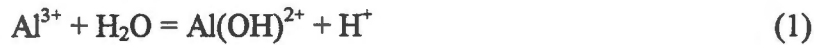
The K_f values for chemical equations 1-4 were known (Table 3.1), but for equations 5-8 the K_f values are not available and must be generated. Conditional equilibrium constants, (^cK_f), were derived from the K_f values of equations 1-4 by employing activity coefficients generated by the Davies equation. Known ^cK_f values for equations 1-4, estimated ^cK_f values for equations 5-8, total concentrations, free concentrations, and activities for each observation were entered into the chemical equilibrium computer program FITEQL (Westall, 1982) to compute the ^cK_f values for equations 5-8. The equilibrium program utilizes an optimization procedure which involves the iterative application of a linear approximation of the chemical equilibrium equations and a linear least squares fit. The computed ^cK_f values and an associated error term were converted to K_f values again by utilizing the Davies equation to correct for ionic strength effects. The K_f values were entered again into GEOCHEM-PC to generate the activity of the aqueous species after taking into account 2-ketogluconic acid complexation in the system. The calculated log

46

(Al^{3+}) were plotted as a function of pH to determine if the chemical model accurately described the complexation occurring in the system.

Chapter IV

Results and Discussion

1. Available P Indicators

Background Characterization

Soil and plant samples used to evaluate P availability indicators were obtained from the Milan and Ames Plantation Experiment Stations of The University of Tennessee, Institute of Agriculture. Research was initiated at Ames to evaluate swine manure as a source of P for crops (O'Dell et al., 1993; O'Dell, 1995). For the Ames soil, methods used for P characterization were: Olsen (Olsen and Sommers, 1982), Mehlich III (Mehlich, 1984), organic P (Bowman, 1989), Chang-Jackson sequential P fractionation (Al-P, Fe-P, Red-P, Ca-P)(Petersen and Corey, 1966), and total P (Bowman, 1988). The results for the samples collected in 1992 showed that concentrations of total P, organic P, Ca-P, Fe-P and Red-P were affected by depth but not by P rate (as swine manure). Organic P at depths of 0-10 and 10-20 averaged 86.7 (20.1%) and 72.7 (16.9%) mg P kg^{-1}. Depth and rate influenced the concentrations of Al-P, Olsen-P, and Mehlich III-P. Olsen at depths of 0-10 and 10-20 cm averaged 5.5 and 3.5 mg P kg^{-1}. Mehlich III at depths of 0-10 and 10-20 cm averaged 8.5 and 3.9 mg P kg^{-1}. Al-P at depths of 0-10 and 10-20 cm averaged 11.8 and 9.4 mg P kg^{-1}. The results for samples collected in 1993 were similar to those of 1992. The concentrations of organic P, Ca-P, Fe-P, and Red-P were affected by depth, but not P rate. Organic P at depths 0-10 and 10-20 cm averaged 102.5 (24.7%) and 91.9 (22.1%) mg P kg^{-1}. Depth and rate influenced the concentration of total P, Al-P, Olsen-P, and Mehlich III-P. Olsen at depths of 0-10 and 10-20 cm

averaged 10.7 and 7.9 mg P kg^{-1}. Mehlich III at depths of 0-10 and 10-20 cm averaged 12.1 and 8.0 mg P kg^{-1}. Al-P at depth of 0-10 and 10-20 cm averaged 13.0 and 9.6 mg P kg^{-1}. For both years, Mehlich III and Olsen extractable P, and Al-P was significantly correlated with plant P content, with Mehlich III producing the highest correlation coefficient (O'Dell, 1995). According to O'Dell (1995), these results suggest that the application of swine manure has a significant influence on available P distribution at different depth increments and plant P concentrations.

The Milan study focused on the effect of conservation tillage systems on the chemical status of soil P (Essington et al., 1994). Soil P in the Milan samples collected in 1992 was characterized as follows: total P (Bowman, 1988), organic P (Bowman, 1989), Chang-Jackson sequential P fractionation (Al-P, Fe-P, Ca-P, Red-P)(Petersen and Corey, 1966), Olsen-P (Olsen and Sommers, 1982), and Mehlich III-P (Mehlich, 1984). Total P averaged 359 mg P kg^{-1}, did not vary with depth in the surface 15 cm, but was significantly influenced by tillage practice and P rate. Organic P was significantly influenced by rate, depth, and tillage practice. Organic P at depths 0-15 cm for the 0, 10, and 30 kg P ha^{-1} rates averaged 18.5%, 22.7%, and 24.4%, respectively. The distribution of inorganic P averaged over depth to 15 cm in NT soil was 6.5% Al-P, 27.3% Fe-P, 30.7% Red-P, and 4.6% Ca-P. The average distribution of P in the surface 15 cm of the DT soil was 5.6% Al-P, 31% Fe-P, 29.7% Red-P, and 4.7% Ca-P. Only the Fe-P was not influenced by tillage practice. Mehlich III-P averaged 4.8 mg P kg^{-1} in the surface 15 cm and was not influenced by tillage. However, Mehlich III-P was influenced by P rate, averaging 2.8 mg P kg^{-1}, 5.1 mg P kg^{-1}, and 6.5 mg P kg^{-1} for 0, 10, and 30 kg P ha^{-1}

treatments. Mehlich III-P was significantly higher in the 0-8 cm depth increment (7.3 mg P kg^{-1}), compared to the 8-15 cm depth increment (averaging 2.3 mg P kg^{-1}). Olsen-P was influenced by tillage, rate, and depth. Olsen-P averaged 4.2 mg P kg^{-1} for NT and DT, and 2.7 mg P kg^{-1}, 6.4 mg P kg^{-1}, and 5.3 mg P kg^{-1} for 0, 10, and 30 kg P ha^{-1} treatments. Olsen-P was higher in the 0-8 cm depth increment (7.0 mg P kg^{-1}) than in the 8-15 cm increment (averaging 2.9 mg P kg^{-1}). These results suggest that even minor tillage can significantly influence total P, bioavailable P, the partitioning of P between organic and inorganic forms, and the distribution of inorganic P in various chemical pools (Essington et al., 1994).

Ames Extractions

Of the four inorganic P fractions examined in the 1994 Ames soil samples, only Al-P was influenced by swine manure applications (Table 4.1). The dominant P fraction was Fe-P (89.9 to 106.1 mg P kg^{-1}). Reductant-P (65.8 to 76.1 mg P kg^{-1}) was also present in high concentrations. Al-P and Ca-P composed from 12 to 26 mg P kg^{-1} of the Ames soil.

Available P in the 1994 Ames samples, as measured by OE1, OE2, and Mehlich III were influenced by swine manure treatments, but Olsen-P was not (Table 4.1). Organic extraction 1 removed higher concentrations of P than OE2 across treatment levels. The concentrations of P measured by the organic acid extractions were much smaller than the available P concentrations measured by the Olsen and Mehlich III methods. The concentration of P extracted by OE1 ranged from 0.4 to 1.0 mg P kg^{-1}, and from 0.2 to 0.5 mg P kg^{-1} by OE2. The Olsen method extracted from 4.4 to 6.5 mg P kg^{-1} and Mehlich III extracted from 2.4 to 6.8 mg P kg^{-1}. Overall, the Olsen method extracted

50

Table 4.1. Available P and inorganic P pools in 0-20 cm depth soil samples, plant P concentrations, and yield measurements from Ames.

	Manure P rate, kg P ha^{-1}			TSP, kg P ha^{-1}
	15	30	60	30
	Available P, mg kg^{-1}			
OE1	1.0a[†]	0.4b	0.5b	0.7ab
OE2	0.5a	0.2b	0.2b	0.4ab
Mehlich III	6.8a	2.4c	2.9bc	4.5b
Olsen	6.5a	4.4a	5.0a	5.8a
	Solid phase P, mg kg^{-1}			
Al-P	25.6a	15.5b	15.6b	18.7b
Fe-P	106.1a	105.8a	89.9a	98.1a
Ca-P	15.1a	13.6a	14.4a	12.1a
Red-P	73.9a	69.1a	76.1a	65.8a
	Plant P, g kg^{-1}			
4 leaf	3.21	2.69	3.00	2.84
10 leaf	3.18	2.67	2.78	3.06
silking	2.92	2.65	2.92	2.83
	Mg ha^{-1}			
Yield	100	89	96	100

† - Means followed by the same letter in the same row are not significantly different at the 0.05 probability level.
OE1 - 10 mM citric acid
OE2 - 5 mM citric acid + 5 mM 2-ketogluconic acid

higher concentrations of P than the organic extractions and Mehlich III. Assuming ligand exchange was involved, it is possible that the HCO_3^- (Olsen) outperformed citrate (OE1 and OE2), 2-ketogluconic acid (OE2), and acetate (Mehlich III) in displacing $H_2PO_4^-$ and HPO_4^{2-} ions from adsorption sites on hydrated oxides of Fe and Al. Conversely, the low concentrations of OE1, OE2, and Mehlich III extractable P, induced by the swine manure treatments, may also be attributed to the concentration of P bound in the organic phase. In the 1992 and 1993 Ames soil samples the % organic P at depths of 0-10 and 10-20 cm was 18.5% and 23.4%, respectively. The addition of organic P (e.g. inositol hexaphosphate, $C_6H_{12}O_6$) by the swine manure to acidic soils will enhance the formation of insoluble salts with Fe and Al oxides (Tisdale et al., 1993). The formation of these persistent salts may influence the quantity of P extracted by Al and Fe complexing agents (e.g. citrate, 2-ketogluconate, F^-, and EDTA). Tran and N'dayegamiye (1995), studied the long-term effect of cattle manure and fertilizer on soil P forms and plant availability. They found manure application to maintain a high level of NaOH-extractable P. They also observed that although manure-P concentrations were relatively low (42 kg P ha^{-1}), organic P pools were maintained with manure treatments. A study by Nair et al. (1995) examining the form of P in soil profiles under dairies, found the A horizon in pasture and forage areas to contain a high organic P fraction (>40% of total P). Consequently, these findings suggest that the extractions may be limited in their assessment of P in soils which are amended with animal manure.

Phosphorus extracted from the Ames soil by OE1 was significantly correlated to that extracted by OE2 (r = 0.95[***])(Table 4.2). OE1-extractable P was also significantly

Table 4.2. Correlation among available P and inorganic P fractions at 0-20 cm depth in the Ames soil.

	OE1	OE2	M3	Olsen	Al-P	Fe-P	Ca-P	Red-P
OE1	1.0	0.95***	0.84***	0.50*	0.85***	0.18	0.28	0.03
OE2		1.0	0.74***	0.36	0.74***	0.04	0.16	-0.15
Mehlich III			1.0	0.45*	0.88***	0.20	0.25	-0.01
Olsen				1.0	0.64**	0.53*	0.002	0.20
Al-P					1.0	0.40	0.23	0.17
Fe-P						1.0	0.19	0.41
Ca-P							1.0	0.60**
Red-P								1.0

*, **, *** Significant at the 0.05, 0.01, and 0.001 probability level.
OE1 - 10 mM citric acid
OE2 - 5 mM citric acid + 5 mM 2-ketogluconic acid

correlated with Mehlich III-P (r = 0.84[***]) and Olsen-P (r = 0.50[*]). In comparison, OE2-P was significantly correlated with only Mehlich III-P (r = 0.74[***]).

The relationship between extractants, which provide a measure of P, and inorganic P fractions may provide information on the extractable P source in a soil. Of the four fractions examined in the Ames soil, only Al-P was significantly correlated with OE1 (r = 0.85[***]) and OE2 (r = 0.74[***])(Table 4.2). Mehlich III-P and Olsen-P were also significantly correlated with Al-P (r = 0.88[***] and r = 0.64[**], respectively). Further, Olsen extractable P was significantly correlated with Fe-P (r = 0.53[*]). These results illustrate the high affinity of the organic acid extractions, as well as Mehlich III and Olsen, for the Al-P pool and suggest that the organic acid extractants are accessing the Mehlich III-P and Olsen-P pools. Therefore, this suggests that OE1 and OE2 are as equally inadequate for assessing plant available P as Mehlich III and Olsen in the Ames soil.

Of the P extractions and inorganic P fractions characterized in the Ames soil, only Mehlich III-P (r = 0.53[*]) at the 4 leaf stage, OE2-P (r = 0.45[*]) at the 10 leaf stage, and Red-P (r = 0.63[*]) at the silking stage were significantly correlated with plant P (Table 4.3). In the Ames soil, none of the available P indices or inorganic P fractions were significantly correlated with yield (Table 4.3).

Milan Extractions

In the Milan soil, all inorganic P fractions except Red-P were influenced by P fertilization rates (Table 4.4). Of the inorganic P fractions characterized, the fractions containing the greatest amounts of P were the Fe-P (69.5 to 183.8 mg P kg^{-1}) and Red-P (98.7 to 110.2 mg P kg^{-1}) fractions. The dramatic increase in Fe-P concentration with

54

Table 4.3. Correlation of available P and inorganic P fractions with plant P and yield from Ames.

	Plant P			Yield
	4 Leaf	10 Leaf	Silking	
	correlation coefficient (r)			
OE1	0.35	0.40	0.43	-0.05
OE2	0.25	0.45*	0.23	-0.004
Mehlich III	0.53*	0.37	0.39	0.15
Olsen	0.18	0.19	0.43	-0.05
Al-P	0.40	0.38	0.41	-0.01
Fe-P	-0.02	-0.23	-0.003	-0.28
Ca-P	0.004	-0.31	0.31	-0.23
Red-P	0.04	-0.28	0.63*	-0.09

*,**,*** Significant at the 0.05, 0.01, and 0.001 probability level.

OE1 - 10 mM citric acid

OE2 - 5 mM citric acid + 5 mM 2-ketogluconic acid

Table 4.4. Available P and inorganic P pools in 0-15 cm depth soil samples, plant P concentrations, and yield measurements from Milan.

	Fertilizer P rate, kg P ha^{-1}		
	0	10	30
	Available P, mg kg^{-1}		
OE1	0.8a[†]	2.0a	7.9b
OE2	1.4a	3.2a	11.4b
Mehlich III	2.3a	3.5a	9.1b
Olsen	2.2a	3.4a	7.3b
	Solid phase P, mg kg^{-1}		
Al-P	13.4a	18.2b	29.5c
Fe-P	69.5a	87.9a	183.8b
Ca-P	14.2a	16.0ab	18.8b
Red-P	98.7a	98.8a	110.2a
	Plant P, g kg^{-1}		
Plant P	2.6	2.8	2.8
	Mg ha^{-1}		
Yield	151	171	179

† - Means followed by the same letter in the same row are not significantly different at the 0.05 probability level.
OE1 - 10 mM citric acid
OE2 - 5 mM citric acid + 5 mM 2-ketogluconic acid

increasing P fertilization rate indicates that Fe-P may be a sink for fertilizer P and may influence P fertility. Concentrations of Al-P (13.4 to 29.5 mg P kg^{-1}) and Ca-P (14.2 to 18.8 mg P kg^{-1}) also increased with fertilizer P rates, and were very small in comparison to the Fe-P and Red-P fractions.

In the Milan soil, OE2 extracted higher concentrations of P than OE1 across all TSP treatments (Table 4.4). Phosphorus concentrations in all extractants were influenced by fertilizer P rates (Table 4.4). Both OE1 and OE2 outperformed the Mehlich III and Olsen extractions. The concentration of P extracted by OE1 ranged from 0.8 to 7.9 mg P kg^{-1}, while that extracted by OE2 ranged from 1.4 to 11.4 mg P kg^{-1}. In comparison, Mehlich III extracted from 2.3 to 9.1 mg P kg^{-1} and Olsen extracted from 2.2 to 7.3 mg P kg^{-1}.

Overall, OE2 extracted the highest concentrations of P from the Milan samples. Both citric acid, and to a lesser extent 2-ketogluconic acid, have been shown to complex with metal cations. There has been little investigation into the mechanism by which 2-ketogluconic acid enhances the solubility of phosphates. However, Duff et al. (1963) showed that 2-ketogluconic acid forms complexes with divalent metal cations. The citrate ion, which has been thoroughly investigated, was found to form complexes with Fe and Al, thereby decreasing phosphate binding to the adsorption site (Struthers and Sieling, 1950). Earl et al. (1979) found 0.1 M citrate removed 91% of the Fe and 88% of the Al from Fe and Al gel, respectively, reducing P sorption by 89% and 88%. Nagarajah et al. (1970) demonstrated that organic anions, such as citrate, were specifically adsorbed at M-OH (M = Al or Fe) surfaces of soil components in a manner analogous to phosphate

adsorption. These workers also suggested that the ability of organic anions to compete with P for sorption sites was at a maximum at a pH equivalent to the pKa of the organic acid. Clearly, the pH of the extraction solution coupled with the pKa values of citric acid (3.13) and 2-ketogluconic acid (2.8), in addition to their known metal complexing capabilities, enhance the P extraction capability of OE2. This was not observed in the Ames soil because the P from the swine manure fertilizer was predominantly in the organic phase and not the inorganic phase. Tran and N'dayegamiye (1995) found the application of mineral P fertilizer generally increased the inorganic P fractions extracted by resin, NaHCO₃, and NaOH. Further, they illustrated that the mineral P fertilizer reduced the NaOH-P fraction, due to mineralization. Based solely on the concentrations of extractable P, these results suggest that the OE2 extracting solution most effectively increases the concentration of P in solution in soil with low organic P concentrations.

The P concentrations extracted in the Milan soil by OE1 was significantly correlated to that extracted by OE2 ($r = 0.99$[***])(Table 4.5). Phosphorus extracted by both the OE1 and OE2 methods were also significantly correlated with that extracted by the Mehlich III ($r = 0.98$[***] for both OE1 and OE2) and Olsen methods ($r = 0.95$[***] for both OE1 and OE2). OE1, OE2, and Mehlich III extractable P were significantly correlated with P in the Al-P and Fe-P fractions (Table 4.5). Citric acid, 2-ketogluconic acid, and components in the Mehlich III extract possess chelating and complexing characteristics towards Al and Fe. The Mehlich III extractant can facilitate Al-P dissociation through the complexation of Al^{3+} by F⁻, and Fe-P through the complexation of Fe^{3+} with EDTA. Jittanoonta (1977) found Mehlich III to be well correlated with Al-P

58

Table 4.5. Correlation among available P and inorganic P fractions at 0-15 cm depth in the Milan soil.

	OE1	OE2	M3	Olsen	Al-P	Fe-P	Ca-P	Red-P
OE1	1.0	0.99***	0.98***	0.95***	0.98***	0.94***	0.56	0.33
OE2		1.0	0.98***	0.95***	0.98***	0.92***	0.53	0.32
Mehlich III			1.0	0.93***	0.95***	0.91***	0.49	0.29
Olsen				1.0	0.96***	0.94***	0.63*	0.16
Al-P					1.0	0.95***	0.66*	0.34
Fe-P						1.0	0.78**	0.33
Ca-P							1.0	0.39
Red-P								1.0

*, **, *** Significant at the 0.05, 0.01, and 0.001 probability level.
OE1 - 10 mM citric acid
OE2 - 5 mM citric acid + 5 mM 2-ketogluconic acid

($r = 0.78$) in 136 Mississippi soils. Citric acid has a large capacity to complex with the metal cations due to its tridentate structure (Stumm and Morgan, 1981). It is also very efficient in displacing $H_2PO_4^-$ from the soil sorbing surfaces (Traina et al., 1987).

Phosphorus extracted by the Olsen bicarbonate method was significantly correlated with P in the Al-P ($r = 0.96^{***}$), Fe-P ($r = 0.94^{***}$), and Ca-P ($r = 0.63^*$) fractions of the Milan soil (Table 4.5). Datta and Khere (1969) reported similar results, with Olsen-P correlated with Al-P ($r = 0.65$) and Fe-P ($r = 0.53$). Debnath and Mandal (1982) also found Olsen-P to be highly correlated with Fe-P ($r = 0.65$) and Al-P ($r = 0.53$). Singh et al. (1968) found significant correlations with Olsen-P and Ca-P ($r = 0.86$) in eleven soils containing low concentrations of Al-P. Reductant-P was not significantly correlated with any measure of available P.

In the Milan soil, none of the available P indices or inorganic P fractions produced a significant correlation with plant P concentrations (Table 4.6). However, OE1-P ($r = 0.68^{**}$), OE2-P ($r = 0.68^{**}$), Mehlich III-P ($r = 0.70^{**}$), and Olsen-P ($r = 0.68^*$) were significantly correlated with yield. Therefore, these results suggest that all four extractions measure the quantity of available P in soil equally well. Of the P measured from chemical pools in the Milan soil, yield was significantly correlated with only Al-P ($r = 0.67^*$) and Fe-P ($r = 0.64^*$). These results further illustrate the plant availability of Al-P and Fe-P.

Summary

The background characterization of solid phase P for the Ames and Milan soils found Fe-P and Red-P to be the dominant inorganic P fractions. In the 1994 Ames soil samples, OE1, OE2, and Mehlich III were influenced by swine manure treatments, but

60

Table 4.6. Correlation of available P and inorganic P fractions with plant P concentration and yield from Milan.

	Plant P	Yield
	correlation coefficient (r)	
OE1	0.08	0.68**
OE2	0.11	0.68**
Mehlich III	0.06	0.70**
Olsen	0.08	0.68*
Al-P	0.16	0.67*
Fe-P	0.09	0.64*
Ca-P	-0.05	0.37
Red-P	-0.09	-0.02

*, **, *** Significant at the 0.05, 0.01, and 0.001 probability level.

OE1 - 10 mM citric acid

OE2 - 5 mM citric acid + 5 mM 2-ketogluconic acid

Olsen was not affected. The difference in extractable P was attributed to the organic P, as a result of the manure fertilizer treatments, and the ability of HCO_3^-, from the Olsen extraction, to effectively exchange with the inorganic phosphate fractions present. OE1 was significantly correlated with Mehlich III, Olsen, and OE2, but OE2 was only correlated with Mehlich III and Al-P. None of the P indices produced significant correlations with plant P or yield.

In the 1992 Milan soil samples, OE1 and OE2 extracted higher concentrations of P than did Mehlich III and Olsen. All extractants showed treatment effects with the increased application rate of fertilizer treatments. Both OE1 and OE2 were significantly correlated to Mehlich III, Olsen, Al-P, and Fe-P. None of the P indices produced significant correlations with plant P. However, OE1, OE2, Mehlich III, Olsen, Al-P, and Fe-P produced significant correlations with yield.

The increased concentration of extractable P and significant correlation to yield in the Milan soil, along with significant correlation to Mehlich III, Al-P, and Fe-P in both soils, suggest that the organic extractions, predominantly OE2, are a feasible alternative to assessing P in acidic soils. Both citric and 2-ketogluconic acid, to a lesser extent, have been shown to effectively enhance P solubilization in soil through metal complexation. Kafkafi et al. (1988) investigating the extraction capabilities of citrate, bicarbonate, acetate, oxalate, and amino acids (phenylalanine and alpha-amino isobutyric), found the citrate ion to extract the highest concentration of P by complexing with Al in the soil. Traina et al. (1986a) also found the citrate ion to be effective in complexing with Al and thereby increasing P solubility. A study by Earl et al. (1979), evaluated the adsorption of

P by synthetic Fe and Al gels in the presence of organic anions (citrate, tartrate, and acetate). The citrate ion (50% reduction), tartrate (20% reduction), and acetate (1% reduction) were markedly different in their capacity to affect P adsorption. Although the mechanism by which 2-ketogluconic acid enhances P solubilization in acidic soils has not been fully described, studies have shown it to also be an effective complexing agent with metals. Duff et al. (1963) demonstrated that 2-ketogluconic acid could increase P solubilization by complexing divalent metal cations. Further, a study by Berrow et al. (1982) found 2-ketogluconic acid to extract higher levels of trace elements and Fe than ammonium acetate and acetic acid. Therefore, findings in this study, as well as current literature, indicate that the organic acid extractions are a possible alternative to the currently employed soil test and thereby justify further investigation.

2. Complexation of Al^{3+} with 2-Ketogluconic Acid

The organic extraction results, as previously described, suggest that 2-ketogluconic acid may enhance P solubilization in soil. However, in soil in which the P fractions are dominate Fe-P and Red-P, and in which the plant available P is dominated by the Fe-P and Al-P fractions the mechanism by which 2-ketogluconic acid solubilizes P has not been characterized. Previous studies suggest an aqueous association of 2-ketogluconic acid with Ca^{2+} and select trace elements, resulting in P solubilization. Correspondingly, the aqueous complexation of Al^{3+} and Fe^{3+} by 2-ketogluconic acid may be significant. In order to examine the influence of 2-ketogluconic acid on the aqueous chemistry of Al^{3+}, the solubility of gibbsite $[Al(OH)_3]$ at 25°C was examined as a function of pH, ionic strength, and 2-ketogluconic acid concentration.

63

Reliable information on the solubility product of gibbsite, $[Ksp = (Al^{3+})/(H^+)^3$ for

the reaction $Al(OH)_3 + 3H^+ = Al^{3+} + 3H_2O]$, is essential to any study of equilibria of

aqueous aluminum ions. In this study, GEOCHEM-PC was utilized to provide activities

for the individual species (Table 4.7). A mean log $Ksp = 8.05 \pm 0.02$ was determined for

gibbsite in the absence of 2-ketogluconic acid. This solubility product is comparable to

those reported by other investigators. Kittrick (1966) and Singh (1972) reported a log

Ksp of 8.0 for gibbsite. Frink and Peech (1962), examined the solubility of gibbsite in

supersaturated and undersaturated $AlCl_3$ solutions, and reported a log Ksp value of 8.5.

May et al. (1979), investigated the solubility of gibbsite in the pH 4 to 9 range at 25°C,

and obtained a log Ksp of 8.11. However, Bloom and Weaver (1982) studying the

influence of reactive surface material on gibbsite solubility, found a mean log Ksp of 7.55

for three acid-treated commercial gibbsite samples. The authors attributed the reduction

in gibbsite solubility to the acid removal of the surface coatings, which contained poorly

crystalline $Al(OH)_3$. Through the years, solubility values calculated from thermodynamic

data for gibbsite have been inconsistent. Verdes et al. (1992) in a review of gibbsite

solubility data from 1920 to 1987, found the solubility measurements to be unreliable and

erratic, particularly in an acid medium, further illustrating the lack of reliable

thermodynamic measurements for gibbsite solubility.

Concentrations of Al, Ca, K, Cl, and C as a function of pH, ionic strength, and in

the presence of different concentrations of 2-ketogluconic acid were measured in solutions

equilibrated with gibbsite (Table 4.7). The calculated log (Al^{3+}) values from GEOCHEM-

PC were plotted as a function of pH (Figure 4.1). The log (Al^{3+}) values vary as a function

Table 4.7. Chemical characteristics of solutions in equilibrium with gibbsite.

I	Al	Ca	K	Cl	C[†]	pH	$p(Al^{3+})$[‡]	log IAP
M				mg L^{-1}				
				supersaturation				
0.063	43.98	0.07	2225	1938	0.05	3.81	3.633	7.80
0.063	44.18	0.28	2205	1923	0.39	3.82	3.629	7.83
0.063	61.17	92.7	2208	1787	319.8	3.89	3.523	8.15
0.113	45.51	0.18	4145	3622	0.25	3.89	3.778	7.89
0.113	45.87	0.04	4250	3602	0.02	3.90	3.778	7.92
0.063	60.34	90.93	2209	1910	284.7	3.90	3.533	8.17
0.063	65.43	118.4	2221	1899	363.8	3.91	3.509	8.22
0.063	63.86	115.8	2239	1950	361.9	3.93	3.522	8.27
0.113	63.84	115.6	4186	3619	371.1	3.94	3.660	8.16
0.113	62.06	111	4127	3611	377	3.96	3.670	8.21
0.113	59.31	89.52	4299	3691	316	3.97	3.693	8.22
0.113	57.72	87.47	3918	3365	297.8	3.99	3.683	8.29
				undersaturation				
0.063	7.29	156	2165	1781	461.5	4.27	4.440	8.37
0.063	2.45	72.52	1850	2002	226.2	4.48	4.901	8.54
0.063	6.90	169.6	2115	2383	514.4	4.50	4.519	8.98
0.063	3.31	87.51	2140	3787	270.6	4.55	4.793	8.86
0.063	6.62	177.9	2186	1773	510.6	4.60	4.523	9.28
0.063	3.18	95.51	2232	1774	285.2	4.65	4.827	9.12
0.113	0.09	0.05	4323	3526	0.02	4.80	6.554	7.85
0.113	0.10	0.06	4210	3453	0.02	4.96	6.556	8.32
0.063	0.07	0.03	2023	1855	0.21	5.11	6.603	8.73
0.063	1.01	86.36	2146	1480	265.2	5.20	5.518	10.08
0.063	1.22	82.16	2322	1857	240.3	5.24	5.489	10.23
0.063	0.83	83.29	2164	1546	259	5.40	5.783	10.42
0.063	1.45	169.6	2111	2732	473.1	5.58	5.771	10.97
0.063	1.64	150.2	2191	1705	457	5.59	5.725	11.05
0.063	1.57	168.5	2135	1752	501.6	5.70	5.909	11.19
0.113	0.58	111.1	4242	3045	361.6	5.70	6.387	10.71
0.113	0.56	112.7	4182	3526	370.3	5.84	6.638	10.88
0.113	0.49	93.73	4199	3415	295.7	5.97	6.937	10.97
0.063	0.24	79.87	2323	1709	249.3	6.15	7.627	10.82
0.063	0.24	77.52	2485	1933	237.8	6.16	7.660	10.82
0.063	0.24	77.72	2206	1763	552.9	6.24	7.311	11.41
0.063	0.44	89.97	2047	1761	296.3	6.26	7.655	11.13
0.113	0.46	96.28	4325	3270	296.5	6.26	7.659	11.12
0.063	0.19	78.28	2269	1665	253.9	6.40	8.445	10.76
0.063	0.77	155.2	2280	2239	482.3	6.40	7.828	11.37
0.063	0.56	113.8	2231	1719	364.1	6.42	8.028	11.23
0.063	0.86	171	2207	1639	511.4	6.43	7.876	11.41
0.063	0.40	90.46	2230	1758	287.9	6.44	8.238	11.08
0.063	0.55	115.9	2144	1758	375.5	6.62	8.700	11.16

† - mg C L^{-1} from 2-ketogluconic acid
‡ - computed by GEOCHEM-PC
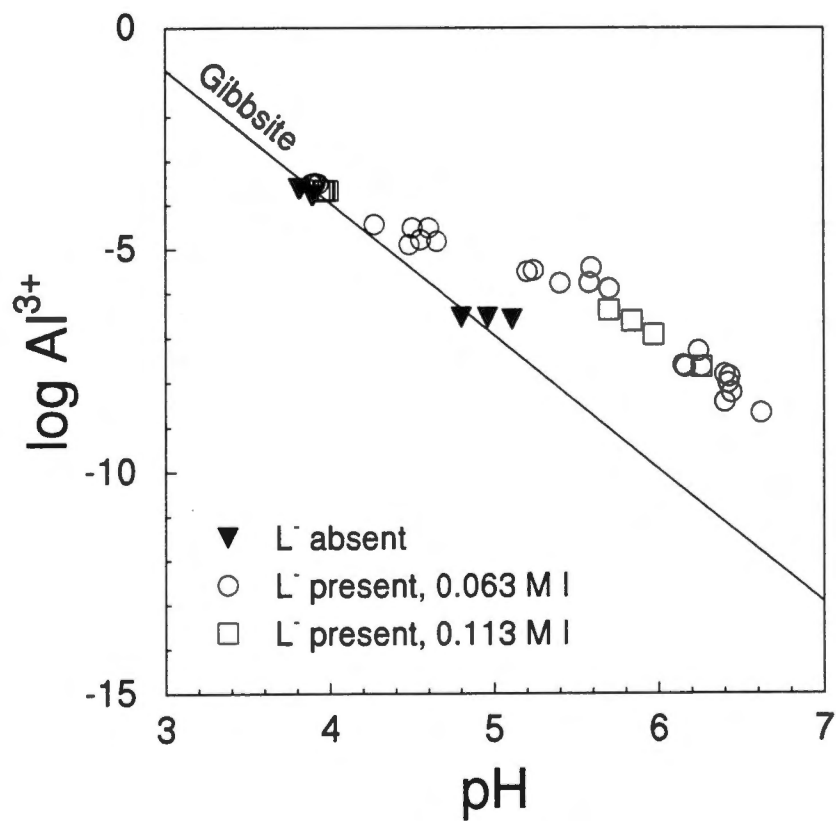
Figure 4.1. Solubility relationships in the gibbsite systems.

of pH and 2-ketogluconate $(C_6H_9O_7^-)$ concentration. Deviation of the data points from the gibbsite stability line, when Al^{3+}-$(C_6H_9O_7^-)$ complexes are not considered in the computations, suggests the formation of Al^{3+}-$(C_6H_{10}O_7)$ complexes. Further, an examination of aqueous Al^{3+} speciation as a function of pH (Figure 4.2) indicates that the Al species, Al^{3+}, $Al(OH)^{2+}$, $Al(OH)_2^+$, and $Al(OH)_3^0$, may play a role in complexing 2-ketogluconic acid. Therefore, the following ion formation reactions were predicted to occur:

$$Al^{3+} + (C_6H_9O_7^-) = Al(C_6H_9O_7)^{2+} \qquad (3)$$

$$Al^{3+} + H_2O + (C_6H_9O_7^-) = Al(OH)(C_6H_9O_7)^+ + H^+ \qquad (4)$$

$$Al^{3+} + 2H_2O + (C_6H_9O_7^-) = Al(OH)_2(C_6H_9O_7)^0 + 2H^+ \qquad (5)$$

$$Al^{3+} + 3H_2O + (C_6H_9O_7^-) = Al(OH)_3(C_6H_9O_7)^- + 3H^+ \qquad (6)$$
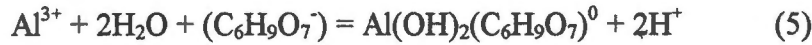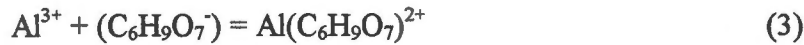
The following relationships are also derived from eq 3-6:

$$Q_0 = (Al(C_6H_9O_7)^{2+}) / (Al^{3+})(C_6H_9O_7^-) \qquad (7)$$

$$Q_1 = (Al(OH)(C_6H_9O_7)^+)(H^+)/(Al^{3+})(C_6H_9O_7^-) \qquad (8)$$

$$Q_3 = (Al(OH)_2(C_6H_9O_7)^0)(H^+)^2/(Al^{3+})(C_6H_9O_7^-) \qquad (9)$$

$$Q_4 = (Al(OH)_3(C_6H_9O_7)^-)(H^+)^3/(Al^{3+})(C_6H_9O_7^-) \qquad (10)$$

where Q is an equilibrium constant. The determination of equilibrium constant for the formation of aqueous species from solubility data is common in experimental geochemistry (Wood and Crerar, 1985; Essington, 1990). The thermodynamic equilibrium constants for the formation of Al^{3+}-2-ketogluconic acid complexes can be ascertained from the solubility data by utilizing a multiple linear regression analysis coupled with an iterative technique. The total measured concentration of aluminum in solution may be written:
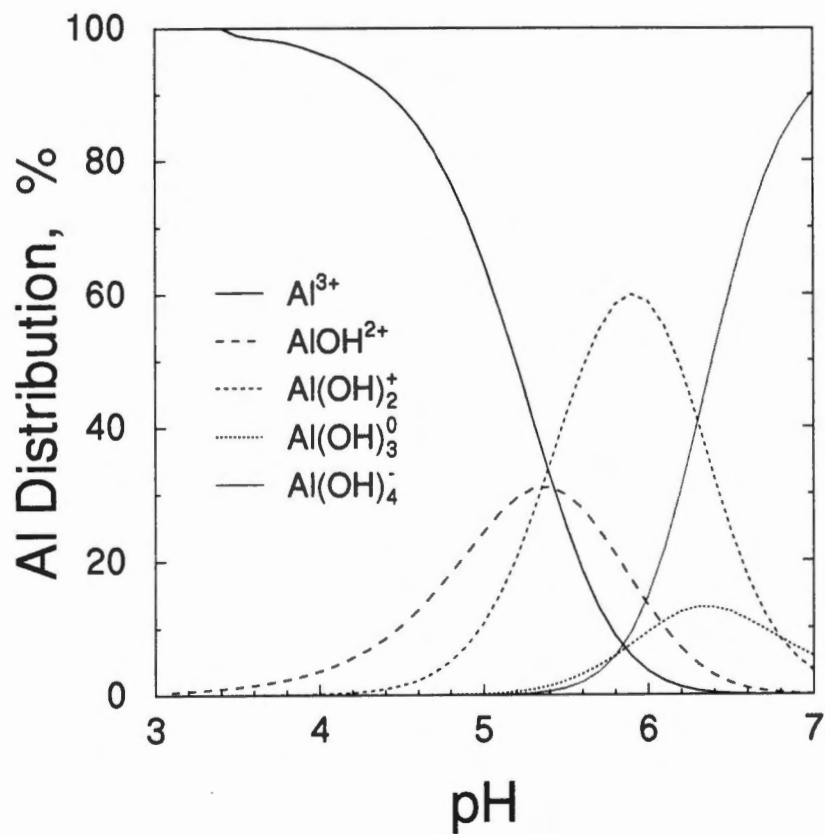
Figure 4.2. Aqueous Al speciation as a function of pH in a 0.05 M KCl solution at 25°C with total Al = 10^{-6} M.

$$Al_T = (Al^{3+})/\gamma_{Al^{3+}} + \sum_{n=1}^{4} \{(Al(OH)_n^{3-n})/\gamma_{Al(OH)_n^{3-n}}\} +$$

$$\sum_{m=0}^{3} \{(Al(OH)_m L^{2-m})/\gamma_{Al(OH)_m L^{2-m}}\} \qquad (11)$$

Equation (11) can be rewritten in terms of (Al^{3+}):

$$Al_T = (Al^{3+})/\gamma_{Al^{3+}} + \sum_{n=1}^{4} \{K_n(Al^{3+})/(H^+)^n \gamma_{Al(OH)_n^{3-n}}\} +$$

$$\sum_{m=0}^{3} \{Q_m(Al^{3+})(L^-)/(H^+)^m \gamma_{Al(OH)_m L^{2-m}}\} \qquad (12)$$

If the activity of Al^{3+} is controlled by gibbsite dissolution, then $(Al^{3+}) = Ksp(H^+)^3$.

Substituting this condition into eq. (12) yields:

$$Al_T = Ksp(H^+)^3/\gamma_{Al^{3+}} + Ksp\sum_{n=1}^{4} \{K_n(H^+)^{3-n}/\gamma_{Al(OH)_n^{3-n}}\} +$$

$$Ksp(L^-)\sum_{m=0}^{3} \{Q_m(H^+)^{3-m}/\gamma_{Al(OH)_m L^{2-m}}\} \qquad (13)$$

By defining Y and Xj as:

$$Y = Al_T/Ksp - (H^+)^3/\gamma_{Al^{3+}} - \sum_{n=1}^{4} \{K_n(H^+)^{3-n}/\gamma_{Al(OH)_n^{3-n}}\} \qquad (14)$$

$$X_0 = (L^-)(H^+)^3/\gamma_{AlL^{2+}} \qquad (15)$$

$$X_1 = (L^-)(H^+)^2/\gamma_{Al(OH)L^+} \qquad (16)$$

$$X_2 = (L^-)(H^+)/\gamma_{Al(OH)_2 L^-} \qquad (17)$$

$$X_3 = (L^-)/\gamma_{Al(OH)_3 L^-} \qquad (18)$$

the expression for total solubility can be written as:

$$Y = Q_0 X_0 + Q_1 X_1 + Q_2 X_2 + Q_3 X_3 + \epsilon \qquad (19)$$

69

where the ion association constants (Q_n values) appear as regression parameters and $\in$ is the random error associated with the multiple linear regression model. In practice, the solution of eq. (19) requires the employment of an iterative refinement procedure, as the activities and activity coefficients are a function of the magnitude of the Q_n values. This analysis can also be performed by the FITEQL program. Preliminary evaluations indicated that both the regression analysis technique and FITEQL provided comparable thermodynamic constants for Al-2-ketogluconate speciation. Therefore, the FITEQL program was employed to derive the equilibrium constants for these systems, based on ease of use. The FITEQL program was employed to estimate ^cQ values (conditional equilibrium constants) from the experimental data (Table 4.8). The Davies equation was then used to convert the log ^cQ values to log Q values. The Q values were averaged for the two ionic strengths, 0.063 M and 0.113 M, and entered into the GEOCHEM-PC thermodynamic data base in order to reassess the gibbsite solubility data (Table 4.9). The log (Al^{3+}) values generated from GEOCHEM-PC were plotted as a function of pH in Figure 4.3. The log (Al^{3+}) values correspond with the gibbsite solubility line, indicating the relevance of the Al-2-ketogluconate species. Figure 4.4 illustrates the distribution of the Al^{3+} in the presence of 2-ketogluconic acid. Figure 4.4 also illustrates reduction in the insignificance of the Al^{3+} species and the hydrolysis products, $Al(OH)^{2+}$, $Al(OH)_2^+$, and $Al(OH)_3^0$ and the dominance of the Al-2-ketogluconate complexes. These results, therefore, illustrate one possible mechanism by which 2-ketogluconic acid increases P solubilization in soil.

Table 4.8. Thermodynamic constants for Al-2-ketogluconate species computed from the gibbsite solubility data.

Species[a]	Ionic Strength, M	$\log {}^cQ^\dagger$	$\log Q^\dagger$	Mean $\log Q \pm$ S.D.[b]
AlL^{2+}	0.063	2.54	3.10	3.03 ± 0.11
	0.113	2.28	2.95	
$Al(OH)L^+$	0.063	-1.71	-0.96	-1.13 ± 0.24
	0.113	-2.19	-1.30	
$Al(OH)_2L^0$	0.063	-5.82	-5.07	-5.18 ± 0.16
	0.113	-6.19	-5.29	
$Al(OH)_3L^-$	0.063	-11.10	-10.53	-10.64 ± 0.15
	0.113	-11.41	-10.74	

$\dagger$ - error ± 0.02

a - $L^- = C_6H_9O_7^-$

b - considers replicate error and the error associated with the minimization procedure of FITEQL.

Table 4.9. Composition of solutions in equilibrium with gibbsite[a].

pH	p[AlL^{2+}][†]	p[AlOHL$^+$]	p[Al(OH)$_2$L^0]	p[Al(OH)$_3$L$^-$]	p[Al^{3+}]	p(Al^{3+})[‡]	log IAP
3.89	3.09	3.57	3.88	5.30	2.65	3.84	7.83
3.90	3.12	3.60	3.91	5.30	2.65	3.83	7.87
3.91	3.04	3.51	3.80	5.20	2.62	3.86	7.87
3.93	3.05	3.51	3.79	5.15	2.63	3.88	7.91
3.94	3.08	3.57	3.85	5.20	2.63	3.98	7.84
3.96	3.08	3.57	3.83	5.14	2.64	4.00	7.88
3.97	3.15	3.61	3.87	5.18	2.66	3.98	7.93
3.99	3.17	3.62	3.85	5.14	2.67	3.97	8.00
4.27	4.10	4.22	4.14	5.17	3.57	5.16	7.65
4.48	4.85	4.74	4.45	5.28	4.04	5.60	7.84
4.50	4.35	4.25	3.96	4.75	3.59	5.48	8.02
4.55	4.79	4.61	4.27	5.02	3.91	5.62	8.03
4.60	4.52	4.31	3.91	4.61	3.61	5.64	8.16
4.65	4.95	4.67	4.22	4.88	3.93	5.81	8.14
5.20	6.55	5.72	4.71	4.82	4.43	7.38	8.22
5.24	6.55	5.69	4.65	4.71	4.35	7.35	8.37
5.40	7.12	6.09	4.89	4.79	4.52	7.94	8.26
5.58	7.32	6.12	4.75	4.46	4.27	8.41	8.33
5.59	7.30	6.08	4.69	4.41	4.22	8.37	8.40
5.70	7.61	6.29	4.78	4.39	4.24	8.73	8.37
5.70	7.99	6.71	5.23	4.81	4.67	9.01	8.09
5.84	8.38	6.97	5.36	4.79	4.69	9.42	8.10
5.97	8.81	7.27	5.52	4.83	4.74	9.75	8.16
6.15	9.69	7.91	5.95	5.11	5.05	10.50	7.95
6.16	9.71	7.94	5.97	5.11	5.05	10.51	7.97
6.24	9.38	7.52	5.49	4.54	4.50	10.55	8.17
6.26	9.75	7.86	5.80	4.84	4.79	10.63	8.15
6.26	9.67	7.84	5.80	4.82	4.77	10.61	8.17
6.40	10.53	8.50	6.30	5.20	5.16	11.35	7.85
6.40	9.89	7.88	5.68	4.58	4.54	11.00	8.20
6.42	10.10	8.05	5.84	4.72	4.69	11.08	8.18
6.43	9.94	7.88	5.66	4.53	4.50	11.07	8.22
6.44	10.31	8.24	6.00	4.86	4.83	11.18	8.14
6.62	10.69	8.45	6.03	4.71	4.69	11.68	8.18

† - concentration
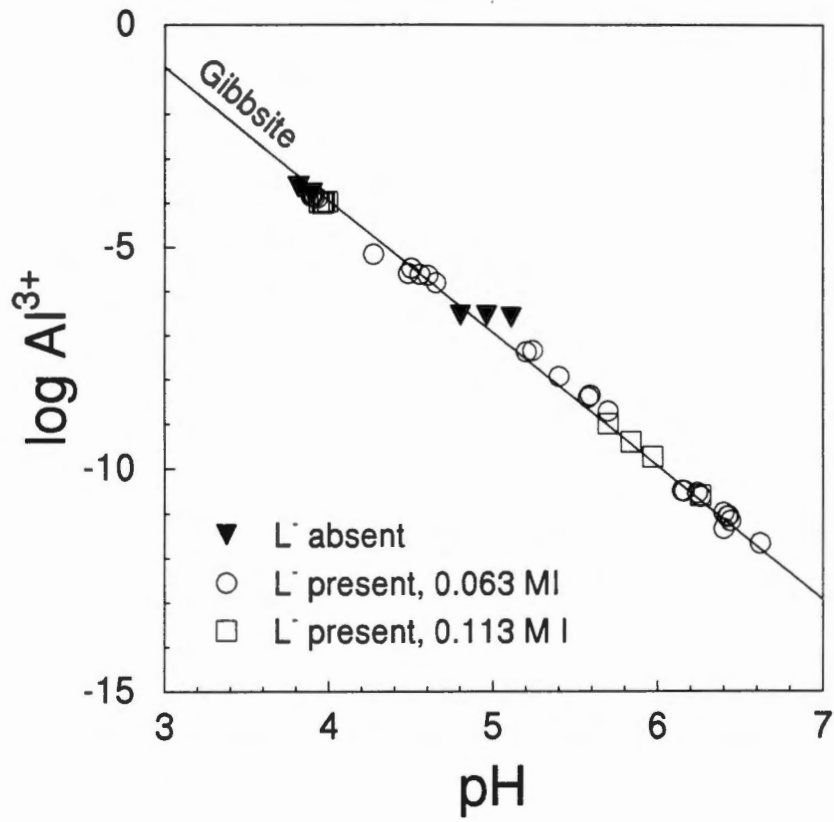
‡ - activity

a - L$^-$ = C$_6$H$_9$O$_7^-$

Figure 4.3. Solubility relationships in the gibbsite systems with Al-2-ketogluconate species considered in the chemical model.
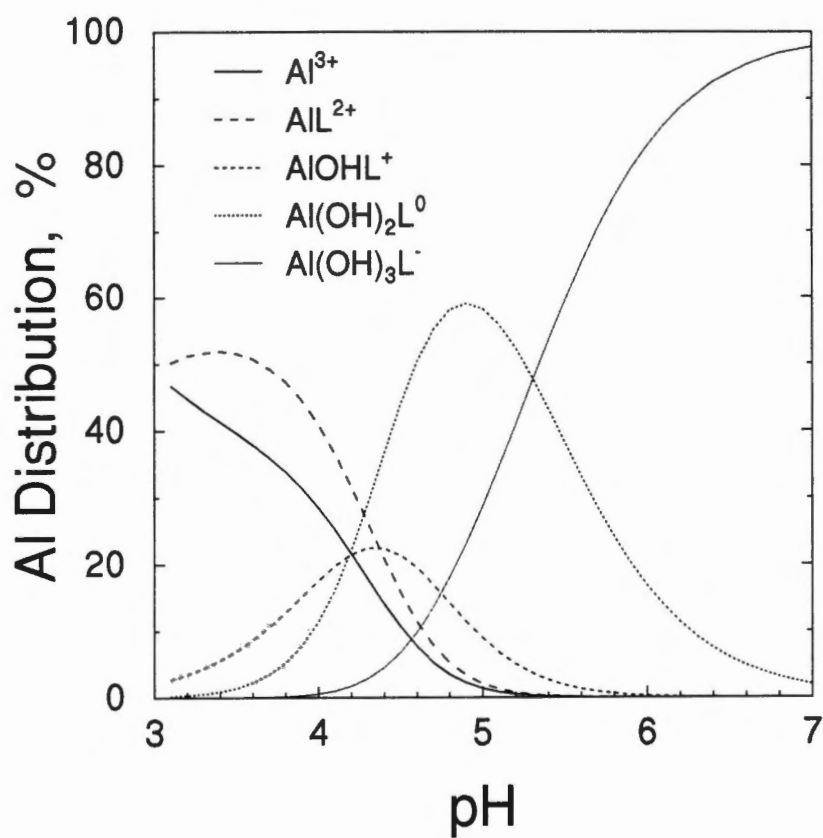
Figure 4.4. Aqueous Al speciation as a function of pH in a solution composed of 0.005 M 2-ketogluconic acid and 0.05 M KCl at 25°C with total Al = 10^{-6} M.

Chapter IV

Summary and Conclusions

Currently employed soil tests for plant available P determinations do not accurately assess P status in loess based soils. These soil tests will often underestimate P availability and produce results which prompt producers to apply P fertilizers. However, crops often show no response to the P fertilization, wasting the producers time and money.

Two soil P extraction procedures that utilize citric and 2-ketogluconic acid (found in the rhizosphere) were developed to assess P availability in loess based soils. The organic acid extraction procedures were developed by evaluating P extractability from a surface soil known to have high concentrations of Mehlich III and Olsen extractable P. The extraction procedures were developed by varying organic acid concentrations (0, 2.5, 5.0, and 10.0 mM), organic acid ratios (10 mM citric acid, 5 mM 2-ketogluconic acid:5 mM citric acid, and 10 mM 2-ketogluconic acid), pH levels (3.5 and 5.5), solid-to-solution ratios (1.0 g/25 ml, 5.0 g/25 ml, and 10.0 g/25 ml), and extraction times (1, 3, and 6 hours). Two extraction procedures were found to maximize P solubility: 1) 10g of soil extracted with 25 ml of 10 mM citric acid at pH 3.5 for 3 hrs (OE1), and 2) 5 g of soil extracted with 25 ml of 5 mM citric acid + 5 mM 2-ketogluconic acid at pH 3.5 for 6 hrs (OE2). The OE1 and OE2 procedures, the Mehlich III and Olsen bicarbonate soil P tests, and the modified Chang-Jackson sequential fractionation procedure, were applied to soil samples collected from a study that examined swine manure as a P fertilizer source (at Ames Plantation) and from a study that investigated the long-term influence of tillage and fertilizer P on corn yield (at Milan Experiment Station). Correlations were then performed

among the extractable P fractions, between the available P pools, plant P and yield. Criteria used to evaluate the effectiveness of each organic acid extraction were: 1) the extraction capabilities of OE1 and OE2 compared to those of Mehlich III, Olsen, and of the modified Chang-Jackson sequential fractionation procedure, 2) correlation with plant P concentrations, and 3) correlation with yield.

In the Ames soil, OE1 was significantly correlated with Mehlich III, Olsen, and Al-P, but OE2 was only correlated with Mehlich III and Al-P. In the Milan soil, OE1 and OE2 were significantly correlated with Mehlich III, Olsen, Al-P, and Fe-P. In the Ames and Milan soils, none of the available P indicators or inorganic P fractions produced a significant correlation with plant P. In the Milan soil, corn yield was significantly correlated with OE1, OE2, Mehlich III, Olsen, Al-P, and Fe-P. In the Ames soil, all P indices produced poor correlations with yield.

The following conclusions were drawn from the results of this study:

1. OE1 and OE2 extracted higher concentrations of P in the Milan soil than in the Ames soil, due to the difference in P fertilizer source.

2. OE1-P was closely related to Mehlich III-P and the Olsen-P in both soils.

3. OE2-P was closely related to Mehlich III-P in both soils and the Olsen-P in the Milan soil.

4. Both OE1-P and OE2-P were correlated with Al-P in both soils and Fe-P in the Milan soil.

5. All extractants were poor indicators of plant P concentrations.

6. All extractants produced greater correlation coefficients with yield in the Milan soil than in the Ames soil.

Overall, both organic extractions were closely related to Mehlich III and to a lesser extent to the Olsen method in their ability to assess plant available P. Therefore, both OE1 and OE2 proved to be inadequate in the assessment of available P for the Ames and Milan soil. However, the high concentration of P extracted by both organic acid extractions from the Milan soil warrants further investigation. In particular OE2 which extracted the highest concentrations of P from the Milan soil. An increase in concentration of both citric acid and 2-ketogluconic acid may further enhance P extractability and provide a better indicator of plant response to fertilizer P.

A solubility study was conducted to elucidate the mechanism by which 2-ketogluconic acid increases P solubilization in soil. The aqueous speciation of 2-ketogluconic acid was investigated by examining the equilibrium solubility of gibbsite as a function of 2-ketogluconic acid concentration, pH, and ionic strength. The solubility results suggested the formation of AlL^{2+}, $AlOHL^+$, $Al(OH)_2L^0$, and $Al(OH)_3L^-$ (where L is the 2-ketogluconate ion). By utilizing the GEOCHEM-PC and FITEQL programs, log K values for the general reaction: $Al^{3+} + L^- + nH_2O = Al(OH)_nL^{2-n} + nH^+$, were determined as follows: AlL^{2+}: log K = 3.0 ± 0.11; $AlOHL^+$: log K = -1.1 ± 0.24; $Al(OH)_2L^0$: log K -5.2 ± 0.16; and $Al(OH)_3L^-$: log K = -10.6 ± 0.15.

LITERATURE REVIEW

Literature Cited

Amer, F., D.R. Bouldin, C.A. Black, and F.R. Duke. 1955. Characterization of soil phosphorus by anion exchange resin adsorption and ^{32}P-equilibration. Plant and Soil. 6:391-408.

Amer, F., S. Mahdi, and A. Alradi. 1969. Limitations in isotropic measurements of labile phosphate in soils. J. Soil Sci. 20:91-100.

Aquino, B.F., and R.G. Hanson. 1984. Soil P supplying capacity evaluated by plant removal and available P extraction. Soil Sci. Soc. Am. J. 48:1091-1096.

Barrow, N.J. 1983. A mechanistic model for describing the sorption and desorption of phosphate by soil. J. Soil Sci. 34:733-750.

Berrow, M.L., M.S. Davidson, and J.C. Burridge. 1982. Trace elements extractable by 2-ketogluconic acid from soils and their relationship to plant contents. Plant and Soil. 66:161-171.

Bingham, F.T. 1962. Chemical soil tests for available phosphorus. Soil Sci. 94:87-95.

Black, C.A. 1957. Soil - Plant Relationships. New York: John Wiley & Sons Inc. pp. 248-286.

Bloom, P.R., and R.M. Weaver. 1982. Effect of the removal of reactive surface material on the solubility of synthetic gibbsite. Clay and Clay Minerals. 30:281-286.

Bowman, R.A. 1988. A rapid method to determine total phosphorus in soils. Soil Sci. Soc. Am. J. 52:1301-1304.

Bowman, R.A. 1989. A sequential extraction procedure with concentrated sulfuric acid and dilute base for soil organic phosphorus. Soil Sci. Soc. Am. J. 53:362-366.

Bowen, G.D., and A.D. Rovira. 1966. Microbial factor in short-term phosphate uptake studies with plant roots. Nature. 211:665-666.

Chiyonobu, T., O. Adachi, and M. Ameyama. 1973. Purification and properties of 2-ketogluconate reductase. Ag. Biol. Chem. 37:2871-2878.

Coleman, N.T., J.T. Thorup, and W.A. Jackson. 1960. Phosphate sorption reactions that involve exchangeable Al. Soil Sci. 90:1-7.

Dalal, R.C., and E.G. Hallsworth. 1977. Evaluation of the parameters of soil phosphorus availability factors in predicting yield response and phosphorus uptake. Soil Sci. Soc. Am. J. 40:541-546.

Datta, N.P., and M.S. Khera. 1969. Phosphorus soil tests based upon correlations with inorganic phosphorus fractions and greenhouse tests. J. Ind. Soc. Soil Sci. 17:191-196.

Debnath, N.C. and S.K. Mandal. 1982. Evaluation of the relationship of inorganic phosphate fractions of soil with available phosphorus determined by some chemical and biological methods. J. Ind. Soc. Soil Sci. 30:489-493.

Duff, R.B., and Webley D.M. 1959. 2-Ketogluconic acid as a natural chelator produced by soil bacteria. Chem. Inds. Lond. 1959:1376-1377.

Duff, R.B., D.M. Webley, and R.O. Scott. 1963. Solubilization of minerals and related materials by 2-ketogluconic acid producing bacteria. Soil Science. 95:105-114.

Dyer, D. 1894. On the analytical determination of probably available "mineral" plant-food in soil. J. Chem. Soc. 65:115-129.

Earl, K.D., J.K. Syers, and J.R. McLaughlin. 1979. Origin of the effects of citrate, tartrate, and acetate on phosphate sorption by soils and synthetic gels. Soil Sci. Soc. Am. J. 43:674-678.

Erlich, H.L. 1981. The geomicrobiology of silica and silicates. *In* H.L. Ehrlich (ed.) Geomicrobiology. Marcel Dekker. pp. 131-135.

Essington, M.E., 1990. Calcium molybdate solubility in spent oil shale and a preliminary evaluation of the association constants for the formation of $CaMoO_4^0(aq)$, $KMoO^-$ (aq), and $NaMoO_4^-(aq)$. Environ. Sci. Technol. 24:214-220.

Essington, M.E., D.D. Howard, and J.M. Williams. 1994. The influence of long-term conservation tillage on soil phosphates. SAAS-Southern Branch ASA. Nashville, TN. Abstracts. p 13.

Frink, C.R., and M. Peech. 1962. Hydrolysis of the aluminum ion in dilute aqueous solutions. Inorg. Chem. 2:473-474.

Goh, T.B., and P.M. Huang. 1984. Formation of hydroxy-Al-montmorillonite complexes as influenced by citric acid. Can. J. Soil Sci. 64:411-421.

Grossl, P.R., and W.P. Inskeep. 1989. Crystal growth of octacalcium phosphate in the presence of organic acids. p.200. *In* Agronomy abstracts. ASA, Madison, WI.

Grossl, P.R., and W.P. Inskeep, 1991. Precipitation of dicalcium phosphate dihydrate in the presence of organic acids. Soil Sci. Soc. Am. J. 55:670-675.

Hardin, S.D. 1988. Effects of rates of applied phosphorus and soil storage temperature on P availability of a low-P loess-derived Alfisol. M.S. Thesis, The University of Tennessee.

Hardin, S.D., D.D. Howard, and J.D. Wolt. 1989. Critical soil phosphorus analysis at selected times throughout the season, of a low-P loess-derived soil as affected by storage temperature. Commun. Soil Sci. Plant Anal. 20:1525-1543.

Halder, A.K., and P.K. Chakrabartty. 1993. Solubilization of inorganic phosphate by *Rhizobium*. Folia Microbiol. 38:325-330.

Hingston, F.J., A.M. Posner, and J.P. Quirk. 1972. Anion adsorption by geothite and gibbsite. J. Soil Sci. 23:177-191.

Howard, D.D., and G.M. Lessman, and B. Hatchcock. 1990. Phosphate and potash fertilization of soybeans produced on loess-derived soils. Univ. of Tenn. Agric. Exp. Stn. Bull. 688.

Ibrikei, H., E.A. Hanlon, and J.E. Recheigl. 1992. Initial calibration and correlation of inorganic phosphorus soil test methods with a bahiagrass field trial. Commun. in Soil Sci. Plant Anal. 23:2569-2579.

Inskeep, W.P., and J.C. Silvertooth. 1988. Inhibition of hydroxyapatite precipitation in the presence of fulvic, humic, and tannic acids. Soil Sci. Soc. Am. J. 52:941-946.

Jayachandran, K., A.P. Schwab, and B.A.D. Hetrick. 1992. Partitioning dissolved inorganic and organic phosphorus using acidified molybdate and isobutanol. Soil Sci. Soc. Am. J. 56:762-765.

Jittanoonta, D. 1977. An evaluation of the new Mehlich soil test method for determining available phosphorus, potassium, calcium, magnesium, and manganese. M.S. Thesis. Mississippi State University.

Kafkafi, U., B. Bar-Yosef, R. Rosenberg, and G. Sposito. 1988. Phosphorus adsorption by kaolinite and montmorillonite: II. Organic Anion Competition. Soil Sci. Soc. Am. J. 52:1585-1589.

Katznelson, H., and B. Rose. 1959. Metabolic activity and phosphate dissolving capability of bacterial isolates from wheat roots, rhizosphere and non-rhizosphere soil. Can. J. Micro. 5:79-85.

Kittrick, J.A. 1966. The free energy of formation of gibbsite and Al(OH) from solubility measurements. Soil Sci. Soc. Am. Proc. 30:595-598.

Klasen, R., S. Bringer-Meyer, and H. Sahm. 1992. Incapability of gluconobacter to produce tartaric acid. Biotechnology and Bioengineering. 40:183-186.

Knudsen, D. 1980. Recommended phosphorus soil tests. North Dakota Agric. Exp. Sta. Bull. 499 Fargo, NC 58105:16-19.

Kucey, R.M.N., H.H. Janzen, and M.E. Leggett. 1989. Microbially mediated increases in plant-available phosphorus. Adv. Agron. 42:199-228.

Kwong, K.F., and P.M. Huang. 1979. The relative of low-molecular-weight, complexing organic acids on the hydrolysis and precipitation of aluminum. Soil Science 128:337-342.

Larsen, S. 1967. Soil phosphorus. Adv. Agron. 19:151-210.

Lawton, K. 1961. Plant nutrition and fertilizer phosphorus. J.R. Van Wazer (ed.) *In* Phosphorus and Its Compounds, Vol. II. Technology, Biological Functions, and Applications. pp.1461-1545.

Lindsay, W.L. 1979. Chemical Equilibria in Soils. New York: John Wiley & Sons Inc.

Lopez-Hernandez, D., G. Siegert, and J.V. Rodriguez. 1986. Competitive adsorption of phosphate with malate and oxalate by tropical soils. Soil Sci. Soc. Am. J. 50:1460-1462.

Lougham, B.C. 1981. Metabolic aspects of the transport of ions by cells and tissues of roots. *In* R. Brouwer et al. (eds.) Structure and Function of Plant Roots. pp. 237-245.

May, H.M., P.A. Helmke, and M.L. Jackson. 1979. Gibbsite solubility and thermodynamic properties of hydroxy aluminum ions in aqueous solution at 25°C. Geochim. Cosmochim. Acta. 43:861-868.

Mehlich, A. 1984. Mehlich 3 soil test extractant: A modification of Mehlich 2 extractant. Comm. Soil Sci. Plant Anal. 15:1409-1416.

Menon, R.G., S.H. Chien, L.L. Hammond, and B.R. Arora. 1990. Sorption of phosphorus by the iron oxide-impregnated filter paper (P_i soil test) embedded in soils. Plant & Soil. 126:287-294.

Moghimi, A., and M.E. Tate. 1978. Does 2-ketogluconate chelate calcium in the pH range 2.4 to 6.4? Soil Biol. Biochem. 10:289-292.

Moghimi, A., M.E. Tate, and J.M. Oades. 1978a. Characterization of the rhizosphere products especially 2-ketogluconic acid. Soil Biol. Biochem. 10:283-287.

Moghimi, A., D.G. Lewis, and J.M. Oades. 1978b. Release of phosphate from calcium phosphates by rhizosphere products. Soil Biol. Biochem. 10:277-281.

Murphy, J., and J.P. Riley. 1962. A modified single solution method for the determination of phosphate in natural waters. Anal. Chim. Acta. 27:31-36.

Nagarajah, S., A.M. Posner, and J.P.Qiurk. 1968. Desorption of phosphate from kaolinite by citrate and bicarbonate. Soil Sci. Soc. Am. Proc. 32:507-510.

Nagarajah, S., A.M. Posner, and J.P. Quirk. 1970. Competitive adsorption of phosphate with polygalacturonate and other organic anions on kaolinite and oxide surfaces. Nature. 228:83-85.

Nair, V.D., D.A. Graetz, and K.M. Portier. 1995. Forms of phosphorus in soil profiles form dairies of South Florida. Soil Sci. Soc. Am. J. 59:1244-1249.

Neijssel, O.M., and D.W. Tempest. 1975. Production of gluconic acid and 2-ketogluconic acid by *Klebsiella aerogenes* NCTC 418. Arch. Microbiol. 105:183-185.

Nelson, W.L., A. Mehlich, and E. Winters. 1953. The development, evaluation, and use of soil tests for phosphorus availability. Agronomy. 4:153-188.

Nye, P.H. 1981. pH changes across the rhizosphere induced by roots. Plant and Soil. 61:7-26.

O'Dell, J.D., M.E. Essington, and D.D. Howard. 1993. Correlation of extractable P with P uptake from swine manure-amended soil. Agron. Abstracts. p. 232.

O'Dell, J.D. 1995. Phosphorus speciation, mineralization, and uptake by corn in a soil amended with liquid swine manure. Ph.D. Dissertation, The University of Tennessee.

Olsen, R.S., and L.E. Sommers. 1982. Phosphorus. *In* A.L. Page et al. (eds.) Methods of Soil Analysis, Part 2, 2nd edition. Agronomy 9:403-446.

Parker, D.R., R.L. Chaney, and W.A. Norvell. 1995. Chemical equilibrium models: applications to plant nutrition research. *In* R.H. Loeppert et al. (eds.), Chemical equilibrium and reaction models. Soil Sci. Soc. Am., Madison, W.I.

Petersen, G.W., and R.B. Corey. 1966. A modified Chang and Jackson procedure for routine fractionation of inorganic soil phosphates. Soil Sci. Soc. Am. Proc. 30:563-565.

Pierre, W.H., and F.W. Parker. 1927. Soil phosphorus studies: II. The concentration of organic and inorganic phosphorus in the soil solution and soil extracts and the availability of the organic phosphorus to plants. Soil Sci. 24:119-128.

Pojasok, T., and B.D. Kay. 1990. Effect of root exudates from corn and bromegrass on soil structural stability. Can. J. Soil Sci. 70:351-362.

Pons, W.A., and J.D. Guthrie. 1946. Determination of inorganic phosphorus in plant materials. Ind. Eng. Chem. Anal. Ed. 18:184-186.

Pratt, P.F., and M.J. Garber. 1964. Correlations of phosphorus availability by chemical tests with inorganic phosphorus fractions. Soil Sci. Soc Am. J. 28:23-26.

Probert, M.E., and I.R. Willett. 1983. The relationship between labile phosphate and Bray P1 extractable phosphate. Comm. Soil Sci. Plant Anal., 14(2):115-120.

Russell, R.S., J.B. Rickson, and S.N. Adams. 1954. Isotope equilibria between phosphate in soil and their significance in the assessment of fertility by tracer methods. J. Soil Sci. 5:85-105.

Ryden, J.C., J.K. Syers, and R.W. Tillman. 1977. Inorganic anion sorption and interactions with phosphate sorption by hydrous ferric oxide gel. J. Soil Sci. 38:211-217.

Sample, E.C., R.J. Soper, and G.J. Racz. 1980. Reactions of phosphate in soils. p.263-310. *In* F.E. Khasawneh et al. (eds.) The Role of Phosphorus in Agriculture. ASA, CSSA, and SSSA, Madison WI.

Schofield, R.K. 1955. Can a precise meaning be given to "available" soil phosphorus? Soils Fert. 18:373-375.

Sharpley, A.N., C.A. Jones, and C. Gray. 1984. Relationships among soil P test values for soils of differing pedogenesis. Commun. in Soil Sci. Plant Anal. 15:985-995.

Sharpley, A.N., D. Curtin, and J.K. Syers. 1988. Changes in water-extractability of soil inorganic phosphate induced by sodium saturation. Soil Sci. Soc. Am. J. 52:637-640.

Sibbesen, E. 1978. An investigation of the anion-exchange resin method for soil phosphate extraction. Plant and Soil. 50:305-321.

Singh, R.N., H. Sinha, and B.P. Sahi. 1968. Fractionation of soil inorganic phosphorus. J. Ind. Soc. Soil Sci. 16:371-377.

Singh, R.N. 1972. The solubility product of gibbsite at 15°, 25°, and 35°C. Soil Sci. Soc. Am. Proc. 38:415-417.

Smith, R.M., and A.E. Martell. 1976. Critical stability constants. Volume 4: Inorganic complexes. Plenum Press, New York, New York.

Sokatch, J.R. 1969. Bacterial Physiology and Metabolism. p.117-119. Academic Press, London.

Sperber, J.I. 1957. Solution of mineral phosphates by soil bacteria. Nature. 180:994-995.

Sperber, J.I. 1958. Incidence of apatite-solubilizing organisms in the rhizosphere and soil. Australian J. Ag. Res. 9:778-781.

Sposito, G. 1984. The Surface Chemistry of Soils. New York: Oxford University Press. pp. 138-147.

Sposito, G. 1989. The Chemistry of Soils. New York: Oxford University Press. pp. 127-145.

Stevenson, F.J. 1986. Cycles of Soil: Carbon, Nitrogen, Phosphorus, Sulfur, Micronutrients. New York: John Wiley and Sons. p. 250.

Stumm, W., and J.J. Morgan. 1981. Aquatic chemistry. 2nd ed. Toronto: John Wiley & Sons.

Struthers, P.H. and D.V. Sieling. 1950. Effect of organic anions on phosphate precipitation by iron and aluminum as influenced by pH. Soil Sci. 69:205-213.

Thomas, G.W., and D.E. Peaslee. 1973. Testing soils for phosphorus. p. 115-132. In L.M. Walsh and J.D. Beaton (ed.) Soil testing and plant analysis. rev. ed. Soil Science Society of America, Madison, WI.

Tinker, P.B. 1975. Soil chemistry of phosphorus and mycorrhizal effects on plant growth. In F.E. Sanders (ed.) Endomycorrhizae. pp. 353-371.

Tisdale, S.L., W.L. Nelson, J.D. Beaton, and J.L. Havlin. 1993. Soil Fertility and Fertilizers. New York: MacMillan Publishing Company. pp. 176-226.

Traina, S.J., G. Sposito, D. Hesterberg, and U. Kafkafi. 1986a. Effects of ionic strength, calcium, and citrate on orthophosphate solubility in an acidic, montmorillonitic soil. Soil Sci. Soc. Am. J. 50:623-627.

Traina, S.J., G. Sposito, D. Hesterberg, and U. Kafkafi. 1986b. Effects of pH and organic acids on organophosphate solubility in an acid, montmorillonitic soil. Soil Sci. Soc. Am. J. 50:45-52.

Traina, S.J., G. Sposito, G.R. Bradford, and U. Kafkafi. 1987. Kinetic study of citrate effects on orthophosphate solubility in an acid, montmorillonitic soil. Soil Sci. Soc. Am. J. 51:1483-1487.

Tran, T.S., J.C. Fardeau, and M. Giroux. 1988. Effects of soil properties on plant-available phosphorus determined by the isotopic dilution phosphorus-32 method. Soil Sci. Soc. Am. J. 52:1383-1390.

Tran, T.S., and A. N'dayegamiye. 1995. Long-term effects of fertilizers and manure application on the forms and availability of soil phosphorus. Can. J. Soil Sci. 75: 281-285.

Van Raij, B., J.A. Quaggio, and N.M. da Silva. 1986. Extraction of phosphorus, potassium calcium, and magnesium from soils by and ion-exchange resin procedure. Commun. in Soil Sci. Plant Anal. 17:547-566.

Verdes, G., R. Gout, and S. Castet. 1992. Thermodynamic properties of the aluminate ion and of bayerite, boehmite, diaspore, and gibbsite. Eur. J. Mineral. 4:767-792.

Violante, A., and P. Violante. 1978. Influence of carboxylic acids on the stability of chlorite-like complexes and on the crystallization of $Al(OH)_3$ polymorphs: Agrochimica 22:335-343.

Wantanabe, F.S., and S.R. Olsen. 1962. Colorimetric determination of phosphorus in water extracts of soil. Soil Sci. 93:183-188.

Watanabe, F.S., and S.R. Olsen. 1965. Test of an ascorbic acid method for determining phosphorus in water and $NaHCO_3$ extracts from soil. Soil Sci. Soc. Proc. 29:677-678.

Webley, D.M., M.E.K. Henderson, and I.F. Taylor. 1963. The microbiology of rocks and weathered stones. J. Soil Sci. 14:102.

Webley, D.M., and R.B. Duff. 1965. The incidence, in soils and other habitats of micro-organisms producing 2-ketogluconic acid. Plant and Soil. 22:307-313.

Westall, J.C. 1982. FITEQL. A computer program for determination of chemical equilibria constants for experimental data. Version 1.2 User's Guide. Oregon State University. Report 82-01.

Williams, E.G., and A.H. Knight. 1963. Evaluation of soil phosphate status by pot experiments, conventional extraction methods and labile phosphate values estimated with the aid of phosphorus-32. J. Sci. Food Agric. 14:555-563.

Wolf, A.M., D.E. Baker, H.B. Pionke, and H.M. Kunishi. 1985. Soil tests for estimating labile, soluble, and algae-available phosphorus in agricultural soils. J. Env. Qual. 14:341-348.

Wood, S.A., and D.A. Crerar. 1985. A numerical method for obtaining multiple linear regression parameters with physically realistic signs and magnitudes: application to the determination of equilibrium constants from solubility data. Geochim. Cos. Acta. 49:165-172.

APPENDICES

Table A.1. Variables used for extraction development and codes for mean separation.

	Variables			
	1	2	3	4
Organic Acid	0 mM L^{-1}	2.5 mM L^{-1}	5.0 mM L^{-1}	10 mM L^{-1}
Organic Acid Ratio	100% citric	5 mM 2-ketogluconic: 5 mM citric	100% 2-ketogluconic	
pH Value	3.5	5.5		
Extraction Time	1 h	3 h	6 h	
Solid:Solution	1.0 g : 25 ml	5.0 g : 25 ml	10.0 g : 25 ml	

Table A.2. Available P concentrations measured by all possible extraction combinations.

Row 1	
Trt	Mean
41133	31.74a[†]
41132	30.947a
41123 (OE1)	30.947a
41113	29.540b
41122	29.183b
42132 (OE2)	25.043c
41112	24.587c
42123	21.293d
42122	20.7d
41232	20.517d
42113	20.48d
31132	19.290e
31122	17.210f
41233	16.580fg
31123	16.487fg
31112	16.323hg
41222	15.653hi
31113	15.440i
41223	15.310i
42112	15.293i
41213	14.447j
31133	14.293j
32132	14.253j
32122	14.170j
42133	14.017j
32112	13.673jk
41212	13.103k
41131	13.003k
21132	12.950k
21122	12.880k
21112	11.853l
31131	11.707l
32131	11.133lm
42131	10.587m
32113	10.573m
41121	10.567m
21131	9.677n
21113	9.66n
32123	9.66n
42121	9.347no
31121	8.943nop
32121	8.923nop
31233	8.783op
42223	8.65opq
31223	8.603opqr
41111	8.583opqr
21123	8.513opqr
42232	8.423pqr

† - Means within a column followed by the same letter are not significantly different at the 0.05 probability level.

90

Table A.2. Available P concentrations measured by all possible extraction combinations (continued).

Row 2	
Trt	Mean
42213	8.37pqr
31222	8.297pqrs
42222	8.157pqrs
32133	7.813qrs
22131	7.747rs
41231	7.483st
42233	6.860tu
31213	6.843tu
21121	6.81tuv
21133	6.783tuv
31232	6.663tuvw
31212	6.587uvw
32111	6.507uvwx
32232	6.507uvwx
42212	6.487uvwx
42111	6.263uvwxy
31111	6.187uvwxy
32212	6.137uvwxy
22112	6.12uvwxy
21232	6.053uvwxy
32222	5.960uvwxyz
21111	5.9vwxyza
21222	5.833wxyza
22122	5.803wxyzab
22121	5.627xyzabc
41221	5.593xyzabcd
32213	5.543yzabcd
32223	5.467yzabcd
22132	5.4yzabcde
32233	5.063zabcdef
21212	5abcdefg
21223	4.92bcdefgh
41211	4.807cdefghi
21213	4.793cdefghi
22111	4.713defghi
21233	4.537efghij
31231	4.25fghijk
43131	4.163ghijkl
32231	4.073hijklm
42231	4.007ijklmn
43121	3.923ijklmno
22232	3.777jklmnop
21231	3.733jklmnopq
31211	3.703jklmnopq
32221	3.7jklmnopq
42221	3.67jklmnopqr
31221	3.527klmnopqrs
43112	3.5klmnopqrs

† - Means within a column followed by the same letter are not significantly different at the 0.05 probability level.

91

Table A.2. Available P concentrations measured by all possible extraction combinations (continued).

	Row 3	
Trt		Mean
22113		3.493lmnopqrs
43122		3.423klmnopqrst
21221		3.383klmnopqrstu
42211		3.35klmnopqrstuv
22222		3.347klmnopqrstuv
22231		3.333klmnopqrstuv
22212		3.333klmnopqrstuv
33121		3.240lmnopqrstuv
22133		3.223lmopnqrstuv
43111		3.203mnopqrstuv
22123		3.093nopqrstuvw
32211		3.08nopqrstuvwx
22213		3.06opqrstuvwxy
43132		3.04opqrstuvwxyz
33111		3.037opqrstuvwxyz
22221		3.037opqrstuvwxyz
22223		2.98opqrstuvwxyza
21211		2.963pqrstuvwxyza
43222		2.963pqrstuvwxyza
43113		2.927pqrstuvwxyzab
33131		2.927pqrstuvwxyzab
43132		2.887pqrstuvwxyzab
43232		2.78qrstuvwxyzabc
43231		2.777qrstuvwxyzabc
43212		2.72rstuvwxyzabc
22233		2.707stuvwxyzabcde
43233		2.707stuvwxyzabcde
43213		2.687stuvwxyzabcde
43221		2.67stuvwxyzabcdef
43223		2.647stuvwxyzabcdef
33113		2.503tuvwxyzabcdefg
43133		2.48tuvwxyzabcdefgh
33122		2.463tuvwxyzabcdefgh
33112		2.443uvwxyzabcdefgh
33132		2.410vwxyzabcdefgh
22211		2.240wxyzabcdefghi
43211		2.187wxyzabcdefghi
23131		2.150wxyzabcdefghi
33123		2.150wxyzabcdefghi
33222		2.133xyzabcdefghi
33213		2.133xyzabcdefghi
33221		2.110yzabcdefghi
33133		2.093zabcdefghi
33232		2.077abcdefghi
33212		2.073abcdefghi
33211		2.057abcdefghi
33223		2.057abcdefghi
23131		2.037abcdefghi

† - Means within a column followed by the same letter are not significantly different at the 0.05 probability level.

Table A.2. Available P concentrations measured by all possible extraction combinations (continued).

Trt	Row 4 Mean
33231	2bcdefghij
23111	1.98bcdefghijk
33233	1.887cdefghijkl
23213	1.853cdefghijklm
23112	1.833defghijklm
23132	1.797defghijklmn
23113	1.797defghijklmn
23122	1.777defghijklmn
23133	1.757efghijklmno
23233	1.723fghijklmnop
23123	1.72fghijklmnop
23233	1.667ghijklmnop
23223	1.663ghijklmnop
23212	1.633ghijklmnopq
23222	1.630ghijklmnopq
23231	1.537hijklmnopqr
23221	1.443ijklmnopqr
11111	1.407ijklmnopqr
11121	1.403ijklmnopqr
23211	1.387ijklmnopqr
11131	1.333ijklmnopqr
11123	1.073jklmnopqr
11122	1.037klmnopqr
11113	1.037klmnopqr
11132	1lmnopqr
11112	.98lmnopqr
11133	.98lmnopqr
11223	.96lmnopqr
11233	.943lmnopqr
11213	.907mnopqr
11232	.853nopqr
11222	.853nopqr
11212	.813opqr
11231	.797pqr
11221	.707qr
11211	.610r

† - Means within a column followed by the same letter are not significantly different at the 0.05 probability level.

APPENDIX B

Table B.1. Fertlizer treatment variables for the tillage-fertilizer P study at the Milan Exeriment Station.

Treatment Number	lbs. P_2O_5 Broadcast	Band N	Treatment P_2O_5
1	0	0	0
2	0	20	0
3	0	20	40
4	40	0	0
5	40	20	0
6	40	20	40
7	80	0	0
8	80	20	40
9	80	20	0
10	120	0	0
11	120	20	0
12	120	20	40

Table B.2. Milan plot plan showing field layout of fertilizer and tillage treatments.

5	9	7	4	10	3	8	2	1	12	11	6	1	5	9	11	8	2	6	10	12	4	3	7
601	602	603	604	605	606	607	608	609	610	611	612	613	614	615	616	617	618	619	620	621	622	623	624

| | | | | | | | | | | No-Till | | Conventional Till | | | | | | | | | | | |

10	11	1	3	9	8	7	4	5	6	2	12	6	12	3	10	9	1	7	2	5	11	8	4
501	502	503	504	505	506	507	508	509	510	511	512	513	514	515	516	517	518	519	520	521	522	523	524

No-Till | Conventional Till

12	1	5	2	3	11	7	8	6	9	4	10
401	402	403	404	405	406	407	408	409	410	411	412

No-Till

11	7	6	9	3	1	12	8	4	10	2	5
301	302	303	304	305	306	307	308	309	310	311	312

No-Till

11	5	8	12	4	1	3	2	6	9	10	7
201	202	203	204	205	206	207	208	209	210	211	212

No-Till

5	3	1	9	6	7	12	2	11	4	8	10
101	102	103	104	105	106	107	108	109	110	111	112

Treatment Number

Plot Number

95

VITA

William Lawrence Holden was born in Mt. Pleasant, Tennessee on April 9, 1970. In May 1988, he graduated from Mt. Pleasant High School in Mt. Pleasant, Tennessee. In May 1992, he received his Bachelor of Sciences degree from Lambuth University, Jackson, Tennessee with a major in Biology. In August 1993, he enrolled in The University of Tennessee, to pursue a Master of Science degree in Plant and Soil Science, which was awarded to him in May of 1996.

143
mg