MONTANA STATE UNIVERSITY

Precipitation of calcium phosphates in the presence of soluble organic matter
by Paul Rudolf Grossl

A thesis submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Crop and Soil Science
Montana State University
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Abstract:
Dicalcium phosphate dihydrate (DCPD) and octacalcium phosphate (OCP) are important P solid phases in soil systems. They both have been recognized as precursor phases to the formation of thermodynamically more stable hydroxyapatite (HAP). Their metastability with respect to HAP may be explained by precipitation kinetics and the influence of dissolved organic C (DOC) on crystal growth. Precipitation rates of DCPD and OCP were measured at respective pHs of 5.7 and 6.0, and 25°C in the absence and presence of organic acids common to soil solutions using a seeded crystal growth method. Initial total soluble (TS) C levels added as humic, fulvic, tannic, and citric acids ranged from 0.26 to 9.0 mM C for DCPD experiments and 20 uM to 2 mM C for OCP experiments. Precipitation of DCPD and OCP was inhibited by adsorption of these organic acids onto crystal seed surfaces blocking sites that act as nuclei for new crystal growth. The efficiency of adsorbed organic acids at inhibiting DCPD and OCP crystal growth is related to their functional group content, size, hydrophobicity, geometry, and orientation on the crystal surface. Of the three Ca phosphate minerals considered, precipitation inhibition increased in the order DCPD < OCP < HAP.

Soil incubation and plant available P experiments were conducted to examine the influence of organic acids on P status of soil systems. For the incubation study, a soil was treated with solutions containing 6 to 8 mM CaTS and PO4TS, with and without 2 mM C as humic acid. Studies examining bioavailability of P to spring wheat (Triticum aestivum L.) and sorghum-sudan grass (Sorghum Sudanese L.) were carried out in controlled environment chambers on soils treated with organic acids. These experiments demonstrated that organic acids were able to increase P solubility and plant availability in soils.

Fulvic acid isolated from a water soluble wheat straw extract was chemically characterized and was most similar to fulvic acids isolated from aquatic systems. It was representative of the hydrophobic, nonhumified fraction of the initial leachate of plant residues and found to inhibit Ca phosphate precipitation. Thus, plant residue breakdown products can enhance P fertilizer efficiency by increasing P bioavailability in soils.

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APPROVAL

of a thesis submitted by

Paul Rudolf Grossl

This thesis has been read by each member of the graduate committee and has been found to be satisfactory regarding content, English usage, format, citations, bibliographic style, and consistency, and is ready for submission to the College of Graduate Studies.

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ABSTRACT

Dicalcium phosphate dihydrate (DCPD) and octacalcium phosphate (OCP) are important P solid phases in soil systems. They both have been recognized as precursor phases to the formation of thermodynamically more stable hydroxyapatite (HAP). Their metastability with respect to HAP may be explained by precipitation kinetics and the influence of dissolved organic C (DOC) on crystal growth. Precipitation rates of DCPD and OCP were measured at respective pHs of 5.7 and 6.0, and 25°C in the absence and presence of organic acids common to soil solutions using a seeded crystal growth method. Initial total soluble (TS) C levels added as humic, fulvic, tannic, and citric acids ranged from 0.26 to 9.0 m*M* C for DCPD experiments and 20 u*M* to 2 m*M* C for OCP experiments. Precipitation of DCPD and OCP was inhibited by adsorption of these organic acids onto crystal seed surfaces blocking sites that act as nuclei for new crystal growth. The efficiency of adsorbed organic acids at inhibiting DCPD and OCP crystal growth is related to their functional group content, size, hydrophobicity, geometry, and orientation on the crystal surface. Of the three Ca phosphate minerals considered, precipitation inhibition increased in the order DCPD < OCP < HAP.

Soil incubation and plant available P experiments were conducted to examine the influence of organic acids on P status of soil systems. For the incubation study, a soil was treated with solutions containing 6 to 8 m*M* Ca_{TS} and PO_{4TS}, with and without 2 m*M* C as humic acid. Studies examining bioavailability of P to spring wheat (*Triticum aestivum* L.) and sorghum-sudan grass (*Sorghum sudanese* L.) were carried out in controlled environment chambers on soils treated with organic acids. These experiments demonstrated that organic acids were able to increase P solubility and plant availability in soils.

Fulvic acid isolated from a water soluble wheat straw extract was chemically characterized and was most similar to fulvic acids isolated from aquatic systems. It was representative of the hydrophobic, nonhumified fraction of the initial leachate of plant residues and found to inhibit Ca phosphate precipitation. Thus, plant residue breakdown products can enhance P fertilizer efficiency by increasing P bioavailability in soils.

CHAPTER 1

INTRODUCTION

When phosphorus fertilizers are applied to soils, chemical processes occur that "fix" or retain P, decreasing phosphate activity. Mechanisms responsible for P fixation are precipitation of P as Ca, Fe, and Al phosphates, adsorption of PO_4 onto oxides, clays, carbonates, and soil organic matter, and immobilization into biomass P. These processes limit the effectiveness of P fertilizer applications; therefore, further applications are necessary to sustain desired crop productivity.

At pHs ranging from near neutral and above, Ca phosphates including dicalcium phosphate dihydrate (DCPD), octacalcium phosphate (OCP), and hydroxyapatite (HAP) are important P solid phases in soils (Lindsay, 1979). Dicalcium phosphate dihydrate and OCP are important reaction products of P fertilizers in soil and are precursors to the formation of thermodynamically more stable HAP. Usually, HAP is predicted to control PO_4 activity in soil solutions (Lindsay, 1979; Stumm and Morgan, 1981); however, DCPD and OCP have been found to persist and control PO_4 activity for considerable lengths of time (Bell and Black, 1970; Fixen et al., 1983; Moreno et al., 1960). The metastability of DCPD and OCP with respect to HAP, whose formation is relatively slow, indicates that precipitation kinetics play a major role in controlling PO_4 levels in soil solutions.

The presence of organic matter may also influence the phosphate chemistry in soil environments. Soluble organic matter was found to be responsible for the persistence of more soluble DCPD and a reduction in the rate of formation of less soluble octacalcium phosphate (OCP) in soils (Moreno et al., 1960). Similarly, numerous studies have demonstrated that the addition of organic amendments, such as sewage sludge and manure, to calcareous soils resulted in higher aqueous PO_4 concentrations than lone additions of inorganic phosphate (O'Connor et al., 1986; Meek et al., 1979; Abbott and Tucker, 1973).

Conservation tillage practices are associated with an accumulation of plant residue and organic C at the soil surface (Dick, 1983; Doran, 1980; Weil et al., 1988). The organic C of plant residues is comprised of: (i) materials present in the initial leachate of plant litter consisting mostly of carbohydrates that degrade rapidly and colored organic acids with molecular weight $<$ 1000 (Thurman, 1985), and (ii) residue humification products consisting of humic and fulvic acids (Shields and Paul, 1973; Stott et al., 1983; Thurman, 1985). These organic constituents appear to play an important role in increasing the efficiency of P fertilizers. Weil et al. (1988) suggested that the increased organic matter under no-till practices may enhance P availability by blocking P adsorption sites on soil colloids. Organic anions commonly found in the rhizosphere compete with phosphate for adsorption sites on oxide and clay surfaces (Nagarajah et al., 1970; Lopez-Hernandez et al., 1986). For example, certain organic molecules (malate and oxalate) are more rapidly adsorbed to soil surfaces than

phosphate, which can improve the phosphate status of the soil (Lopez-Hernandez et al., 1986).

Organic acids commonly found in soil solutions have also been shown to inhibit the precipitation of Ca phosphates (Inskeep and Silvertooth, 1988a). The probable mechanism of inhibition is adsorption of organic acids onto crystal surfaces, which block sites acting as nuclei for new crystal growth. In natural environments, potential inhibitors of crystal growth such as soluble organic matter are ubiquitous. Consequently, precipitation inhibition may be an important mechanism in explaining the metastability of more soluble Ca phosphate solid phases (DCPD and OCP) with respect to more thermodynamically stable HAP.

The objectives of this investigation were to: (i) examine the effects of naturally occurring organic acids on DCPD and OCP precipitation; (ii) determine mechanisms of inhibition by which these organic ligands (humic, fulvic, tannic, and citric acids) influence the rates of DCPD and OCP precipitation; (iii) examine the role of precipitation inhibition of Ca phosphates in soils and its effect on P availability to plants; and (iv) chemically characterize fulvic acid isolated from a water soluble extract of wheat straw.

CHAPTER 2

PRECIPITATION OF DICALCIUM PHOSPHATE DIHYDRATE IN THE PRESENCE OF ORGANIC ACIDS

Introduction

Dicalcium phosphate dihydrate (DCPD) is an important reaction product of P fertilizer applications to calcareous soils and has been recognized as a precursor in the formation of thermodynamically more stable hydroxyapatite (HAP). Although HAP is generally predicted to control PO_4 activity in soil solutions (Lindsay, 1979; Stumm and Morgan, 1981), equilibrium PO_4 levels are frequently regulated by DCPD and octacalcium phosphate (OCP), both metastable to HAP (Moreno et al., 1960; Fixen et al., 1983; Bell and Black, 1970). Since DCPD and OCP can persist and control PO_4 activity in soils for considerable lengths of time (Moreno et al., 1960; Bell and Black, 1970) and formation of HAP is relatively slow, precipitation kinetics may be paramount in determining PO_4 levels in soils.

Phosphate chemistry in soil environments may also be influenced by the presence of organic matter. Additions of organic amendments (sludge and manure) to calcareous soils reportedly increase PO_4 solubility over time more than lone additions of inorganic phosphate (O'Connor et al., 1986; Meek et al., 1979; Abbott and Tucker,

1973). Similarly, Moreno et al. (1960) found that soluble organic matter was responsible for the persistence of DCPD and the reduction in the rate of formation of OCP in calcareous soils. Inskeep and Silvertooth (1988a) discovered that HAP precipitation was inhibited by organic acids common to soil environments. These organic acids inhibited HAP precipitation rates by adsorbing onto crystal seeds that act as nuclei for new crystal growth. Organic constituents may inhibit formation of thermodynamically stable calcium phosphates, thereby maintaining higher PO_4 levels in soils.

To better understand control of PO_4 activity in soils, and since there have been no investigations on the direct effect of soil organic acids on DCPD precipitation rates, the objectives of this study were to: (i) examine the effect of several soil organic acids (humic, fulvic, tannic, and citric) on DCPD precipitation; and (ii) determine the mechanism of inhibition by these organic acids on DCPD precipitation rates.

Materials and Methods

Organic Acids

The water soluble organic acids used in the DCPD precipitation experiments consisted of a peat humic acid obtained from the International Humic Substances Society (IHSS), a fulvic acid isolated from a water soluble extract of wheat straw (WSE-FA) using methods described by Thurman and Malcolm (1981) and Leenheer (1981), and commercially purchased tannic and citric acids (J.T. Baker Chemical Company, Phillipsburg, NJ). Elemental analysis of the WSE-FA was performed by

Huffman Laboratories (Golden, CO; Table 1). Total acidity and carboxylic acid functional group content were determined using barium hydroxide and calcium acetate methods, respectively (Schnitzer, 1982). Stock solutions of the above organic acids containing 0.01 *M* C_{TS} (TS=total soluble) were prepared in distilled-deionized water not more than two days prior to conducting experiments and were stored at 5°C.

Table 1. Elemental composition and functional group content of IHSS peat humic acid and wheat straw extract fulvic acid.

Element/functional group	Fulvic	Humic
	g kg^{-1}	
C	550.0	565.0
O	357.0	354.0
H	52.0	41.0
N	8.5	37.1
S	4.6	7.0
P	<1.0	0.2
Ash	24.4	9.0
	mmol c g^{-1}	
COOH	5.5	4.2
∅−OH	0.7	4.6

Crystal Growth Experiments

The DCPD precipitation experiments were conducted in a flat-bottomed, water-jacketed 600 cm^3 vessel covered with a removable plexiglass lid containing entry ports for a stirrer, burette tip, pH and Ca ion electrodes, and N_2 gas bubbler.

The DCPD precipitation rates were measured at constant pH = 5.7 and temperature = 25°C using a seeded crystal growth method (Marshall and Nancollas, 1969). Starting supersaturated solutions consisted of 400 mL of 13.0 m*M* Ca_{TS}, 11.3 m*M*

PO_{4TS}. These solutions also contained 20 m*M* KCl as a background ionic strength commonly found in soil solutions. The level of initial supersaturation with respect to DCPD was 3.5 (ion activity product/solubility product (K_{sp}) where log $K_{sp} = -6.57$ (Bennett and Adams, 1976)). Solutions were stirred with a three-bladed polyethylene propeller driven by an overhead motor at 6.17 rev s^{-1}. Humidified N_2 gas was bubbled through solutions at 3.3 cm^3s^{-1} to purge CO_2(g). Solutions were brought to pH 5.7 by dropwise addition of 0.1 *M* HCl and 0.1 *M* KOH and were allowed to stabilize for 30 minutes prior to the introduction of 300 mg DCPD seed crystals. The DCPD seed crystals were prepared by aging Baker reagent grade DCPD under N_2(g) for four weeks. Seed crystals had a surface area (S) = 3.0 m^2g^{-1} as measured by a triple point N_2-Brunauer-Emmett-Teller adsorption isotherm. Total dissolution of three DCPD seed crystal subsamples resulted in a Ca/P ratio = 0.980 $\pm$ 0.005 (theoretical Ca/P for DCPD is 1.0). DCPD seed crystals were also characterized by X-ray diffraction and scanning electron microscopy (SEM). X-ray diffraction patterns displayed only those peaks characteristic of DCPD (Smith, 1967).

A pH-stat titrator maintained constant solution pH by addition of 0.4993 *M* KOH which was continuously recorded throughout all experiments. Five mL samples were periodically collected during each precipitation experiment, filtered through prewashed 0.2 micron filters (Gelman GA-8, Gelman Sciences, Inc., Ann Arbor, MI), and analyzed for Ca_{TS} by atomic absorption spectroscopy and PO_{4TS} using a modified Murphy-Riley colorimetric method (Harwood et al., 1969). Seed crystals were collected after several experiments and analyzed by X-ray diffraction and SEM to

confirm the formation of DCPD. Average stoichiometries for the change in Ca_{TS}, PO_{4TS}, and [OH^-] during precipitation were also close to values predicted from the overall stoichiometry for DCPD precipitation at pH 5.7 (Eq. [1]; Table 2).

Table 2. Experimental and predicted stoichiometric ratios based on DCPD precipitation at pH 5.7.

Element ratio	Experimental	Theoretical
Ca/P	1.01 ± 0.07†	1.00
Ca/OH	1.07 ± 0.03	1.06
P/OH	1.07 ± 0.06	1.06

† ± values are standard deviations (three experiments).

Experiments run in the presence of organic acids had C_{TS} levels ranging from 1.1 to 7.8 m*M* as fulvic acid, 0.26 to 2.05 m*M* as humic acid, 0.9 to 9.0 m*M* as tannic acid, and 0.5 to 8.0 m*M* as citric acid. Final C_{TS} values were measured using a C-analyzer (Dohrmann CD-80, Santa Clara, CA). Calcium ion activity was measured before and after each experiment with a Ca^{2+} ion electrode (Radiometer #f2112, Copenhagen, Denmark) to determine the degree of complexation of Ca^{2+} with organic acids. Experiments were also run at 13, 19, 25, and 32°C to calculate an Arrhenius activation energy (E_a) for DCPD precipitation.

Determination of Rate Constants

The overall stoichiometry predicted for the DCPD precipitation reaction at pH 5.7 and 25°C is:

$$Ca^{2+} + 0.94\ H_2PO_4^- + 0.06\ HPO_4^{2-} + 2H_2O = CaHPO_4 \cdot 2H_2O + 0.94H^+ \quad [1]$$

The forward rate constant (k_f) was determined using the second order rate law expression for the rate of formation of DCPD (Marshall and Nancollas, 1969),

$$\text{Rate} = \frac{-d[Ca^{2+}]}{dt} = \frac{d[DCPD]}{dt} = k_f S [Ca^{2+}][HPO_4^{2-}]\gamma_2^2 \qquad [2]$$

where brackets represent concentrations, γ_2 is the divalent ion activity coefficient determined from the Davies equation, and S is the surface area of DCPD seed crystals (2.28 m^2L^{-1}). The rate of precipitation was determined by monitoring $[Ca^{2+}]$ over time ($-d[Ca^{2+}]/dt$) which is equal to the DCPD precipitation rate, d[DCPD]/dt.

The stoichiometry of the precipitation reaction (Eq. [1]) can be used to relate the loss in soluble Ca to the loss in soluble PO_4 over time:

$$Ca_{TS_0} - Ca_{TS_x} = PO_{4TS_0} - PO_{4TS_x} \qquad [3]$$

where the subscripts 0 and x denote concentrations at time = 0, and concentrations at time = x. Eq. [3] can be solved for the concentration of HPO_4^{2-} utilizing distribution coefficients (α) for the Ca^{2+} and $H_2PO_4^-$ species:

$$[HPO_4^{2-}]_x = \alpha_{H_2PO_{4_x}^-} \left[\frac{[HPO_4^{2-}]_0}{\alpha_{H_2PO_{4_0}^-}} - e \left[\frac{[Ca^{2+}]_0}{\alpha_{Ca_0^{2+}}} - \frac{[Ca^{2+}]_x}{\alpha_{Ca_x^{2+}}} \right] \right] \qquad [4]$$

($e = \gamma_1 \cdot \gamma_2^{-1} \cdot 10^{-7.2} \cdot 10^{pH}$). Substituting Eq. [4] into Eq. [2] results in the expression:

$$\frac{d[Ca^{2+}]_x}{a[Ca^{2+}]_x^2 + b[Ca^{2+}]_x} = k_f S dt \qquad [5a]$$

where $a = \frac{\alpha_{H_2PO_{4_x}^-}}{\alpha_{Ca_x^{2+}}} \cdot e \cdot \gamma_2^2$, [5b]

$$b = \alpha_{H_2PO_{4_x}^-} \cdot \gamma_2^2 \left[\frac{[HPO_4^{2-}]_0}{\alpha_{H_2PO_{4_0}^-}} - e \frac{[Ca^{2+}]_0}{\alpha_{Ca_0^{2+}}} \right] \quad [5c]$$

which upon integration yields

$$\frac{1}{b} \ell n \frac{a[Ca^{2+}]_x}{a[Ca^{2+}]_x + b} = k_f St + C \quad [6]$$

where C = constant of integration.

The gamma and alpha terms used to derive "a" (Eq. [5a]) and "b" (Eq. [5b]) in Eq. [6] were calculated over the course of the reaction using the chemical equilibrium model GEOCHEM (Sposito and Mattigod, 1979; Parker et al., 1987) from analytically determined Ca_{TS}, PO_{4TS}, K_{TS}, and Cl_{TS} input values and thermodynamic constants listed in Inskeep and Silvertooth (1988b). In a typical precipitation experiment at 25°C in the absence of organic acids, ionic strength declined from 0.07 to 0.06 (approximately 14%). This results in small changes in γ_1 from 0.80 to 0.81 and γ_2 from 0.41 to 0.43 (1 and 4% changes, respectively). Changes in γ_1, γ_2, and the distribution coefficients used in Eq. [5b] and [5c] as a function of time resulted in slight changes in a and b (< 10%). Although these changes are relatively minor, a and b were calculated over the course of the reaction to reflect changes in solution composition.

Calcium concentrations were calculated using the relationship

$$[Ca^{2+}]_x = [Ca^{2+}]_0 - 1.06 \left([OH^-]_x - [OH^-]_0 \right) \quad [7]$$

based on the overall stoichiometry for DCPD precipitation at pH 5.7 (Eq. [1]), where $[Ca]_o$ = initial free Ca^{2+} concentration at time = 0, calculated by GEOCHEM. This method allowed for the determination of an essentially endless number of Ca^{2+} data points (we used 15 to 25) obtained from continuous plots of OH^- vs. time, compared to only 4 to 8 Ca^{2+} values actually measured from subsamples withdrawn from the reaction vessel. Values calculated using Eq. [7] differed by an average of only 7% from those determined by GEOCHEM (values obtained from three experiments). Further justification for using Eq. [7] was that Ca/OH ratios determined experimentally were consistent with the ratio predicted from the overall stoichiometry of DCPD precipitation (Eq. [1]; Table 2).

Forward rate constants were determined from the slopes of integral vs. time plots (Eq. [6]). For example, the initial 14 to 16 points representing the first 12 to 20 minutes of DCPD precipitation were used to calculate k_f for experiments run in the absence of organic acids at 25°C. During this time, the majority of DCPD precipitation had occurred, as indicated by the loss of Ca_{TS} and PO_{4TS} and the addition of $[OH^-]$ (Figure 1).

Carbon Adsorption

Adsorption data for tannic and citric acids were determined by measuring C_{TS} on samples collected at the end of precipitation experiments using a C-analyzer. A separate C adsorption study was established using the humic and fulvic acids to confirm their adsorption onto DCPD crystal seed surfaces. These experiments were

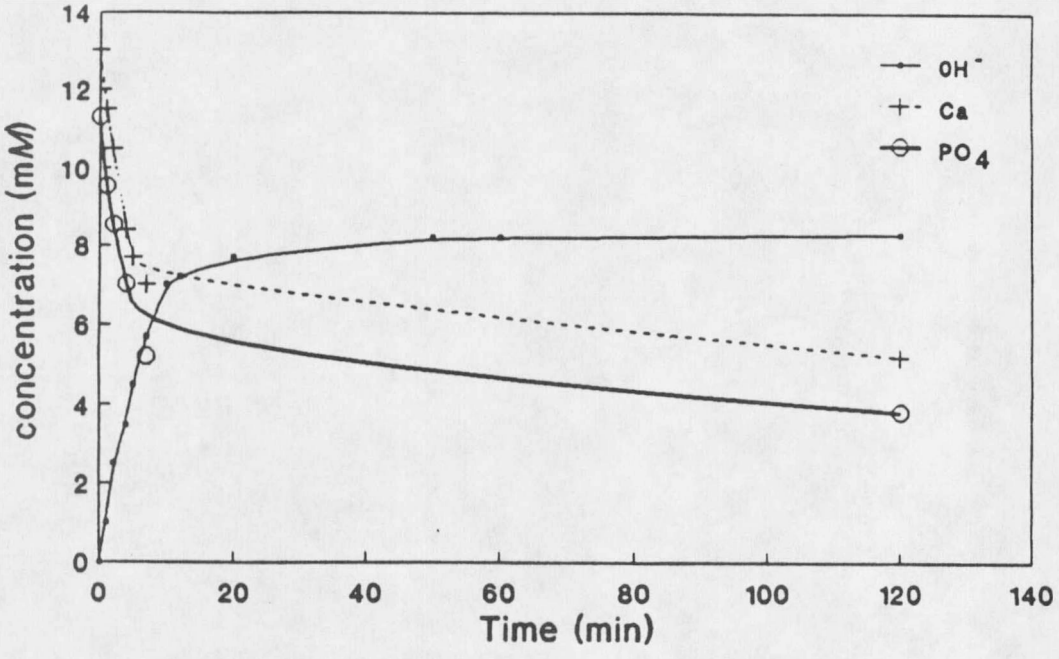


Figure 1. Total soluble concentrations of Ca and PO_4 and addition of OH^- as a function of time during the precipitation of DCPD in the absence of organic acids.

performed in a water-jacketed 400 cm^3 flat-bottomed vessel using the same system described for DCPD crystal growth experiments. Treatment solutions consisting of 250 ml of 0, 0.25, 0.5, 1.0, 2.0, and 5.0 m*M* C as fulvic and humic acids in the presence of 20 m*M* KCl were brought to pH 5.7 and allowed to stabilize for 30 minutes. An aliquot (50 mL) of treatment solution was collected for C analysis prior to the addition of 170 mg of DCPD seed crystals. After addition of seed crystals, a pH-stat titrator maintained constant pH by adding 0.5 *M* HCl to the solutions. Experiments were allowed to proceed for 2 hours; however, equilibrium was achieved within 30 minutes. At the end of each experiment, solutions were collected and centrifuged at 13,170 g for 30 minutes for C and Ca analysis. DCPD seed crystals were also collected by filtration (0.2 micron Gelman GA-8 filters) to further verify organic acid adsorption onto DCPD seed crystals by X-ray photoelectron spectroscopy (XPS). Carbon analysis was performed by measuring UV absorbance at 230 nm (Inskeep and Silvertooth, 1988a). The amount of C adsorbed was determined by difference between C values measured before and after seed inoculation.

Results and Discussion

The Arrhenius activation energy (E_a) for DCPD precipitation was calculated from the slope of the linear ($r^2 = 0.98$) plot of $\ell n\ k_f$ (Eq. [6]) vs. 1/T (K) for experiments run at 13, 19, 25, and 32°C. The k_f values ranged from 2.6 $L^2mol^{-1}m^{-2}s^{-1}$ at 13°C to 14.06 $L^2mol^{-1}m^{-2}s^{-1}$ at 32°C. The calculated E_a for DCPD precipitation at pH 5.7 was 64.4 kJ mol^{-1}, greater than E_a values reported for diffusion controlled

processes (16 to 20 kJ mol^{-1}), and indicative of a surface controlled precipitation mechanism (Lasaga, 1981; Marshall and Nancollas, 1969).

Experiments run at all C_{TS} levels of humic acid and from 1.1 to 3.3 m*M* C_{TS} as fulvic acid were characterized by an induction period prior to DCPD precipitation. For example, recorder output for DCPD precipitation experiments measured in the presence of 0.5 m*M* C as humic acid (Figure 2) displayed an induction period lasting about 60 minutes, succeeded by a rapid increase in the rate of DCPD precipitation. No induction periods were noted in experiments conducted in the absence of organic acids. Induction periods were longer in the presence of humic acid than in fulvic acid, ranging from 2.0 to 632 minutes and 1.5 to 10 minutes for respective additions of 0.26 and 2.05 m*M* C_{TS} as humic acid, and 1.1 to 3.3 m*M* C_{TS} as fulvic acid (Table 3). The rate of DCPD precipitation during the induction periods was characterized by calculating forward rate constants (Eq. [6]) that decreased from 4.0 to 0.02 with additions of humic acid (0.26 to 2.0 m*M* C_{TS}), and from 2.0 to 0.5 with fulvic acid (1.1 to 3.3 m*M* C_{TS} (Table 3)).

Similar induction periods have been reported for precipitation of calcium sulfate dihydrate in the presence of inorganic and organic additives (Sarig and Mullin, 1982; Liu and Nancollas, 1975). Liu and Nancollas (1975) suggested that the occurrence of induction periods followed by rapid crystal growth is the result of incorporation of the additives into the growing crystals. They suggested that the most active crystal growth sites are "poisoned" by adsorption of added foreign molecules; however, some lower energy sites remain available for crystal growth to proceed at a very slow rate. After

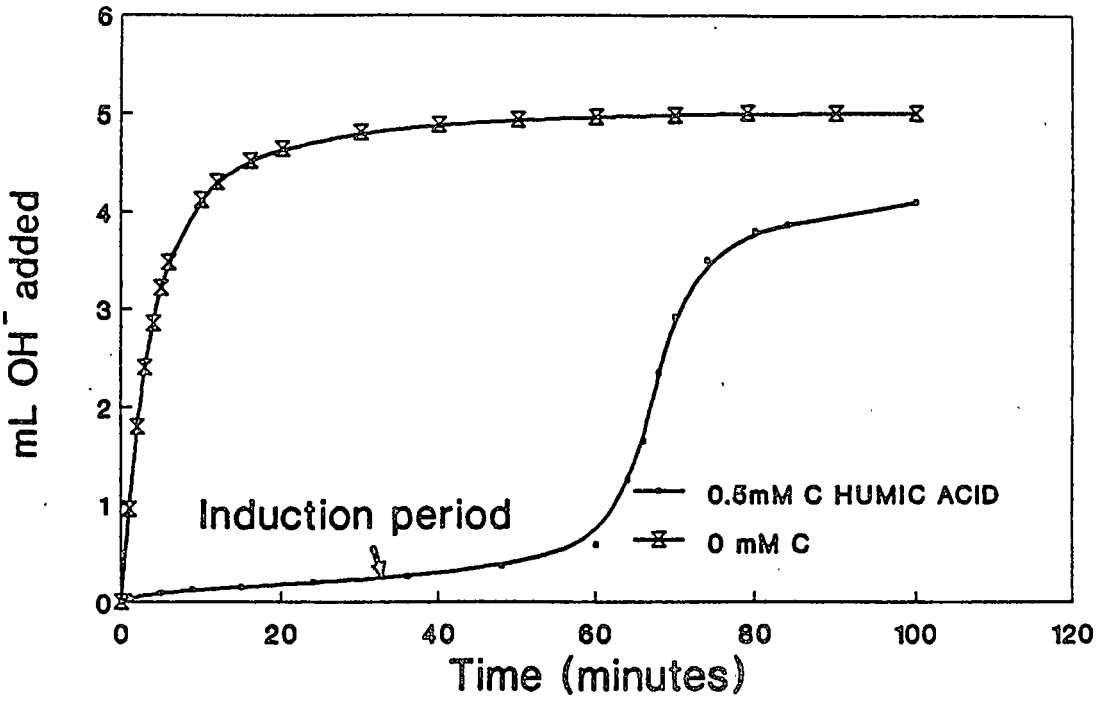


Figure 2. Volume of KOH added to maintain constant pH at 5.7 during DCPD precipitation experiments in the absence and presence of 0.5 m*M* C_{TS} as humic acid.

a period of slow precipitation, the adsorbed foreign molecules become incorporated into the growing crystals and precipitation resumes at a rate similar to systems run in the absence of additives (Liu and Nancollas, 1975).

Table 3. Induction times and forward rate constants (k_f) during induction for precipitation of DCPD in the presence of organic acids.

Organic acid	Initial C_{TS}	Induction time	k_f during induction period
	(m*M*)	(sec)	($L^2mol^{-1}m^{-2}s^{-1}$)
Humic	0.26	120	4.00
	0.51	600	0.48
	0.77	3720	0.08
	1.02	6480	0.07
	2.05	37920	0.02
Fulvic	1.10	90	2.00
	2.20	180	1.70
	3.30	600	0.51
	5.60	n.i.†	--
	7.80	n.i.	--
Tannic	0.9 - 9.0	n.i.	--
Citric	0.5 - 8.0	n.i.	--

† n.i. = no induction period.

DCPD seed crystals were collected during and after induction in an experiment with 0.7 m*M* C as humic acid. SEM micrographs of samples collected during the induction period reveal poorly defined DCPD crystal structure (Figure 3). Seed crystals collected after induction reveal well defined DCPD platelets growing from coated DCPD seed crystals (Figure 4). These observations are consistent with measured precipitation rates before and after induction and we hypothesize that DCPD

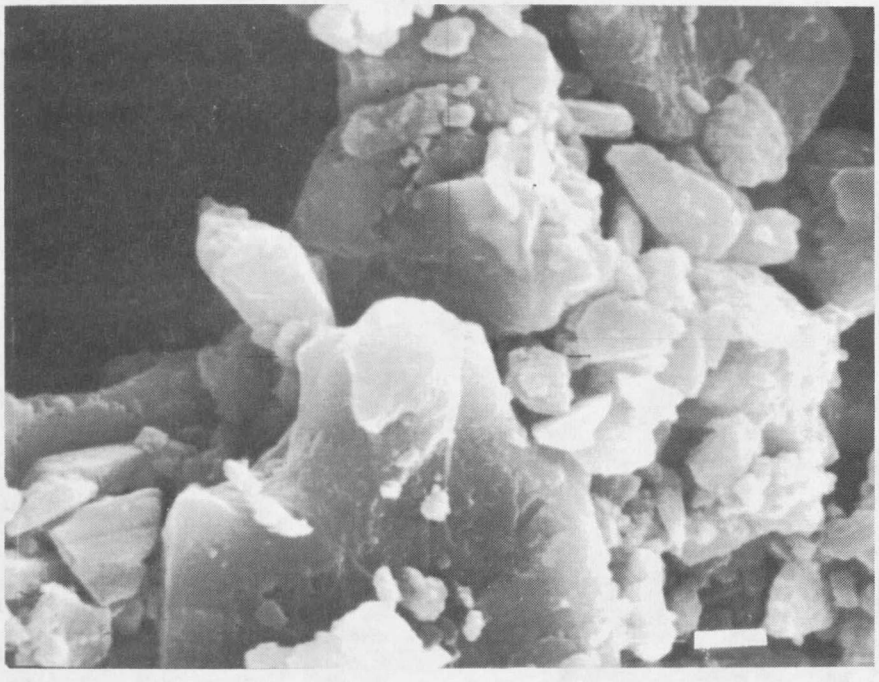


Figure 3. SEM micrograph of DCPD crystals collected during induction period for DCPD precipitation in the presence of 0.7 m*M* C_{TS} as humic acid (bar = 1 micron).

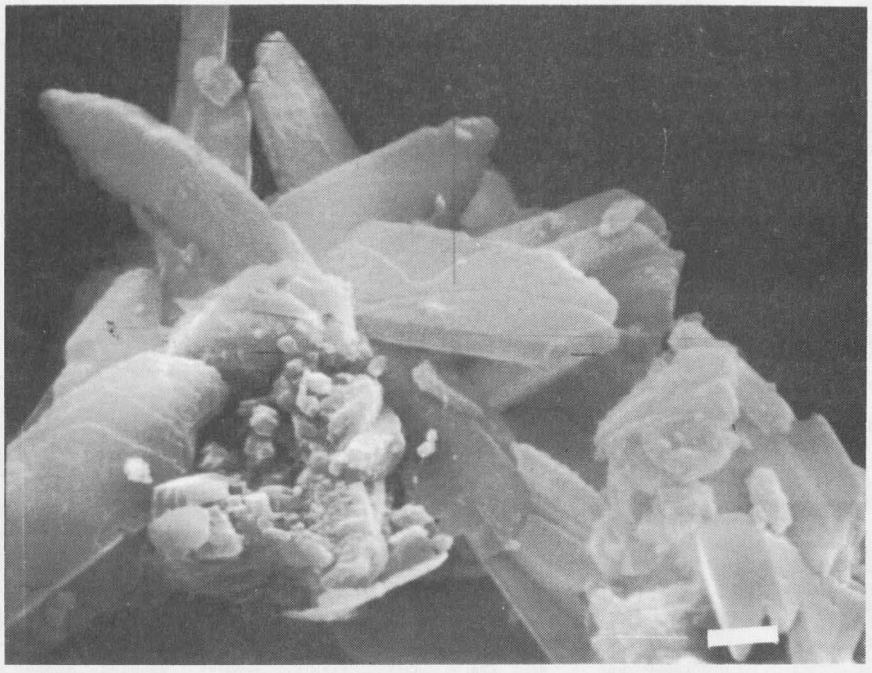


Figure 4. SEM micrograph of DCPD crystals collected at the end of DCPD precipitation experiment in the presence of 0.7 m*M* C_{TS} as humic acid (bar = 1 micron).

precipitation has the ability to overgrow adsorbed humic and fulvic acids, incorporating these into the solid phase.

Forward rate constants (k_f) were calculated using Eq. [6] to quantify DCPD precipitation rates after induction periods. The k_f for DCPD precipitation in the absence of organic acids at pH 5.7 and 25°C was 9.7 $\pm$ 0.5 $L^2mol^{-1}m^{-2}s^{-1}$ (average of three experiments, Table 4). Inhibition of DCPD precipitation by organic acids was determined by comparing k_f values for precipitation experiments run in the presence of organic acids to the k_f determined in their absence (Table 4). Crystal growth was inhibited by all organic acids used in this study, as indicated by the decrease in k_f values with increasing amounts of soluble C (Table 4).

Humic and citric acids were the most effective inhibitors of DCPD precipitation per mole of soluble C added where 2 m*M* C_{TS} additions of both acids inhibited crystal growth by 89%. Additions of 3.3 m*M* total soluble C as fulvic acid and 9.0 m*M* C as tannic acid inhibited crystal growth by 86 and 78%, respectively (Figure 5; Table 4). Experiments in the presence of humic acid were not conducted beyond 2 m*M* C_{TS} since greater C_{TS} levels would have resulted in induction periods exceeding 10 hours. The most complete inhibition was demonstrated by fulvic acid, which inhibited DCPD precipitation by 99% at 7.8 m*M* C_{TS} (Figure 5).

Mechanism of DCPD Crystal Growth Inhibition

Previous research on the precipitation kinetics of HAP in the presence of organic additives has found that precipitation was inhibited by C adsorption onto seed crystal surfaces blocking sites for new crystal growth (Inskeep and Silvertooth, 1988a; Aoba

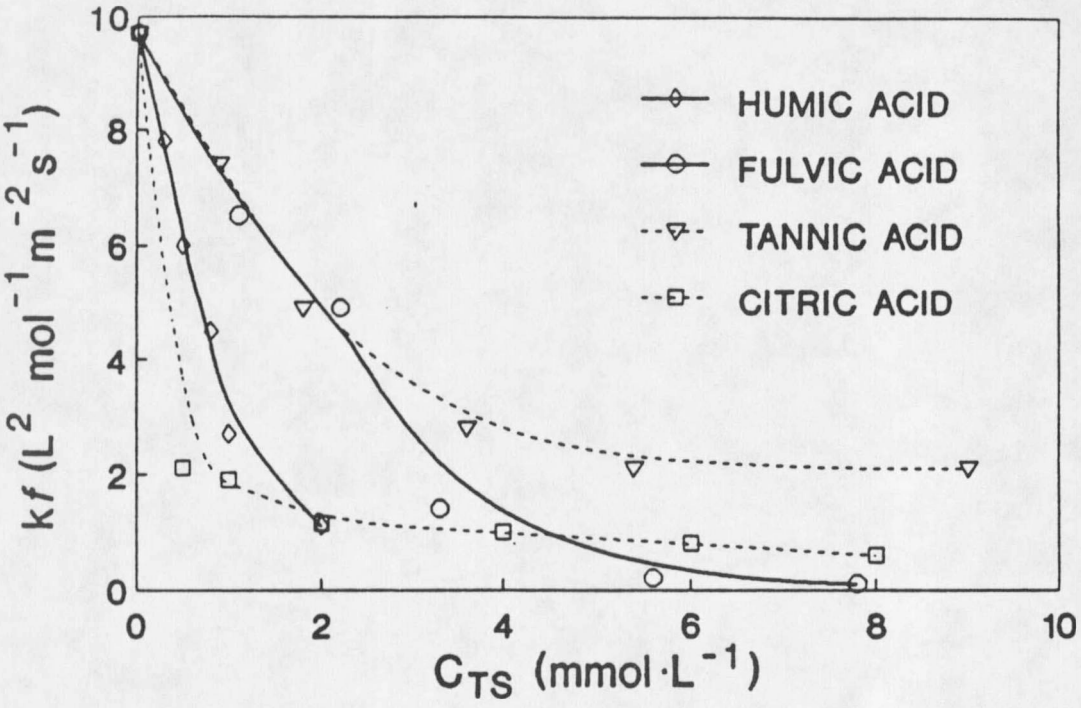


Figure 5. Decrease in forward rate constants with additions of C_{TS} as humic, fulvic, tannic, and citric acids.

and Moreno, 1985). In the current study, adsorption of C onto DCPD seed crystals was demonstrated in the presence of humic, fulvic, tannic, and citric acids (Figure 6) and confirmed by X-ray photoelectron spectroscopy (XPS) surface analysis of DCPD crystals (data not shown).

Table 4. Functional group content, k_f ($L^2mol^{-1}m^{-2}s^{-1}$), and percent of inhibition of DCPD precipitation in the presence of organic acids.

Organic acid	Initial C_{TS}	Total COOH functional groups	Total O functional groups	k_f	% Inhibition of DCPD precipitation
	- - - - - - - - - mmol L^{-1} - - - - - - - - -			$L^2mol^{-1}m^{-2}s^{-1}$	%
None	--	--	--	9.7 ± 0.05	--
Humic	0.26	0.023	0.048	7.8	20
	0.51	0.045	0.100	6.0	38
	0.80	0.069	0.140	4.5	54
	1.02	0.091	0.190	2.7	72
	2.05	0.150	0.380	1.1	89
Fulvic	1.10	0.130	0.150	6.5	33
	2.20	0.260	0.300	4.9	49
	3.30	0.390	0.450	1.4	86
	5.60	0.670	0.750	0.2	98
	7.80	0.930	1.050	0.1	99
Tannic	0.09	0.120	0.410	7.4	24
	1.80	0.240	0.830	4.9	49
	3.60	0.470	1.660	2.8	71
	5.40	0.710	2.480	2.1	78
	9.00	1.180	4.140	2.1	78
Citric	0.50	0.250	0.330	2.1	78
	1.00	0.500	0.670	1.9	80
	2.00	1.000	1.330	1.1	89
	4.00	2.000	2.670	1.0	90
	6.00	3.000	4.000	0.8	92
	8.00	4.000	5.330	0.6	94

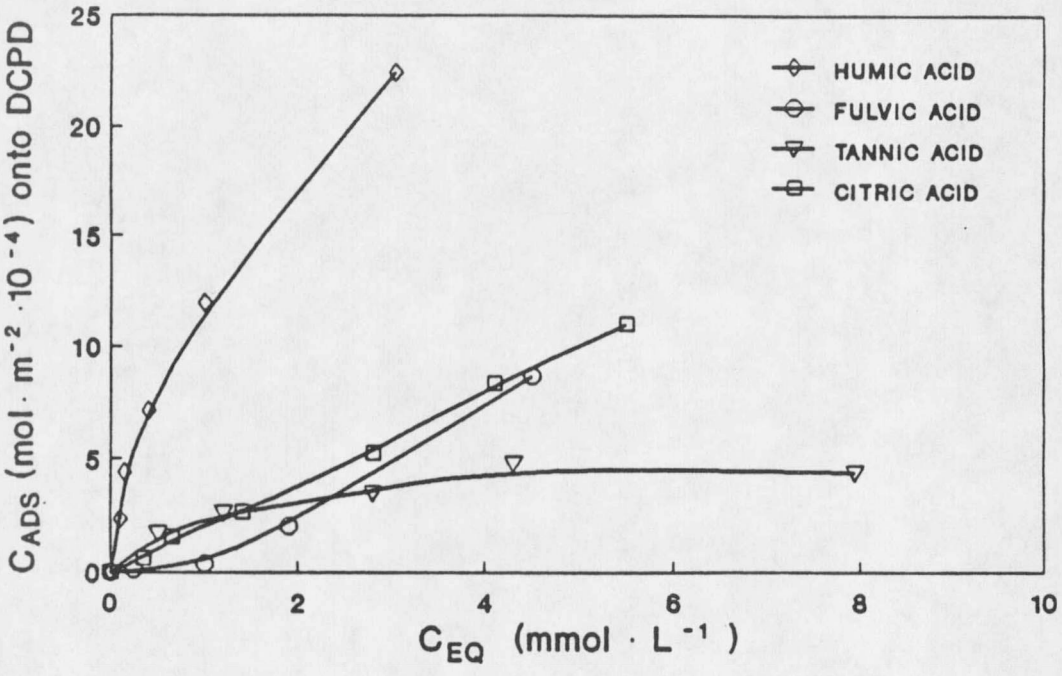


Figure 6. C adsorbed as humic, fulvic, tannic, and citric acids onto DCPD seed surfaces as a function of equilibrium levels.

The relative efficiencies of these organic acids can be described in terms of their ability to inhibit DCPD precipitation per mole C_{TS} added. The order of efficiency of these organic acids, as reported above, was citric > humic > fulvic ≃ tannic (Figure 5). However, since inhibition of DCPD crystal growth is related to adsorption phenomena, then the total amount of C adsorbed and the degree of surface coverage of the adsorbate on the DCPD surface are important factors in determining the level of inhibition. These factors are related to the functional group content of the organic acids and their geometry (Inskeep and Silvertooth, 1988a; Kresak et al., 1977; Meyer and Nancollas, 1973). At pH 5.7, the majority of COOH functional groups should be dissociated and the adsorption of organic acids is probably due to COO^- binding with Ca^{2+} present at kinks and dislocations on DCPD surfaces (Inskeep and Silvertooth, 1988a; Kresak et al., 1977; Meyer and Nancollas, 1973).

The amount of C adsorbed onto DCPD as humic acid per equilibrium C (C_{eq}) far exceeded that of the other organic acids (Figure 6), which explains why it was so efficient at inhibiting DCPD precipitation per mole C_{TS} added. Although citric acid was the most effective at inhibiting DCPD growth per mole C_{TS} (Figure 5), it was adsorbed onto DCPD to about the same extent as fulvic and tannic acids. Therefore, some factor other than the total amount of C adsorbed must also be involved in the adsorption mechanism leading to DCPD precipitation inhibition. It is proposed that the efficiency of these organics is also related to the geometry of the molecule on the DCPD crystal surface, specifically whether the molecule lays flat or perpendicular to the crystal surface. If all of the organic acids had identical geometric configurations

at DCPD surfaces, the plot of k_f ($L^2mol^{-1}m^{-2}s^{-1}$) versus C adsorbed (mol m^{-2}) (Figure 7) should be constant for all molecules. This is not the case. Instead, the order of efficiency per mole C adsorbed is citric > fulvic = tannic > humic (Figure 7). Citric acid probably provides the best surface coverage and subsequent blockage of available growth sites per mole C adsorbed since it is smaller than the other organic acids and has the highest COOH content per mole C (0.5:1), allowing more surface binding per unit C. Conversely, surface coverage was probably not as great for humic acid per unit C adsorbed. It is a much larger molecule and has the lowest COOH content per mole C (0.09:1). Therefore, much of the adsorbed molecule may lie perpendicular to the DCPD surface and would not be as effective at blocking available crystal growth sites.

Complexation of soluble Ca^{2+} by organic ligands can also reduce precipitation rates by lowering the free Ca^{2+} concentration in solution (Eq. [2]). When the formation constants for Ca-ligand complexes are known, the effect of complexation is accounted for directly by calculating the amount of free Ca^{2+} available for precipitation (Eq. [2]). For example, the complexation of Ca with citrate was determined with GEOCHEM using formation constants for H-citrate and Ca-citrate obtained from Smith and Martell (1976). Values for $[Ca^{2+}]_0$ used to solve the rate equation reflect the amount of Ca complexed at time zero. For the citric acid experiments, the total soluble Ca complexed by citrate species ranged from 0.6 to 9.5%. However, when Ca formation constants are not known, as is the case for humic, fulvic, and tannic acids, the calculated rate constants may reflect contributions from both the reduction

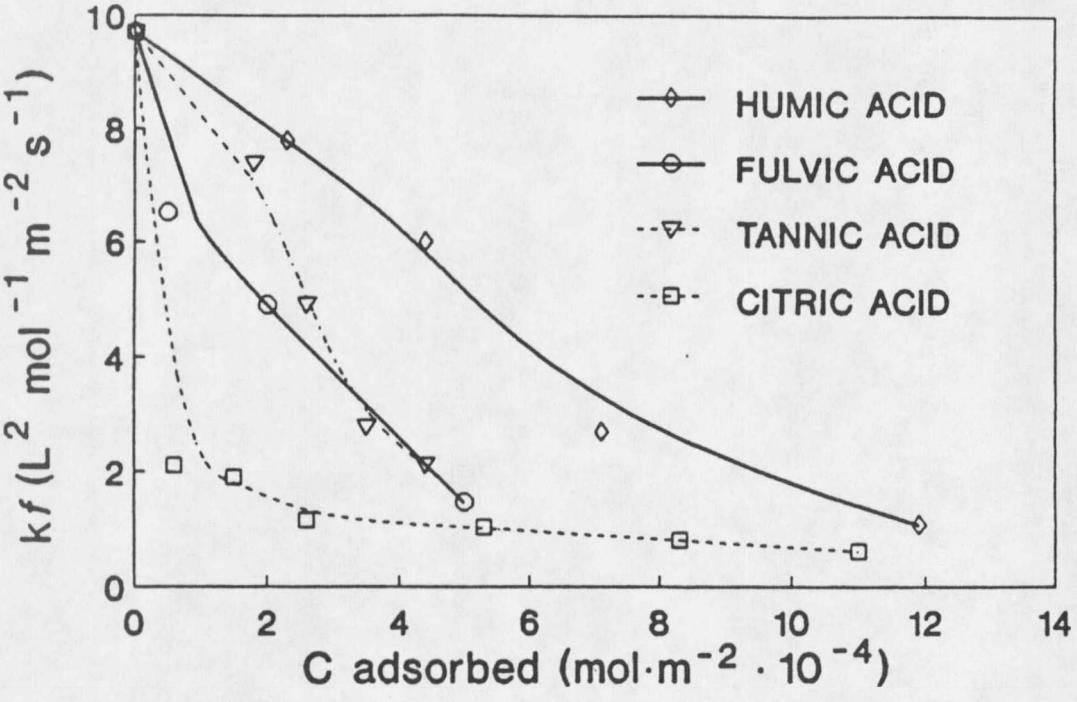


Figure 7. Decrease in forward rate constants with increasing levels of C adsorbed onto DCPD crystal seed surfaces in the presence of humic, fulvic, tannic, and citric acids.

in free Ca^{2+} and the inhibition due to adsorption of C constituents onto seed crystals. Estimations of the amount of Ca complexed by humic, fulvic, and tannic acids must be used for determining the relative contribution of these two mechanisms to the decrease in forward rate constants. At the highest level of humic acid (2 m*M* C_{TS}), only 6.0% of the total soluble Ca was complexed as determined by Ca ion electrode measurements. This does not account for the amount of inhibition (89%) observed in the presence of 2 m*M* C as humic acid, nor does it explain the induction period that occurred prior to DCPD precipitation for the same treatment. Calcium ion electrode measurements were unstable in the presence of tannic and fulvic acids and therefore were not used to calculate Ca^{2+} activities. However, even at the highest C_{TS} levels (assuming that all COOH functional groups complexed Ca), tannic acid would complex about 4.5% of the soluble Ca^{2+} and fulvic acid would complex about 3.5%. This would account for only 3.5% of the inhibition of DCPD crystal growth by both. In summary, Ca complexation with these organic acids played only a minor role in inhibiting DCPD crystal growth.

Conclusions

Humic, fulvic, tannic, and citric acids all inhibited the rate of DCPD crystal growth. These organic acids inhibited DCPD precipitation by adsorbing onto DCPD surfaces, thus blocking sites for new crystal growth. The amount of C adsorbed onto DCPD and its efficiency at inhibiting DCPD precipitation was related to the functional group content and size of the organic acids. Precipitation of DCPD overgrew humic

and fulvic acids adsorbed onto DCPD crystal seeds after an induction period which was proportional to the amount of C adsorbed. Precipitation kinetics of HAP (Inskeep and Silvertooth, 1988a) and OCP (Grossl and Inskeep, 1989) in the presence of similar organic acids have not demonstrated overgrowth of adsorbed C onto their respective crystal surfaces. The ability of DCPD precipitation to overgrow humic and fulvic acids (present at higher C_{TS} levels and adsorbed onto smaller surface area DCPD seed crystals than conditions for HAP and OCP experiments) may explain the formation of DCPD and subsequent metastability with respect to OCP and HAP in soils receiving additions of fertilizer P.

CHAPTER 3

KINETICS OF OCTACALCIUM PHOSPHATE CRYSTAL GROWTH IN THE PRESENCE OF ORGANIC ACIDS

Introduction

Precipitation of Ca phosphates plays an important role in the fate of phosphate applied as P fertilizers in soils (Sample et al., 1980), in the mineralization of bones and teeth (Tung and Brown, 1983; Nancollas, 1979), and in the formation of scale deposits on heat transfer surfaces restricting the use of evaporative techniques in desalination technology (Nancollas, 1979).

In geochemical environments, octacalcium phosphate (OCP) has been recognized both as an important reaction product of P fertilizer addition to calcareous soils, and as a precursor solid phase in the formation of thermodynamically more stable hydroxyapatite (HAP). It is an intermediate Ca phosphate phase in the formation sequence proceeding from dicalcium phosphate dihydrate (DCPD) to HAP. Generally, HAP is expected to control PO_4 activity in soil solutions and natural waters (Lindsay, 1979; Stumm and Morgan, 1981); however, PO_4 equilibrium levels are frequently supersaturated with respect to HAP and appear to be regulated by more soluble solid phases, such as OCP (Fixen et al., 1983; O'Connor et al., 1986; Bell and Black,

1970). The fact that OCP can persist and control PO_4 levels for considerable lengths of time, and that HAP formation is relatively slow, suggests that precipitation kinetics play a major role in determining PO_4 levels in soil solutions, sediments, and natural waters.

The presence of organic matter in soils may influence dissolved phosphate concentrations. Numerous studies have demonstrated that the addition of organic amendments, such as sewage sludge and manure, to calcareous soils results in higher aqueous PO_4 concentrations than lone additions of inorganic phosphate (O'Connor et al., 1986; Meek et al., 1979; Abbott and Tucker, 1973). Organic acids commonly found in soil solutions have been shown to inhibit the precipitation of DCPD (Grossl and Inskeep, 1991) and HAP (Inskeep and Silvertooth, 1988a). The probable mechanism of inhibition is adsorption of organic acids onto crystal surfaces, which block sites acting as nuclei for new crystal growth. The capability of organic acids to inhibit crystal growth varies with both the structure and geometry of the organic acids (Meyer and Nancollas, 1973; Grossl and Inskeep, 1991) and the precipitating phase. For example, precipitation rates of HAP and calcite are strongly inhibited by low levels (2.8 to 437 u*M*) of dissolved organic C (DOC) present as humic and fulvic acids (Inskeep and Bloom, 1986; Inskeep and Silvertooth, 1988a). However, much higher levels of DOC (0.26 to 7.8 m*M*) are necessary to show inhibition of DCPD precipitation (Grossl and Inskeep, 1991). In addition, DCPD and other more soluble solid phases, such as gypsum, can overgrow adsorbed organic constituents during crystal growth (Grossl and Inskeep, 1991; Cody and Cody, 1988). In natural environments,

potential inhibitors of crystal growth including cations and inorganic and organic anions are ubiquitous. Consequently, the inhibition of crystal growth is an important mechanism in the metastability of aqueous systems with respect to more thermodynamically stable solid phases.

The objectives of this investigation were to: (i) examine the effect of humic, fulvic, citric, and tannic acids on OCP precipitation using a constant composition seeded crystal growth method (Heughebaert et al., 1986; Heughebaert and Nancollas, 1984); and (ii) establish the mechanism of inhibition by which these organic acids influence the rate of OCP crystal growth. An understanding of the relative rates and the level of inhibition for crystal growth of DCPD, OCP, and HAP may help explain the metastability of many natural waters with respect to thermodynamically stable Ca phosphate minerals such as HAP.

Materials and Methods

Octacalcium phosphate (OCP) precipitation experiments were conducted in a flat-bottomed, water-jacketed 400 cm^3 vessel covered with a removable plexiglass lid containing entry ports for a stirrer, burette tips, pH and Ca ion electrodes, and an N_2 gas bubbler. OCP precipitation rates were measured at constant pH = 6.0 and temperature = 25 $\pm$ 0.1 °C using a seeded crystal growth constant composition method (Heughebaert and Nancollas, 1984; Heughebaert et al., 1986). Starting reaction solutions were prepared from analytical reagent grade chemicals (J.T. Baker Chemical Company, Phillipsburg, NJ) in deionized-distilled water. They consisted of 4.4 m*M*

total soluble (TS) Ca and 3.3 m*M* PO_{4TS} made from $CaCl_2 \cdot 2H_20$ and KH_2PO_4, respectively. They also contained 0.01 M KCl as a background electrolyte to simulate ionic strengths commonly encountered in natural waters. Activities of ionic species in the supersaturated solutions were calculated using total soluble (TS) concentration data as input to GEOCHEM, a chemical speciation program (Sposito and Mattigod, 1979; Parker et al., 1987), and thermodynamic constants listed in Inskeep and Silvertooth (1988b). Starting reaction solutions were supersaturated with respect to OCP and just undersaturated with respect to DCPD. The degree of supersaturation (DS), defined here as IAP/Ksp (where IAP is the ion activity product equal to $(Ca^{2+})^4(PO_4^{3-})^3(H^+)$ and Ksp is the solubility product for OCP at 25°C equal to 2.5 x 10^{-50} $(mol\ L^{-1})^8$ (Heughebaert et al., 1986)), was $10^{2.47}$ for all experiments except those designed to vary DS.

Solutions were stirred with a three-bladed polyethylene propeller driven by an overhead motor at 6.17 revolutions s^{-1}. Humidified N_2 gas was bubbled (200 mL min^{-1}) continuously through the reaction solutions to purge CO_2. Starting solutions were brought to pH 6.0 by dropwise addition of 0.1 *M* KOH and allowed to stabilize for at least 30 minutes prior to inoculation with 50 mg OCP seed crystals, thereby initiating OCP precipitation. OCP seed crystals used for these experiments were prepared by precipitation of OCP on seed crystals donated by Dr. M.S Tung (National Bureau of Standards, Washington, D.C.) using the seeded crystal growth constant composition method at 37°C. Seed crystals had a surface area = 14.4 m^2g^{-1} as determined by a triple point N_2-Brunauer-Emmett-Teller adsorption isotherm. Total

dissolution of three OCP subsamples revealed a Ca/P ratio of 1.34 $\pm$ 0.02 (theoretical ratio = 1.33). X-ray powder diffraction patterns for seed crystals exhibited only those peaks characteristic of OCP (Lehr et al., 1967).

A constant composition method was used to maintain constant pH, Ca, and PO_4 during precipitation (Heughebaert and Nancollas, 1984; Heughebaert et al., 1986). This method utilizes mechanically coupled buret controlled by a pH-stat titrator (System-1, Metrohm Brinkman Instruments, Westbury, NY) which simultaneously add titrant solutions to offset changes in H^+, Ca and PO_4 concentrations during OCP precipitation. Titrant solution concentrations in one buret contained 12.0 m*M* Ca_{TS} and 20 m*M* KCl; the other buret contained 9.0 m*M* PO_{4TS} and 13.0 m*M* KOH (Ca/P ratio of titrant solutions = 1.33). The titrant volume added to the reaction vessel during each experiment was monitored and recorded, and used as a direct measure of OCP precipitation rates. The recorder outputs for several representative experiments are displayed in Figure 8, where mLs of titrant added (one buret) are plotted as a function of time. The relationship between mLs titrant added versus time was linear ($r^2 > 0.97$), as determined by evaluating 10 to 20 data points selected from the chart output for each experiment.

Five mL samples were periodically collected during each precipitation experiment, filtered through prewashed 0.2 micron filters (Gelman GA-8, Gelman Sciences, Inc., Ann Arbor, MI) and analyzed for Ca_{TS} by atomic absorption spectroscopy and PO_{4TS} using a modified Murphy-Riley colormetric method (Harwood et al., 1969). Analysis of these samples indicated that OCP was the only precipitating phase (Ca/P =1.33) and

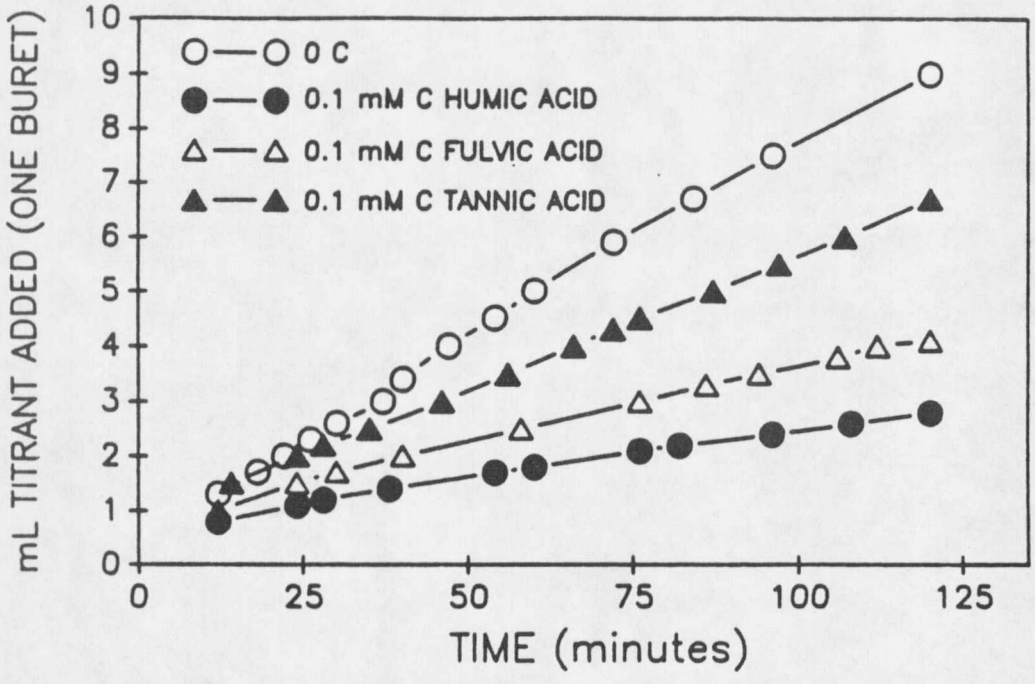


Figure 8. Recorder output for octacalcium phosphate precipitation experiments, showing mLs of titrant added (one buret) as a function of time.

that the chemical composition of reaction solutions remained constant during the course of each experiment. Specifically, average Ca_{TS} and PO_{4TS} levels of 65 samples collected during OCP precipitation experiments were 4.49 ± 0.05 m*M* and 3.32 ± 0.13 m*M*, respectively. Seed crystals were collected after several experiments and analyzed by X-ray diffraction confirming the formation of OCP. Experiments were also run at 20, 25, 30 and 35°C to determine an apparent Arrhenius activation energy (E_a) for OCP precipitation. Rate constants and an overall rate order for OCP precipitation were calculated using the following rate expression:

$$\text{Rate} = d[\text{OCP}]/dt = kS(IAP^{1/8} - Ksp^{1/8})^n \qquad [1]$$

where k is the rate constant ($L^7m^{-2}mol^{-6}s^{-1}$), S is the OCP seed crystal surface area (m^2L^{-1}), and n the overall reaction order (Heughebaert et al., 1986). To calculate the reaction order, it was necessary to conduct experiments at different supersaturation levels with Ca_{TS} = 3.7, 4.0, 4.4, and 5.0 m*M* (Ca/PO_{4TS} ratio kept constant at 1.33), resulting in respective DS values of $10^{2.05}$, $10^{2.22}$, $10^{2.47}$, and $10^{2.78}$.

Organic Acid Experiments

The organic acids used for OCP precipitation experiments included a peat humic acid obtained from the International Humic Substances Society (IHSS), a fulvic acid isolated from a water soluble extract of wheat straw (WSE-FA) (Inskeep and Grossl, 1989), and commercially obtained tannic and citric acids (J.T. Baker reagent grade). Stock solutions of these organic acids were prepared (0.01 *M* C_{TS}) in distilled-deionized water not more than two days prior to conducting experiments and stored at 5°C. The elemental composition of the WSE-FA was determined by Huffman

Laboratories (Golden, CO), and total acidity and carboxylic acid content of the humic acid and WSE-FA were measured using methods described by Schnitzer (1982) (Table 1, Chapter 2). Experiments run in the presence of organic acids had C_{TS} ranging from 0.02 to 2.0 m*M* as humic acid, 0.03 to 2.0 m*M* as fulvic acid, 0.1 to 2.0 m*M* as tannic acid, and 0.03 to 1.0 m*M* as citric acid. Calcium ion activity was measured at the start of each experiment with a Ca^{2+} ion electrode (Radiometer #f2112, Copenhagen, Denmark) to determine the degree of complexation of Ca^{2+} with organic acids.

Adsorption of humic and tannic acids onto OCP seed crystals was determined from samples collected at the end of precipitation experiments using UV absorbance at 230 nm to measure final equilibrium C (Inskeep and Silvertooth, 1988a). The amount of C adsorbed (C_{ADS}) as humic and fulvic acids onto OCP crystals was determined by the difference between C values measured before and after seed inoculation.

Adsorption of citric and tannic acids onto OCP was measured in a separate experiment conducted in a 400 cm^3 water-jacketed vessel using the same system described for OCP crystal growth experiments. Prior to the addition of citric and tannic acids, 70 mg of OCP seed crystals were added to 150 mL of 0.01 *M* KCl and allowed to equilibrate for at least 18 h. Solution pH was maintained at 6.0 by the addition of 0.04 *M* HCl. Carbon was then added every 50 min from stock solutions containing 0.1 *M* C as tannic and citric acids (total volume added did not exceed 8 mL). Total soluble C levels ranged from 0.5 m*M* to 5.0 m*M*. Before each C addition a 3 mL aliquot was removed, filtered through 0.2 micron filters (Gelman GA-8),

and analyzed for C using a C-analyzer (Dohrmann CD-80, Santa Clara, CA). Total soluble Ca was also measured on samples collected prior to addition of C treatments to determine the amount of OCP dissolution which occurred during the adsorption experiment. All volume additions and removals were recorded throughout experiments in order to establish the moles of C at equilibrium (C_{EQ}) in the reaction vessel.

The following linear form of the Langmuir adsorption isotherm was used to describe adsorption of humic, fulvic, tannic, and citric acids onto OCP seed crystals (Langmuir, 1918; Kresak et al., 1977):

$$C/Q = 1/KN + C/N \qquad [2]$$

where C = concentration at equilibrium (mol L^{-1}), Q = moles of C adsorbed per unit surface area of OCP (m^2L^{-1}), K = the affinity that adsorbate molecules have for adsorption sites on OCP seed crystals (L mol^{-1}), and N = capacity factor describing the maximum amount of C that can be adsorbed onto OCP (mol m^{-2}).

The change in the rate constant for OCP precipitation (k) as a function of C_{TS} (mol L^{-1}) and C_{ADS} (mol m^{-2}) was linearized by plotting X/I versus X, where X = C_{TS} or C_{ADS}, and I is related to the inhibition of OCP precipitation in the presence of added C,

$$I = 1 - k/10^{34.93} \qquad [3]$$

where k is the rate constant in the presence of added C, and $10^{34.93}$ is the rate constant for OCP precipitation without organic acids. Once these plots were established, it was possible to statistically compare linear regressions to determine if there were significant treatment differences ($\alpha = 0.05$) among the organic acid treatments (Snedecor and Cochran, 1980). Linear regressions had r^2 values > 0.92.

From the linear regression equations, "best fit" curves to the experimental data were developed for the relationships of k versus C_{TS} (Figure 10), C_{ADS} versus C_{EQ} (Figure 11), and k versus C_{ADS} (Figure 12). For example, for the relationship between k and C_{TS} (Figure 10), a k value could be calculated for any value of C_{TS} using

$$k = -10^{34.93} (C_{TS}/(mC_{TS} + b) - 1) \quad [4]$$

where m = slope and b = y-intercept. Eq. [4] was derived by substitution of Eq. [3] into the linear expression

$$C_{TS}/I = mC_{TS} + b \quad [5]$$

and subsequent rearrangement. Curves for C_{ADS} versus C_{EQ}, and k versus C_{ADS} were established in the same manner.

Results

The overall rate order for OCP precipitation at 25°C and pH 6.0 was determined using Eq. [1]. OCP precipitation rates were $10^{-8.90}$, $10^{-8.51}$, $10^{-7.97}$, and $10^{-7.61}$ (mol $L^{-1}s^{-1}$) for respective DS values equal to $10^{2.05}$, $10^{2.22}$, $10^{2.47}$, and $10^{2.78}$. To calculate the order of reaction (n), natural log values of the above reaction rates were plotted as a function of $\ln(IAP^{1/8} - Ksp^{1/8})$, the slope of this plot being equal to n (Figure 9). This plot was linear with an $r^2 = 0.98$ and yielded a slope (n) = 7, in agreement with Heughebaert et al. (1986). By knowing n for OCP precipitation, it was possible to determine the rate constants (k) using Eq. [1] for experiments conducted at different temperatures. The Arrhenius activation energy (E_a) for OCP precipitation was calculated from the slope of the linear ($r^2 = 0.99$) plot of ln k versus

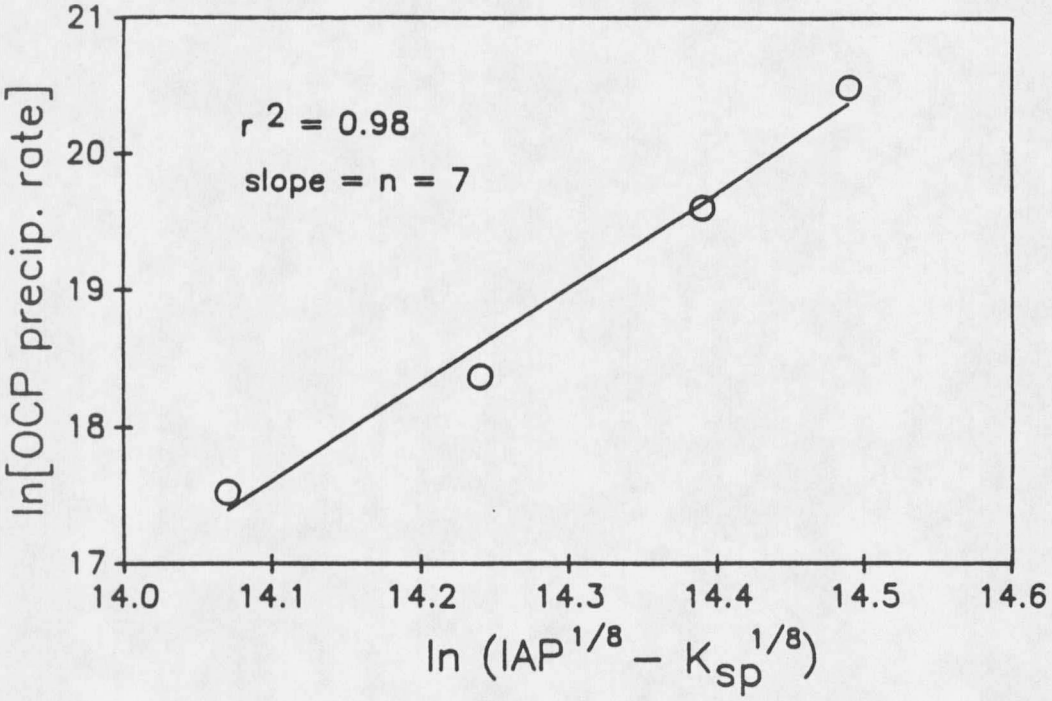


Figure 9. Log-log plot of octacalcium phosphate precipitation rate as a function of $(IAP^{1/8} - K_{sp}^{1/8})$. Slope corresponds to reaction rate order (n) = 7 (Eq. [1]).

temperature^{-1} (K) for experiments run at 20, 25, 30, and 35°C. The *k* values ranged from $10^{34.56}$ $L^7m^{-2}mol^{-6}s^{-1}$ at 20°C to $10^{35.90}$ $L^7m^{-2}mol^{-6}s^{-1}$ at 35°C. The E_a for OCP precipitation at pH = 6.0 was calculated to be 164.2 kJ mol^{-1}. However, *n* may not equal 7 for OCP precipitation at temperatures other than 25°C. For example, Heughebaert and Nancollas (1984) calculated $n = 4$ for OCP precipitation at 37°C. Therefore, we also determined an E_a from the linear plot ($r^2 = 0.94$) of ln rate (mol $L^{-1}s^{-1}$) versus temperature^{-1} (K), where rates were equal to $10^{-9.07}$, $10^{-8.98}$, $10^{-7.37}$, $10^{-7.03}$ mol $L^{-1}s^{-1}$ at 20, 25, 30, and 35°C, respectively. The E_a was 230 kJ mol^{-1}. In either case, E_a values are indicative of a surface controlled precipitation mechanism (Lasaga, 1981).

Influence of Organic Acids on OCP Precipitation

The rate constant (*k*) for OCP crystal growth in the absence of organic acids at pH 6.0 and 25°C was $8.5 \times 10^{34} \pm 0.9 \times 10^{34}$ $L^7mol^{-6}m^{-2}s^{-1}$ (average of three experiments). Inhibition of OCP precipitation by organic acids was determined by comparing *k* values for experiments run in the presence and absence of organic acids (Table 5). Humic, fulvic, tannic, and citric acids were all effective at inhibiting the precipitation of OCP. The effectiveness of these organic acids can be described by their ability to inhibit OCP precipitation per mole of C_{TS} added (Figure 10). The most effective inhibitor was humic acid, followed by fulvic and tannic acids. There was no significant difference between inhibition of OCP precipitation by citric and fulvic acids, and citric and tannic acids. In the presence of 1.0 m*M* C_{TS} as humic and fulvic acids, inhibition of OCP precipitation was nearly complete (99 and 97%, respectively). At

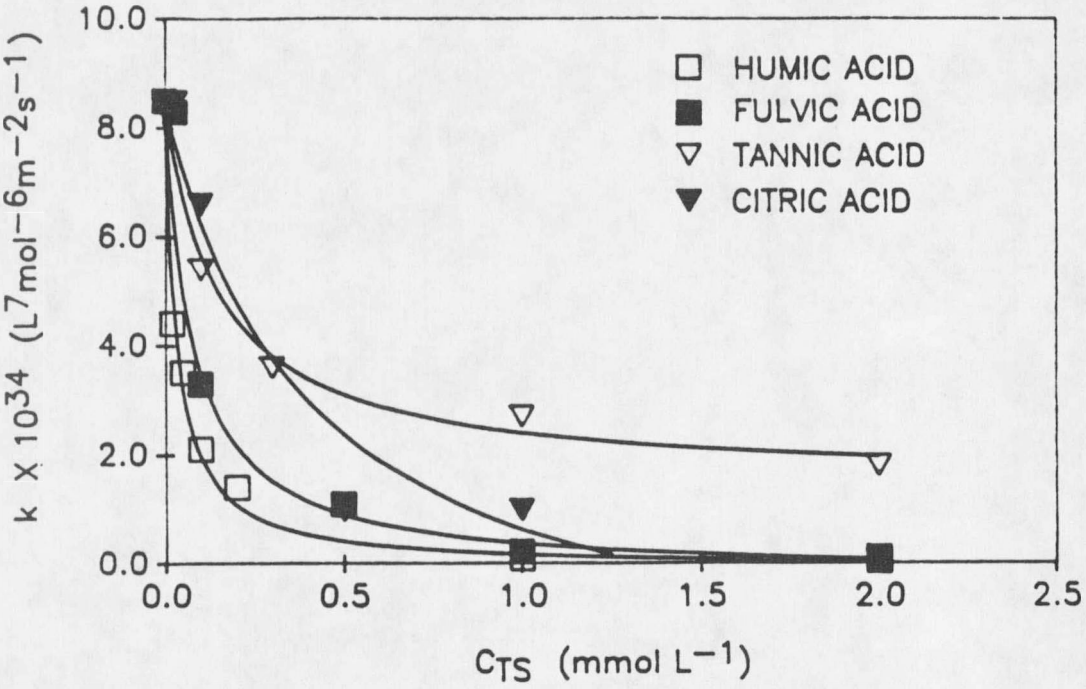


Figure 10. Rate constants (*k*) for octacalcium phosphate crystal growth as a function of total soluble C added as humic, fulvic, tannic, and citric acids.

the same level of C, citric acid inhibited OCP precipitation by 89%, while the presence of tannic acid resulted in 68% inhibition (Table 5).

Table 5. Rate constants (k), and percent inhibition of octacalcium phosphate precipitation in the presence of organic acids.

Organic acid	Initial total soluble C	$k \times 10^{34}$†	Inhibition of OCP precipitation
	(mmol L^{-1})	($L^7 mol^{-6} m^{-2} s^{-1}$)	(%)
None	--	8.5 ± 0.9	--
Humic	0.02	4.400	48.0
	0.05	3.500	59.0
	0.10	2.100	75.0
	0.20	1.400	84.0
	1.00	0.084	99.0
	2.00	0.041	99.5
Fulvic	0.03	8.300	0.0
	0.10	3.300	60.0
	0.50	1.100	87.0
	1.00	0.230	97.0
	2.00	0.120	99.0
Tannic	0.10	5.400	36.0
	0.30	3.600	57.0
	1.00	2.700	68.0
	2.00	1.800	78.0
Citric	0.03	9.600	0.0
	0.10	6.600	22.0
	0.50	1.000	88.0
	1.00	1.000	88.0

† Exponent indicates each value should be multiplied by 10^{34}.

All of these organic acids were adsorbed onto OCP seed crystals (Figure 11). The order of their adsorption (mol C m^{-2}) onto OCP surfaces per C_{EQ} (mmol C L^{-1}) was tannic > humic = fulvic > citric. Adsorption data for all of these organic acids followed the Langmuir adsorption isotherm (Eq. [2]) (r^2 = 0.99 for humic and fulvic acids and r^2 = 0.98 for tannic and citric acids; Table 6). The affinity parameters for humic and fulvic acids were similar and greater than those for tannic and citric acids. However, the capacity factor for tannic acid was about three times greater than the values determined for the other organic acids (Table 6).

Table 6. Langmuir adsorption parameters for the adsorption of humic, fulvic, tannic, and citric acids onto octacalcium phosphate.

Adsorbate	Affinity†	Adsorption capacity*	Correlation coefficient (r^2)
	L/mol C	mol C/m^2	
Humic acid	9112	2.24 x 10^{-4}	0.99
Fulvic acid	10020	2.08 x 10^{-4}	0.99
Tannic acid	2790	6.0 x 10^{-4}	0.98
Citric acid	1700	2.3 x 10^{-4}	0.98

† K in Eq. [2]
* N in Eq. [2]

To compare the efficiency of organic acid adsorption on inhibition of OCP crystal growth, k ($L^7 mol^{-6} m^{-2} s^{-1}$) was plotted as a function of moles of C_{ADS} onto OCP. The ability of adsorbed humic, fulvic, and citric acids to inhibit OCP precipitation did not differ significantly (Figure 12). However, these three organics were more efficient than tannic acid at inhibiting OCP crystal growth per mole of C_{ADS}. For example, evaluation of Langmuir adsorption capacity constants (mol C m^{-2}) (Table 6) indicates

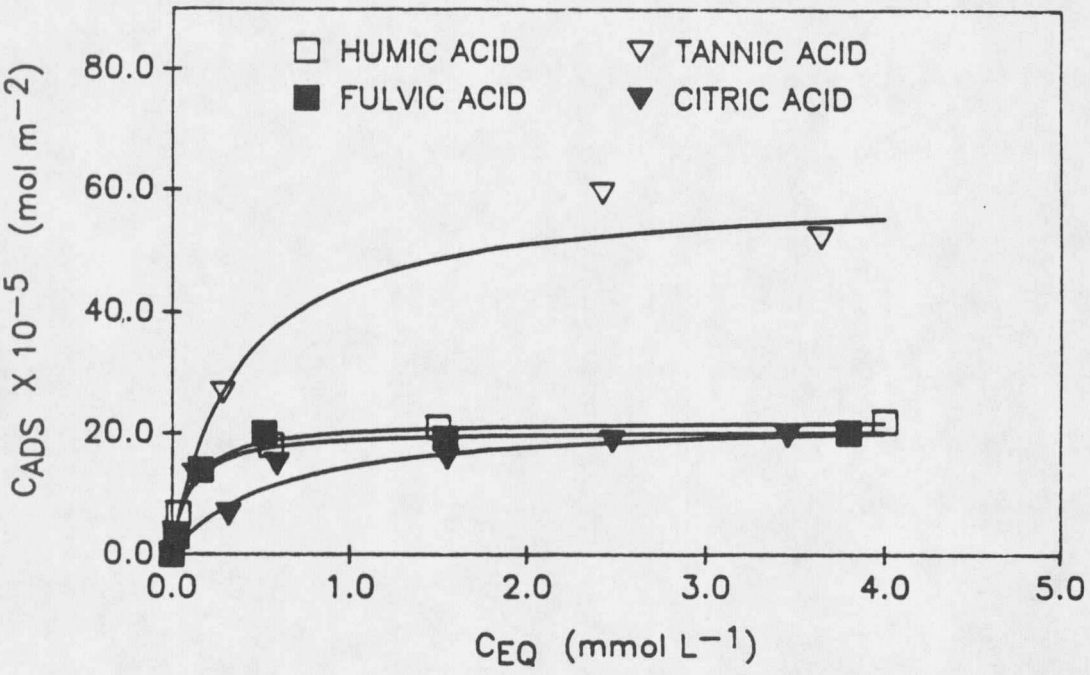


Figure 11. Adsorption of C as a function of equilibrium C levels of humic, fulvic, tannic, and citric acids.

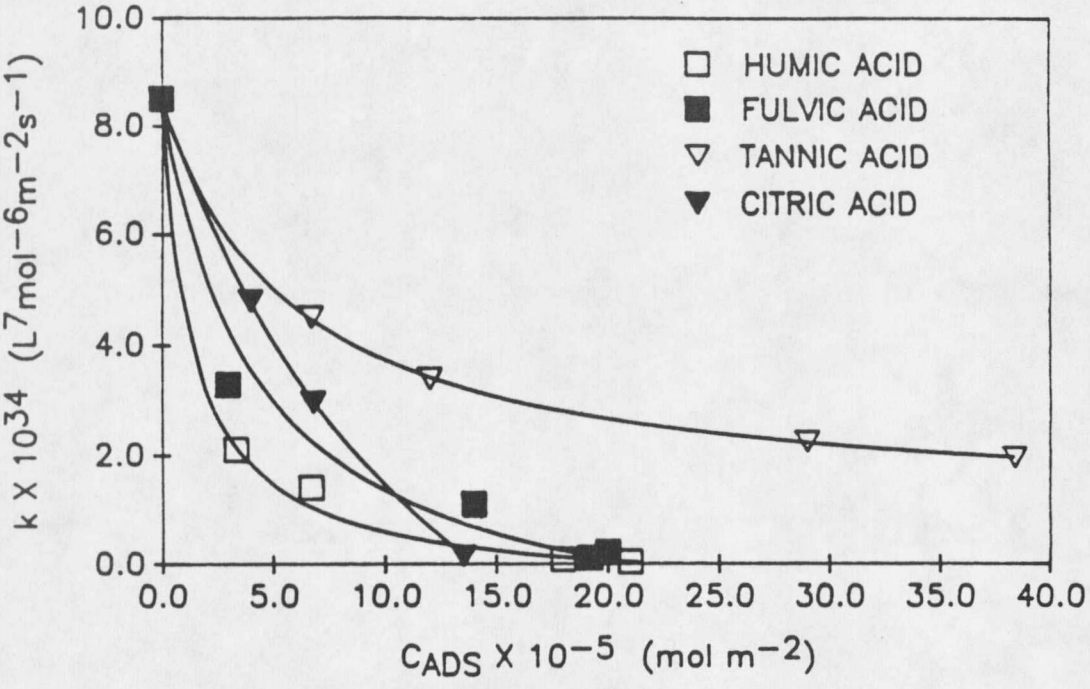


Figure 12. Rate constants (*k*) for octacalcium phosphate (OCP) crystal growth as a function of C adsorbed as humic, fulvic, tannic, and citric acids onto OCP crystal surfaces.

that maximum coverage of OCP by humic, fulvic, and citric acids corresponds with complete inhibition of crystal growth, while maximum coverage by tannic acid results in approximately 80% inhibition (Figure 12). Langmuir capacity parameters (Table 6) were recalculated as nm^2 C per nm^2 of OCP surface area using assumptions presented in Inskeep and Silvertooth (1988a). The amount of monolayer surface coverage corresponding to complete inhibition was approximately 2 nm^2 C per nm^2 OCP for humic, fulvic, and citric acids. Surface coverage for maximum inhibition by tannic acid was 6 nm^2 C per nm^2 OCP. It is clear that the effective inhibitors required less C coverage for complete inhibition of OCP precipitation. However, compared to HAP (surface coverage $\simeq$ 0.5 nm^2 C per nm^2 HAP (Inskeep and Silvertooth, 1988a)), it took more C coverage per unit area to obtain complete inhibition for OCP precipitation.

Discussion

Earlier investigations on the precipitation kinetics of DCPD and HAP in the presence of organic acids commonly found in soil solutions and natural waters have reported that precipitation was inhibited by C adsorption onto seed crystal surfaces (Grossl and Inskeep, 1991; Inskeep and Silvertooth, 1988a). These organic acids block crystal growth by adsorbing onto and "poisoning" active crystal growth sites, present at kinks and dislocations on the seed surface (Aoba and Moreno, 1985; Kresak et al., 1977; Meyer and Nancollas, 1973). The ability of these organic acids to inhibit precipitation is related to their functional group content, size, geometry, and their

orientation on the seed surface (Kresak et al., 1977; Meyer and Nancollas, 1973; Inskeep and Silvertooth, 1988a; Grossl and Inskeep, 1991).

Humic acid was the most effective inhibitor of OCP crystal growth per C_{TS} added and was also an efficient inhibitor per mole of C_{ADS} onto OCP surfaces. The affinities (Table 6) of humic and fulvic acids for OCP crystal seed surfaces were similar, suggesting that their adsorption involves the same adsorption sites on OCP crystal surfaces and the same adsorbate functional groups. At pH 6.0 the majority of carboxylic acid (COOH) functional groups should be dissociated and the adsorption of organic acids is probably due to COO^- binding with Ca^{2+} present at active growth sites (Inskeep and Silvertooth, 1988a; Grossl and Inskeep, 1991; Kresak et al., 1977). However, the carboxyl functional group content is not the only factor governing the effectiveness of adsorbed organics at inhibiting OCP precipitation. If it were, then citric acid ($COOH/C_{TS}$ ratio = 0.5) and tannic acid ($COOH/C_{TS}$ = 0.13) should be more effective inhibitors than humic acid ($COOH/C_{TS}$ = 0.09). This is not the case; therefore, some other factor must be responsible for the dissimilar behavior observed between humic acid, and citric and tannic acids. The hydrophobic nature (Thurman, 1985) of humic and fulvic acids may lead to an energetically favorable entropy driven adsorption process that results in more complete surface coverage of active growth sites and consequently greater inhibition of OCP precipitation. Although such an entropically driven mechanism has not been conclusively demonstrated for the adsorption of humic substances onto Ca phosphate solid phase surfaces, it has been confirmed for the adsorption of salivary proteins onto the surface of HAP (Moreno et al., 1982; Moreno et al., 1984). The suggested reactions responsible for this

process involved the loss of secondary structure and H-bonding of salivary proteins, and displacement of water molecules from the adsorbate and HAP surface.

The difference in adsorption behavior between tannic and citric acids can be explained by size and functional group differences that may determine whether the molecules lie flat or perpendicular to the crystal surface. Although the amount of C adsorbed as tannic acid onto OCP exceeded that of citric acid (Figure 11), citric acid was more efficient at inhibiting OCP precipitation per mole of C adsorbed. Citric acid probably provides better surface coverage and subsequent blockage of available growth sites per mole of C adsorbed than tannic acid, since it is smaller and has the highest COOH content per mole C. Conversely, surface coverage was probably not as great for tannic acid, a polyphenolic compound (Cody and Cody, 1988), per mole C. Much of the adsorbed tannic acid molecule may lie perpendicular to the OCP surface and would not be as effective at blocking available crystal growth sites. Perhaps if OCP crystal growth experiments were conducted at higher pH values, tannic acid would be a greater inhibitor per C adsorbed since more phenolic functional groups would be deprotonated resulting in greater surface binding per unit C (Cody and Cody, 1988).

Complexation of soluble Ca^{2+} by organic ligands can also reduce precipitation rates by lowering the free Ca^{2+} concentration in solution and consequently the degree of supersaturation with respect to the precipitating solid phase. The level of Ca complexation was calculated directly for citric acid using GEOCHEM, since the formation constants for H-citrate and Ca-citrate complexes (Smith and Martell, 1976) are known. Thus, the amount of citric acid complexed with Ca^{2+} was accounted for and is reflected in the rate constant determined using Eq. [1]. To estimate the amount

of Ca complexed by humic acid, a Ca-ion electrode was employed, indicating that at the highest level of humic acid (2m*M* C_{TS}) only 2% of the total soluble Ca was complexed, which does not account for the amount of inhibition observed (99.5%) at this level of humic acid. The Ca-ion electrode measurements were unstable in the presence of fulvic and tannic acids and were therefore not used to calculate Ca^{2+} activities. However, even at the highest C_{TS} levels (assuming that all COOH functional groups complexed Ca), fulvic and tannic acids would complex 5 to 6% of the soluble Ca^{2+}, accounting for only 5% and 8% of the inhibition of OCP precipitation by fulvic and tannic acid, respectively. In summary, under the current experimental conditions, Ca complexation was not a significant factor in the inhibition of OCP crystal growth.

Using results from precipitation experiments of DCPD (Grossl and Inskeep, 1991), HAP (Inskeep and Silvertooth, 1988a), and OCP (this study), we developed a conceptual model to describe the inhibition of Ca phosphate precipitation rates by organic ligands in geochemical environments. A hypothetical aqueous solution used for calculation of precipitation rates was comprised of 5 m*M* Ca_{TS} and PO_{4TS}, and consequently, supersaturated with respect to DCPD, OCP, and HAP. Other conditions, such as pH = 6.0, seed surface area = 2.4 m^2L^{-1}, and ionic strength adjustment with 10 m*M* KCl, were the same as those for OCP experiments described in the current study. A pH value of 6.0 was also an intermediate value between DCPD and HAP experiments. Use of pH values > 7.0 may result in overestimation of DCPD and OCP precipitation rates since values would deviate from the conditions

under which the rate equations were developed. Precipitation rates for HAP and DCPD were calculated using the following rate equations:

$$Rate_{HAP} = k_f S(Ca^{2+})(PO_4^{3-})$$

$$Rate_{DCPD} = k_f S(Ca^{2+})(HPO_4^{2-})$$

where k_f = forward rate constant ($L^2mol^{-1}m^{-2}s^{-1}$) equal to 173 for HAP and 9.7 for DCPD, S = crystal seed surface area (m^2L^{-1}), and brackets represent activities. Using these assumptions, the rate of precipitation increases in the order DCPD > OCP > HAP (Table 7), suggesting that when geochemical environments are supersaturated with respect to these three solid phases, DCPD would form faster than OCP and HAP. Additions of soluble organic constituents will further reduce these rates (Table 7) by blocking active crystal growth sites on seed surfaces. At a constant level of soluble C, the percent of inhibition is greatest for HAP and lowest for DCPD (Table 7). In addition, DCPD has the ability to overgrow adsorbed humic and fulvic acids noted by an increase in precipitation rate after an induction period (Grossl and Inskeep, 1991).

Principles of chemical kinetics and reaction pathways can be used to conceptually explain Ca phosphate precipitation. In one case, a supersaturated solution of Ca and PO_4 can precipitate several solid phases by a parallel reaction pathway:

$$Ca^{2+} + H_xPO_4^{x-3} \xrightarrow{R_1\ (Step\ 1)} DCPD$$
$$\xrightarrow{R_2\ (Step\ 2)} OCP \qquad [6]$$
$$\xrightarrow{R_3\ (Step\ 3)} HAP$$

Based on the rates calculated above, $R_1 >> R_2 > R_3$, and reaction steps 2 and 3 are strongly inhibited by soluble C at levels realistic for many geochemical environments.

Step 1 is inhibited to a lesser extent and can overgrow adsorbed C; thus, DCPD would potentially form first and remain as a metastable solid phase prior to the precipitation of OCP and HAP.

Table 7. Precipitation rates for dicalcium phosphate dihydrate (DCPD), octacalcium phosphate (OCP), and hydroxyapatite (HAP) in the absence and presence of humic acid, and percent inhibition of precipitation by humic acid.†

	OC	0.2 m*M* C as humic acid	Inhibition of precipitation
Ca phosphate solid phase	- - Precipitation rate (mol $L^{-1}s^{-1}$) - -		(%)
DCPD	8.7×10^{-6}	7.2×10^{-6}	17
OCP	3.4×10^{-8}	5.6×10^{-9}	83
HAP	7.7×10^{-11}	1.1×10^{-11}	85

† Solution conditions were, Ca_{TS} and PO_{4TS} = 5.0 m*M*, pH = 6.0, seed surface area = 2.4 m^2L^{-1}, and ionic strength adjustment with 10 m*M* KCl.

In the second case, when a series reation pathway is considered, DCPD would form first from a supersaturated solution successively followed by the precipitation of OCP and HAP.

$$Ca^{2+} + H_xPO_4^{x-3} \xrightarrow{R_1\ (Step\ 1)} DCPD \xrightarrow{R_2\ (Step\ 2)} OCP \xrightarrow{R_3\ (Step\ 3)} HAP \quad [7]$$

Since R_2 and $R_3 << R_1$, steps 2 and 3 are rate limiting for the formation of thermodynamically stable HAP. Inhibition of steps 2 and 3 by soluble C further reduces R_2 and R_3 relative to R_1, again increasing the period of metastability where DCPD is the predominant Ca phosphate solid phase.

Conclusions

Humic, fulvic, citric, and tannic acids all inhibited the rate of OCP crystal growth. Inhibition of OCP precipitation was due to adsorption of these organic acids onto OCP crystal seed surfaces resulting in blockage of active crystal growth sites. The amount of C adsorbed onto OCP, and the ability to inhibit OCP formation, is related to the adsorbate's functional group content, size, geometry, and orientation on the adsorbent. Humic acid was the most effective inhibitor of OCP crystal growth per mole C_{TS} added. Humic and fulvic acids had similar adsorption behavior onto OCP. This may be related to their hydrophobic nature resulting in an energetically favorable entropy driven adsorption reaction with more complete coverage of active crystal growth sites. Although the amount of C adsorbed as tannic acid onto OCP exceeded the amount adsorbed as the other organics, it was the least effective inhibitor of OCP precipitation. This may be due to the orientation of tannic acid molecules at the OCP surface, resulting in less surface poisoning per mole of C adsorbed.

Soluble C constituents representative of soil solutions and natural waters are most effective at inhibiting HAP precipitation, successively followed by inhibition of OCP and DCPD. Inhibition of precipitation by foreign ions may explain the metastability of OCP and DCPD solid phases with respect to thermodynamically stable HAP, which is often observed in geochemical environments.

CHAPTER 4

SOIL PHOSPHATE SOLUBILITY AND PLANT AVAILABILITY IN THE PRESENCE OF SOLUBLE ORGANIC MATTER

Introduction

When phosphorus fertilizers are applied to soils, chemical processes occur that "fix" or retain P, decreasing PO_4 activity. Mechanisms responsible for P fixation are precipitation of P as Ca, Fe, and Al phosphates and adsorption of PO_4 onto oxides, clays, carbonates, and soil organic matter (Sample et al., 1980). These processes limit the effectiveness of P fertilizer applications and therefore further applications are necessary to sustain desired crop productivity.

Organic matter appears to play an important role in increasing the efficiency of P fertilizers. Weil et al. (1988) suggested that the increased organic matter under no-till management practices may enhance P availability by blocking P adsorption sites on soil colloids. Other studies have shown that organic anions commonly found in the rhizosphere compete with phosphate for adsorption sites on oxide and clay surfaces (Nagarajah et al., 1970; Lopez-Hernandez et al., 1986). Certain organic molecules (i.e., malate and oxalate) are more rapidly adsorbed to soil surfaces than phosphate, which can improve the phosphate status of the soil (Lopez-Hernandez et al., 1986).

Organic constituents are also known to hinder the precipitation of insoluble Ca phosphates. Moreno et al. (1960) reported that soluble organic matter was responsible for the persistence of the more soluble dicalcium phosphate dihydrate (DCPD) and a reduction in the rate of formation of less soluble octacalcium phosphate (OCP) in soils. Similarly, the application of organic amendments (sludge and manure) to calcareous soils was found to increase PO_4 solubility over time more than lone additions of inorganic P (Abbott and Tucker, 1973; Meek et al., 1979; O'Connor et al., 1986). Results of O'Connor et al. (1986) in field and greenhouse soils suggest that soluble organic matter was influencing the rate of transformation of Ca phosphate solid phases.

The precipitation experiments described in Chapters 2 and 3 were all conducted with pure Ca phosphate minerals under controlled laboratory conditions. The objectives of this study were to: (i) conduct incubation studies on soil systems to determine if precipitation inhibition by soluble organic ligands could explain slow PO_4 transformations in soil, and (ii) investigate the availability of P to spring wheat (*Triticum aestivum* L. Pondera) and sorghum-sudan grass (*Sorghum sudanese* L. Sordan 79) in calcareous soils treated with and without organic acids.

Materials and Methods

Soil Incubation Experiments

Two completely randomized factorial soil incubation experiments (A and B) were conducted in 50 mL polypropylene centrifuge tubes. For Experiment A, 5 g of a ground and sieved (< 1.0 mm) calcareous Grail clay loam soil (collected from the top 15 cm of the profile) was treated with a 25 mL solution containing 6 m*M* total soluble

(TS) Ca and PO_{4TS} and 20 m*M* KCl, which was added as a background electrolyte. The soil was also treated with and without 2.0 m*M* C as peat humic acid obtained from the International Humic Substances Society (IHSS), and with and without 20 mg pure DCPD seed crystals. Each treatment was replicated twice. Prior to the addition of treatments, we washed the soil successively with declining solution concentrations of Ca and K. In order to condition the cation exchange sites with Ca and K, the last wash contained Ca and K concentrations equal to their concentrations in the treatment solutions. Treatments were shaken at 120 oscillations per minute for 2 and 14 days, centrifuged, and the supernatants were filtered through 0.2 micron Gelman filters (Gelman Sciences, Inc., Ann Arbor, MI). The solutions were then analyzed for Ca_{TS}, K_{TS}, PO_{4TS}, Cl^-, and pH. Calcium and K concentrations were measured by atomic absorption spectroscopy, PO_4 was measured colormetrically using a modified Murphy-Riley technique (Harwood et al., 1969) and Cl^- by ion chromatography. The activities of Ca^{2+} and $H_2PO_4^-$ were calculated by entering the elemental concentration data into GEOCHEM (Sposito and Mattigod, 1979; Parker et al., 1987). The results were statistically evaluated at the 5% level of significance (Steele and Torrie, 1980) using Turbostat, a computer software program (Turbostat, 1986).

The conditions for Experiment B differed from A in that 3 g of the < 50 micron fraction of the same soil was now treated with 8 m*M* Ca_{TS} and PO_{4TS}, 100 mg of pure DCPD seed crystals, and treatments were replicated three times. In addition, after removal of treatment solutions from soils sampled at 2 days, fresh treatment solutions were again added to these soils, and at 14 days the supernatant was collected and analyzed. Procedures for solution analysis were the same as those described for Experiment A.

Plant Available Phosphate Experiments

Two completely randomized factorial design experiments (A and B) were conducted in a controlled environment chamber. The photosynthetically active light radiation in the chamber was 300 uE m^{-2} s^{-1} and average relative humidity was equal to 70%.

Experiment A investigated the effect of organic acid C sources on P uptake of spring wheat (*Triticum aestivum* L. Pondera) and consisted of the following treatments:

1. Three P levels (0, 25, and 50 kg ha^{-1} P added as monocalcium phosphate).
2. Four C treatments (0 C, tannic acid (J.T. Baker Chemical Company, Phillipsburg, NJ), commercial humic acid (Fluka Chemical Corporation, Ronkonkona, NY), and a mixed solution containing equivalent C levels as oxalic, citric, and succinic acids (J.T. Baker)). Carbon treatments were added with each watering. These watering solutions contained approximately 3 m*M* C.

These treatments were applied to two calcareous soils, a Brocko silt loam and Cushman sandy loam, both of which tested low in available P (< 10 mg kg^{-1} P extracted with $NaHCO_3$ (Olsen and Sommers, 1982)). Treatments were replicated three times, each replicate consisting of a soil:sand ratio equal to 2:1, 750 g of which was added to each pot. Sand was added to ensure even infiltration and percolation. Each treatment received N and K equal to 224 and 34 kg ha^{-1}, respectively.

Each pot was initially seeded with 20 plants and thinned to 12 plants after seed emergence. The plants were watered as needed every 3 to 4 days and maintained near field capacity (21% for the Brocko and 25% for the Cushman soil). Plants received

16 hours of daylight (21°C) and 8 hours of darkness (12°C). The plants were harvested at Feekes stage 6 (first node of stem visible) and a final harvest at Feekes stage 10 (in "boot") (Nelson et al., 1988). Harvested plant tissue was dried at 45°C for 48 hours. Dried tissue was evaluated for weight (g), P concentration (mg kg^{-1}), and for P uptake (mg pot^{-1}). Plant tissue was solubilized using a nitric/perchloric acid digest and P was measured colormetrically (Harwood et al., 1969). Results were statistically analyzed ($\alpha = 0.05$) using Turbostat (Turbostat, 1986).

Experiment B investigated the effect of organic acid C sources on P uptake of sorghum-sudan grass (*Sorghum sudanese* L. Sordan 79). This experiment consisted of the following treatments:

1. Two P levels (0 and 75 kg ha^{-1} P added as monocalcium phosphate).
2. Three C treatments (0 C, tannic acid (J.T. Baker), and commercial humic acid (Fluka)). These treatments were added with each watering containing approximately 3 m*M* C.

These treatments were replicated three times and applied to a calcareous, low P (< 6 mg kg^{-1} P extracted with $NaHCO_3$ (Olsen and Sommers, 1982)) Grail clay loam soil. Twelve kilograms of soil were added to each pot and brought to field capacity (29% water content) with the C containing watering solutions. A blanket application of 300 kg ha^{-1} N was added to each replication. Porous ceramic cups were placed 15 cm from the surface running lengthwise in each pot for collection of soil solutions by vacuum extraction.

Thirty-five seeds were planted in each pot, and after plant establishment seedlings were thinned back to 28. Plants received 16 hours of daylight (22°C) and 8 hours of

darkness (21°C). Plants were harvested four times during the course of the experiment (Table 8). For each harvest, seven above ground whole plants were collected, dried for 48 hours at 45°C, and measured for dry weight, P concentration, and P uptake. Results were statistically analyzed using the same methods described for Experiment A.

Table 8. Harvest times (wks) with respective growth stages for sorghum-sudan grass.

Harvest	Time after planting (wks)	Growth stage description
1	4	2-4 leaves present
2	8	3-6 leaves present
3	14	5-7 leaves present
4	23	6-8 leaves present, grain heads formed

Results and Discussion

Soil Incubation Experiments

When the calcareous Grail clay loam was subjected to oversaturated Ca phosphate solutions containing 6 mM Ca_{TS} and PO_{4TS}, with and without humic acid, all the solutions were undersaturated with respect to DCPD but oversaturated with respect to OCP after 2 and 14 days (Figure 13). Calcium phosphate potential ($0.5pCa^{2+} + pH_2PO_4^-$), a measure of P solubility, was significantly higher for C treatments at two days; however, there were no treatment differences at 14 days (Table 9; Figure 13). The addition of pure DCPD seed crystals tended to reduce the oversaturation with respect to OCP.

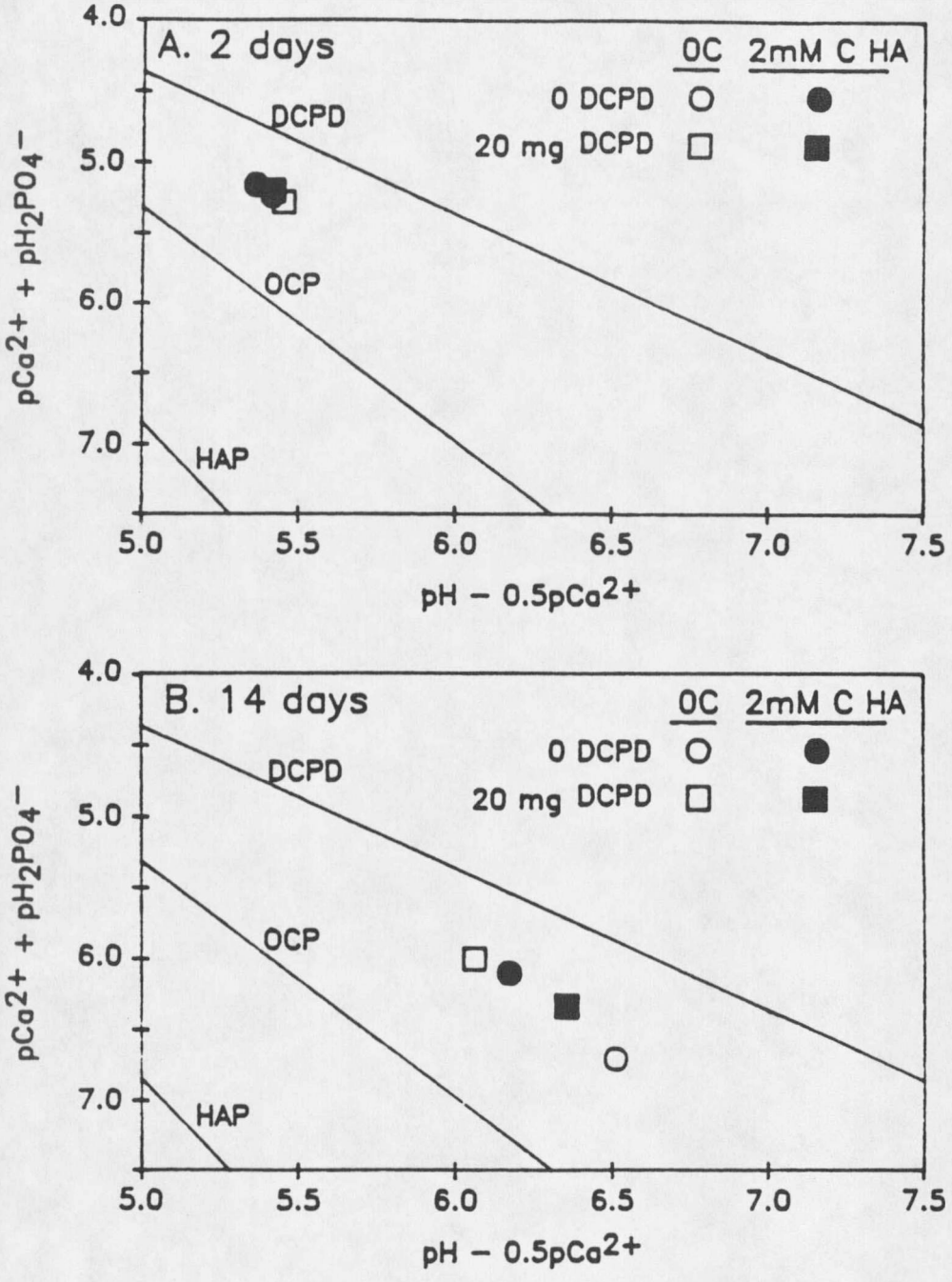


Figure 13. Ca phosphate potential for samples treated with 25 mL of 6 m*M* Ca_{TS} and PO_{4TS}, with and without 2 m*M* C as humic acid and with and without 20 mg DCPD crystals added. Soil solutions were collected after (A) 2 days and (B) 14 days.

Table 9. Average Ca phosphate potential values for samples treated with 6 m*M* total soluble Ca and PO_4, with and without humic acid, and with and without DCPD added.

Treatment		Ca phosphate potential ($0.5\ pCa^{2+} + pH_2PO_4^-$)	
m*M* C added as humic acid	mg DCPD added	2d	14d
0	0	5.236†	6.708 N.S.
0	20	5.281	6.004
2	0	5.160	6.102
2	20	5.200	6.340
LSD (0.05)		0.103	0.688

† Significant treatment differences at $P < 0.05$.
N.S. No significant treatment differences.

When the same soil was treated with solutions more oversaturated with respect to DCPD (8 mM Ca_{TS} and PO_{4TS}), the samples collected at 2 and 14 days were all supersaturated with respect to DCPD (Figure 14). Addition of 2 m*M* C as humic acid significantly increased Ca phosphate potential of samples collected at 2 days only when 100 mg of pure DCPD seed crystals were present (Table 10; Figure 14). In contrast, addition of humic acid significantly increased Ca phosphate potential of samples collected at 14 days only in the absence of DCPD seed crystals (Table 10). The presence of DCPD also tended to reduce the oversaturation with respect to DCPD. The humic acid probably adsorbed onto the DCPD crystal seeds, blocking active crystal growth sites and consequently inhibiting DCPD precipitation (Grossl and Inskeep, 1991).

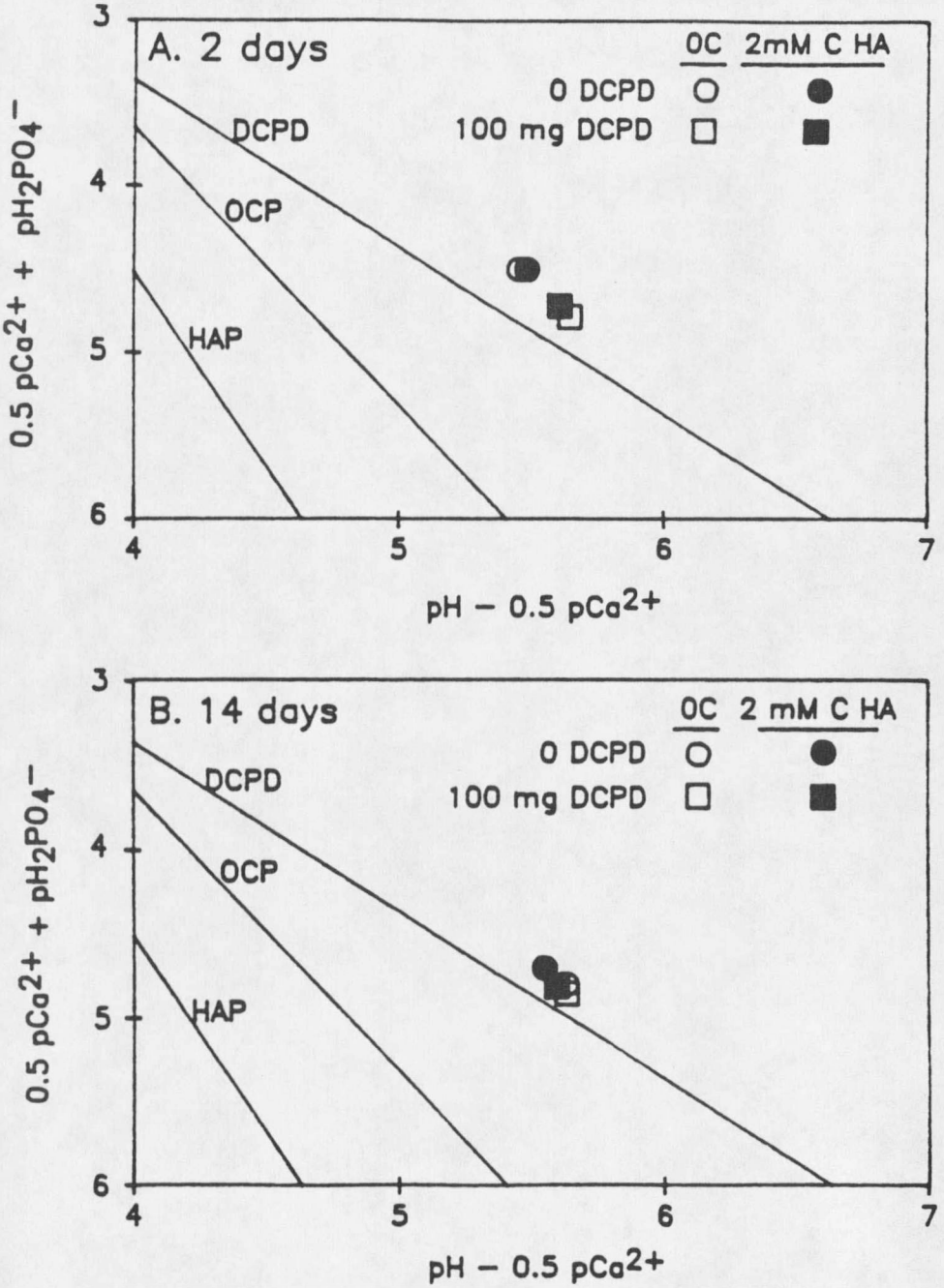


Figure 14. Ca phosphate potential for samples treated with 25 mL of 8 m*M* Ca_{TS} and PO_{4TS}, with and without 2 m*M* C as humic acid and with and without 100 mg DCPD crystals added. Soil solutions were collected after (A) 2 days and (B) 14 days.

Table 10. Average Ca phosphate potential values for samples treated with 8 m*M* total soluble Ca and PO_4, with and without humic acid, and with and without DCPD added.

Treatment		Ca phosphate potential ($0.5\ pCa^{2+} + pH_2PO_4^-$)		
m*M* C added as humic acid	mg DCPD added	2d	14d	14d*
0	0	4.500†	4.801†	4.464†
0	100	4.790	4.858	4.384
2	0	4.501	4.706	4.303
2	100	4.721	4.815	4.269
LSD (0.05)		0.030	0.091	0.043

* Soil solution was replaced with new treatment solution at 2 days and collected at 14 days.

† Significant treatment differences at $P < 0.05$.

The treatments where solutions were replaced at 2 days and collected at 14 days had significantly greater Ca phosphate potentials with humic acid both in the absence and presence of DCPD seed crystals (Table 10; Figure 15). Precipitation of DCPD may be the predominant force controlling PO_4 solubility for this treatment, since soil adsorption sites should be saturated with PO_4 from solutions removed at 2 days.

Overall, the addition of humic acid slightly increased PO_4 solubility in calcareous soils. We speculate that the humic acid is adsorbed either onto Ca phosphate surfaces and also onto clay and oxide surfaces, hence preventing the precipitation of Ca phosphate minerals and the adsorption of PO_4 onto solid phase surfaces. Thus, soluble organic C constituents present in soil solutions may block fixation of fertilizer P and consequently prolong the availability of plant available P.

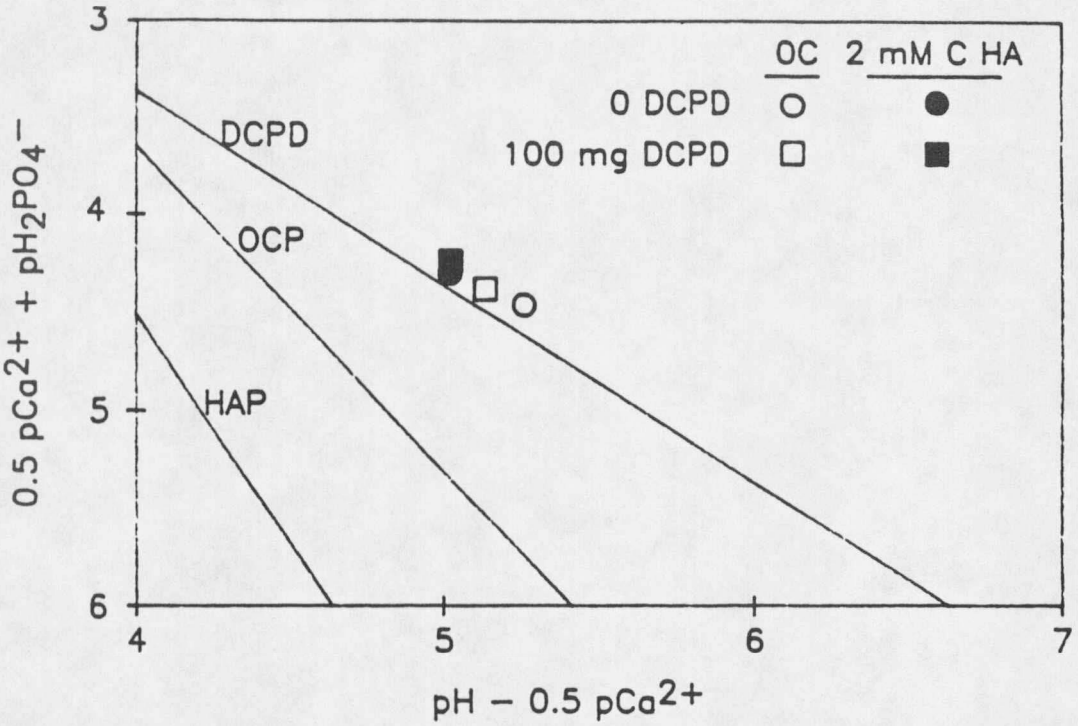


Figure 15. Ca phosphate potential for treatment solutions (25 mL of 8 m*M* Ca_{TS} and PO_{4TS}, with and without 2 m*M* C as humic acid and with and without 100 mg DCPD crystals added). Soil solution was replaced with new treatment solution at 2 days and collected at 14 days.

Plant Available Phosphate Experiments

Spring wheat. Significant increases in plant dry weights were noted with the addition of increasing P levels for the Brocko soil at both harvests and for the Cushman soil only at harvest 1 (Tables 11 and 12). Plant P concentrations and P uptake increased significantly with increasing levels of P added for both soils and both harvests (Tables 11 and 12). These results are to be expected since more P would be available for plant growth and P uptake. A significant increase in dry weight due to C added as tannic acid was observed for the Brocko soil at harvest 1 (Table 13). Addition of tannic acid and the mixed C treatment significantly increased P uptake of plants grown on the same soil collected at harvest 1 (Table 13). A significant interaction between P and C treatments was also observed for harvest 1 on the Brocko soil. The greatest effect on plant dry weights was at high P treatments when solutions were potentially oversaturated with respect to OCP (Figure 16). Overall, the above parameters tended to show the greatest response to tannic acid treatments (Figure 16). At harvest 2, a significant increase in P uptake due to C was again noted with the addition of tannic acid to the Brocko soil (Table 13). For the Cushman soil, significant positive responses due to C were only observed for samples from harvest 2, where dry weights were greatest for the humic acid treatment (Table 13). These data suggest that the addition of soluble organic C, especially as tannic acid, may increase plant available P. However, it is not known whether the response is physiological, or actually due to the adsorption of these organic acids onto Ca phosphate crystal seed surfaces inhibiting the precipitation of P solid phases.

Table 11. P treatment responses (mean) for Brocko silt loam.

Harvest	P level (kg ha^{-1})	Dry wt. (g)	P conc. (mg kg^{-1})	P uptake (mg pot^{-1})
1	0	1.67†	1100†	1.97†
	25	1.90	2520	4.78
	50	2.06	3020	6.22
	LSD (0.05)	0.07	347	0.73
2	0	3.82†	950†	3.64†
	25	3.95	1540	6.06
	50	4.14	2000	8.26
	LSD (0.05)	0.24	141	0.43

† Significant treatment differences at $P < 0.05$.

Table 12. P treatment responses (mean) for Cushman sandy loam.

Harvest	P level (kg ha^{-1})	Dry wt. (g)	P conc. (mg kg^{-1})	P uptake (mg pot^{-1})
1	0	1.33†	1200†	1.60†
	25	1.37	1770	2.42
	50	1.42	2190	3.11
	LSD (0.05)	0.05	407	0.56
2	0	2.19†	1540†	3.36†
	25	1.87	3670	6.88
	50	2.00	4440	8.74
	LSD (0.05)	0.13	219	0.49

† Significant treatment differences at $P < 0.05$.

Table 13. Dry weights and P uptake for spring wheat grown in the presence of organic acids.

Harvest	Organic acid	Brocko soil		Cushman soil	
		Dry weight	P uptake	Dry weight	P uptake
		(g)	(mg kg^{-1})	(g)	(mg kg^{-1})
1	0	1.85†	3.81†	1.37 N.S.	2.08 N.S.
	Mixed C*	1.82	4.90	1.36	2.62
	Tannic	1.95	4.88	1.38	2.56
	Humic	1.88	3.71	1.40	2.24
	LSD (0.05)	0.08	0.84	0.06	0.65
2	0	3.92 N.S.	6.09†	1.91†	6.59†
	Mixed C	3.80	5.20	1.90	5.69
	Tannic	4.07	6.65	1.98	6.43
	Humic	4.10	6.01	2.29	6.61
	LSD (0.05)	0.28	0.50	0.16	0.56

* Mixed C = equal parts C as citric, oxalic, and succinic acids.
† Significant treatment differences at $P < 0.05$.
N.S. No significant treatment differences.

Sorghum-sudan grass. In Experiment B, the addition of 75 kg ha^{-1} P significantly increased sorghum-sudan grass dry weights, concentration of P in whole plant tissue, and the amount of P taken up by plants for all harvests (Figures 17, 18, 19). Phosphate concentrations decreased in plant tissue with time (Figure 18), most likely due to dilution of P by increased carbohydrate production (Jarrell and Beverly, 1981). We were expecting that this experiment would give us some insight into whether or not C sources were effective at inhibiting the rate of P fixation, thus extending the period that P is available to plants. Unfortunately, there were no significant increases in dry weights, P concentrations, and P uptake due to C treatments (Table 14). If such

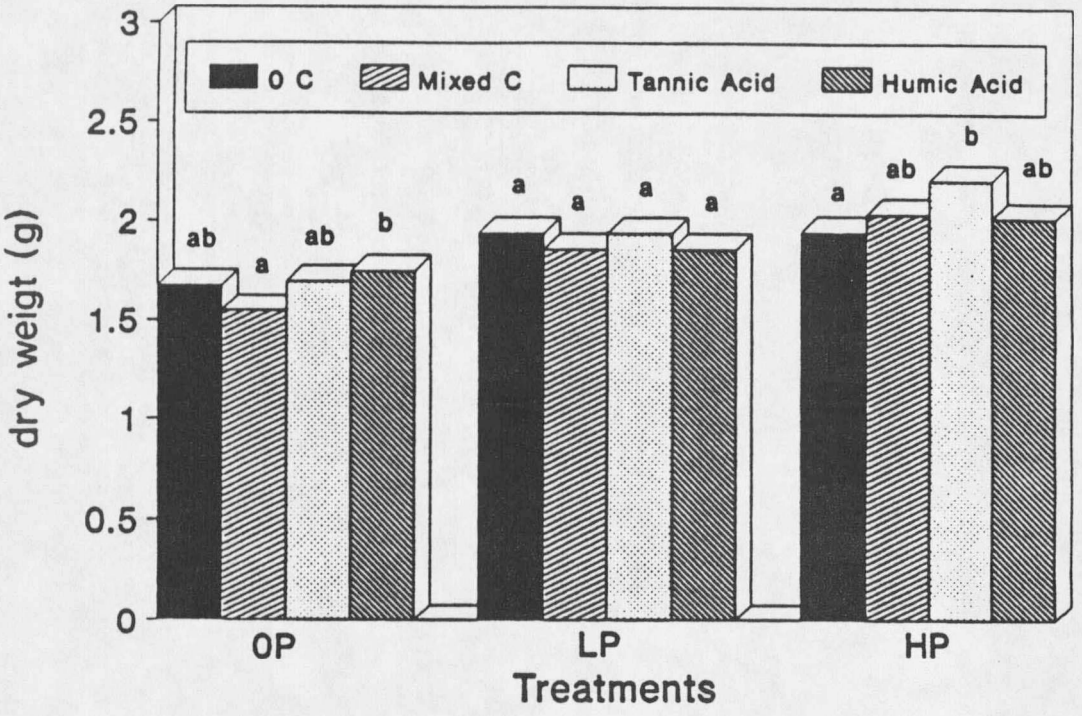


Figure 16. Average dry weights of spring wheat at three levels of added P (OP, LP=25 kg P ha^{-1}, HP=50 kg P ha^{-1}) and four treatments of added soluble C. Same letters above bars within a given P treatment indicate that C treatments are not significantly different at the 5% level of probability.

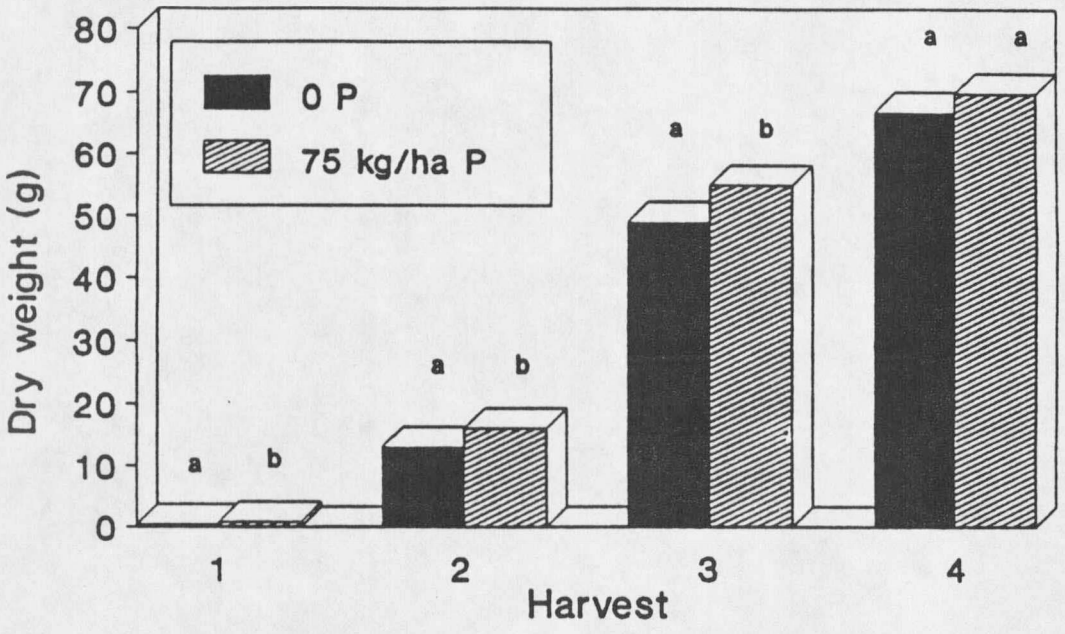


Figure 17. Average dry weights of sorghum-sudan grass at two levels of P and four harvests. Same letters above bars of a given harvest indicate that P treatments do not differ significantly at the 5% level of probability.

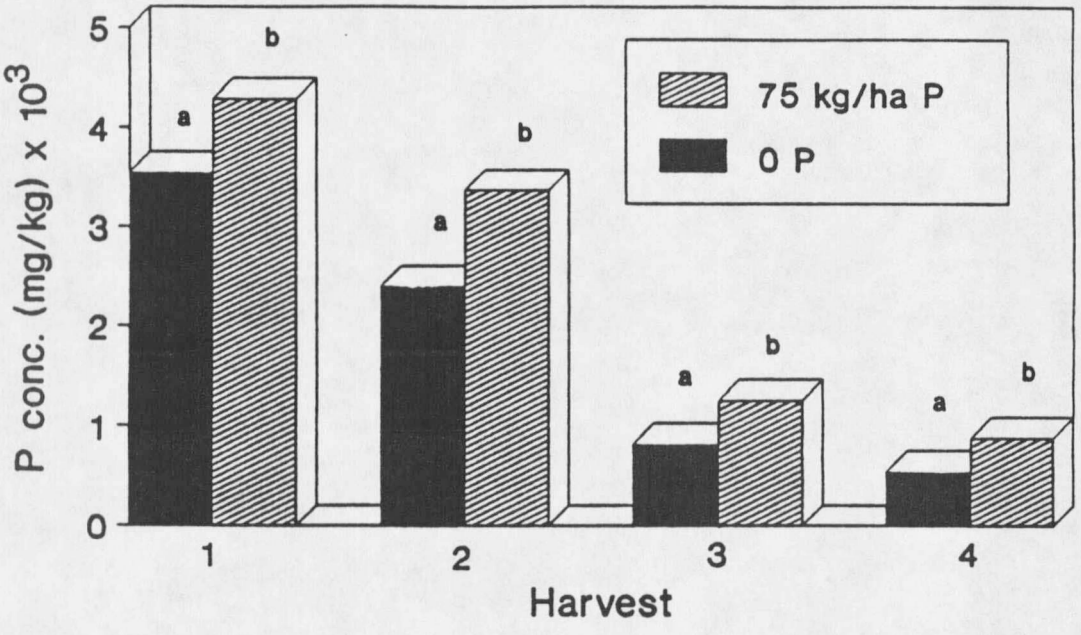


Figure 18. Average P concentrations of sorghum-sudan grass at two levels of P and four harvests. Same letters above bars within a given harvest indicate that P treatments do not differ significantly at the 5% level of probability.

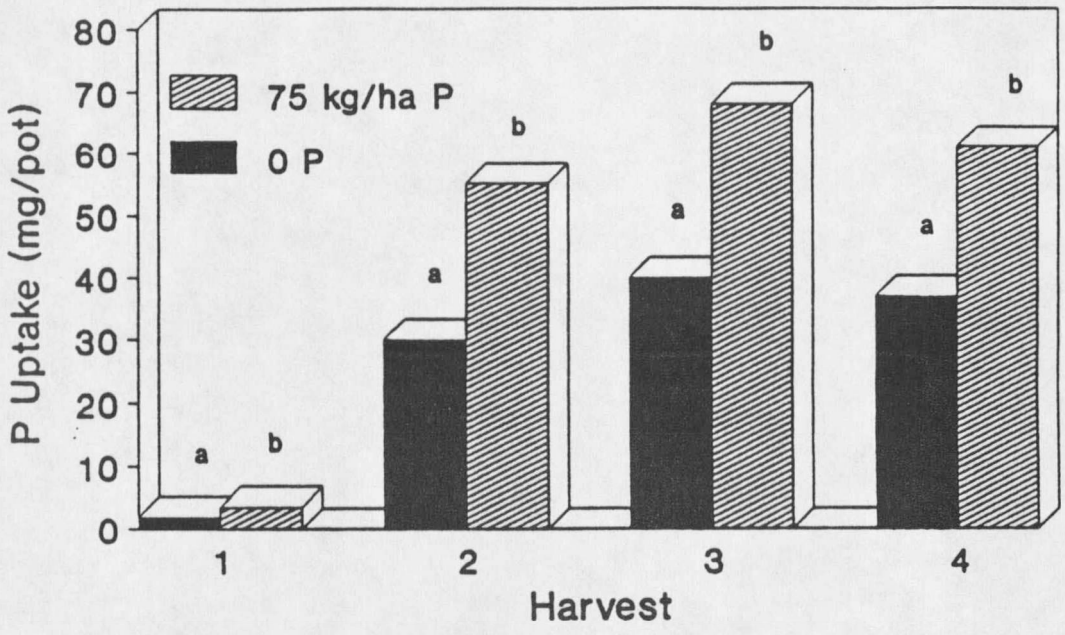


Figure 19. Average P uptake of sorghum-sudan grass at two levels of P and four harvests. Same letters above bars within a given harvest indicate that P treatments do not differ significantly at the 5% level of probability.

differences had been observed, then soil solutions would have been chemically analyzed and ion activity products calculated to determine which calcium phosphate minerals were controlling PO_4 activities, and also determine if inhibition of Ca phosphate precipitation by soluble C results in higher PO_4 levels in soil solutions.

Table 14. Sorghum-sudan grass dry weights, P concentrations, and P uptake for all four harvests, in the presence of organic acids.

Harvest	C treatment	Dry weights	P concentration	P uptake
		(g)	(mg kg^{-1})	(mg pot^{-1})
1	O C	0.65 N.S.	3940 N.S.	2.58 N.S.
	Humic Acid	0.70	3730	2.69
	Tannic Acid	0.70	4070	2.88
	LSD (0.05)	0.12	720	0.59
2	O C	13.77 N.S.	2920 N.S.	40.69 N.S.
	Humic Acid	14.92	2840	43.08
	Tannic Acid	14.65	2890	43.11
	LSD (0.05)	1.53	340	5.46
3	O C	57.95†	1020 N.S.	59.51 N.S.
	Humic Acid	50.98	1040	52.75
	Tannic Acid	47.22	1060	50.47
	LSD (0.05)	7.32	240	11.24
4	O C	67.12 N.S.	690 N.S.	45.57 N.S.
	Humic Acid	68.55	710	49.21
	Tannic Acid	70.62	750	52.10
	LSD (0.05)	15.16	130	8.61

† Significant treatment differences at $P < 0.05$.
N.S. No significant treatment differences.

Conclusion

The results from soil incubation experiments and the plant available P experiment with spring wheat suggest that organic C constituents may prevent the fixation of soluble phosphate from P fertilizers. This is probably accomplished by inhibiting the precipitation of phosphate minerals and/or the adsorption of phosphate onto oxide and clay surfaces. Inhibition of phosphate fixation can potentially increase the bioavailability of P, thus resulting in greater efficiency of P fertilization.

CHAPTER 5

CHARACTERIZATION OF FULVIC ACID ISOLATED FROM A WATER SOLUBLE EXTRACT OF WHEAT STRAW

Introduction

The addition of plant residues to soils is beneficial in conserving water, reducing soil erosion, stabilizing soil temperatures, and enhancing the nutrient status of soil systems (Steiner and Tanaka, 1990; Grossl and Inskeep, 1990; Larson et al., 1978; Skidmore and Siddoway, 1978; Hayes and Kimberlin, 1978; Power and Legg, 1978). Plant residues rapidly decompose upon incorporation into soil. The water soluble fraction of residues is the first portion to undergo decomposition, successively followed by structural carbohydrates and lignin (Parr and Papendick, 1978; Stott and Martin, 1990). Of the total C added as crop residues, about 60 to 80% is converted to CO_2 and returned to the atmosphere in one year, 5 to 15% is incorporated into microbial biomass, and the remainder is stabilized as new humus (Stott and Martin, 1990). Humic substances are found in most natural environments and are important because of their involvement in mineral weathering, mobilization and transport of metal ions, sorption of pesticides, formation of stable soil aggregates, and the cation exchange capacity of soils (Stevenson, 1982).

The interstitial waters of soil dissolve a large fraction of plant litter, with 25 to 40% of the plant organic matter being solubilized by water in 24 hours (Thurman, 1985). In addition, plant residue leachate may contribute significantly to the dissolved organic carbon (DOC) content in groundwaters and streams (Thurman, 1985). The DOC of the initial leachate of plant material is comprised of simple carbohydrates (oligimers) and colored organic acids that are less than 1000 molecular weight and similar to fulvic acids found in aquatic systems (Thurman, 1985).

Presently, there is very little information on the chemical nature of the DOC from plant residues, especially on the hydrophobic acid portion. This material may play an important role in soil chemical processes; for example, it has been shown to inhibit the formation of insoluble Ca phosphate solid phases (Grossl and Inskeep, 1991; Inskeep and Grossl, 1991) that may result in greater efficiency of P fertilizers. It may also be a precursor to aquatic humic substances. The objectives of this study were to: (i) isolate the fulvic acid portion of a water soluble extract of wheat straw (*Triticum aestivum L.*), (ii) chemically characterize the wheat straw extract fulvic acid (WSE-FA), and (iii) compare this material to fulvic acids originating from other sources in order to better understand the nature of DOC from wheat straw residues.

Materials and Methods

Isolation of the Fulvic Acid Portion of a Water Soluble Extract of Wheat Straw

Forty liters of a 3% (w/w) wheat straw solution (30 g ground wheat straw per liter distilled-deionized water (DD-H_2O)) were prepared, stirred (6.17 revolutions s^{-1})

for two hours using an overhead motor-driven polyethylene propeller, and then allowed to stand for 24 hours. The solution was centrifuged (13,170 g for 30 minutes) to remove coarse debris and filtered through 0.45 micron filters (Gelman Sciences, Inc., Ann Arbor, MI) using a high pressure filtration system (Millipore Corporation, Bedford, MA). The filtered solution was acidified with concentrated HCl to pH 1 and allowed to stand for 24 hours. The solution was then filtered through 0.45 micron filters (Gelman) to separate the "humic acid" precipitate from the soluble wheat straw extract (WSE).

The WSE-FA was collected on a 1L glass chromatography column (Spectrum Medical Industries, Inc., Los Angeles, CA) packed with Amberlite XAD-8 resin (Rohm and Haas Company, Philadelphia, PA). Prior to the addition of the WSE to the column, the resin was precleaned with 0.1 *N* NaOH and soxlet extracted with acetone and methanol similar to preparative techniques reported by Thurman and Malcolm (1981). To ensure that the resin was clean, about 100 L of DD-H_2O was passed through the column, specifically until no C (<0.5 mg L^{-1} C) could be detected by a C-analyzer (Dohrmann CD-80, Santa Clara, CA). The hydrophobic WSE-FA concentrate, collected on the column, was washed with several bed volumes of DD-H_2O to remove Cl^- and was removed from the column with 0.1 *N* NaOH. The isolated WSE-FA was passed through a 0.1 L column (Spectrum) containing Bio Rad AG-MG-50 cation exchange resin (Bio Rad Laboratories, Richmond, CA), and the hydrogen saturated fulvic acid solution was freeze dried and the final product stored under desiccation. Samples were collected and analyzed for soluble C during each step

of the separation and isolation process to determine percent of initial C in each fraction.

Characterization of the WSE-FA

The elemental content (C, H, O, N, P, S, and ash) of the WSE-FA was determined by Huffman Laboratories (Golden, CO). Total acidity and carboxyl functional group content were measured using the barium hydroxide and calcium acetate exchange methods, respectively (Schnitzer, 1982).

The molecular weight (MW) of the WSE-FA was estimated using three techniques: (i) 4-point vapor osmometry (VPO) using tetrahydrafuran as a solvent (Huffman Labs, Golden, CO; Aiken and Malcolm (1987)); (ii) gel-filtration using prepacked progel-TSK columns (Supelco, Inc., Bellefonte, PA) and polyethylene glycols as molecular weight markers; and (iii) ultrafiltration where a solution containing 0.8 m*M* C as WSE-FA was passed through YCO5 (500 MW), YM1 (1000 MW) and YM3 (3000 MW) filters (Amicon, Division of W.R. Grace and Co., Beverly, MA).

A UV-visible absorption spectrum was recorded on the WSE-FA dissolved in 0.05 *N* $NaHCO_3$, which also allowed determination of an E_4/E_6 ratio by comparing absorbance values at 465 and 665 nm (Schnitzer, 1982). The cross-polarization magic angle spinning (CPMAS) ^{13}C-NMR spectrum was performed by the Colorado State University Regional NMR Center, Fort Collins, CO. The Fourier transform infrared (FTIR) spectrum was measured on WSE-FA packed in KBr pellets.

Results and Discussion

The DOC content for the water soluble extract of wheat straw (WSE) was 840 ± 40 mg L^{-1}. Nine percent of the WSE was the humic acid precipitate, 75% was the hydrophilic acid fraction that was not retained on the column containing the Amberlite XAD-8 resin, and the remaining 16% was the hydrophobic fulvic acid portion which was freeze dried, yielding a total of 4.7 g (Table 15). In comparison, Caine (1982) as cited by Thurman (1985) reported a DOC of 500 mg/L for the leachate of willow leaves, birch leaves, and sedge (equal weight plant matter and water), of which 50% was comprised of very water soluble simple carbohydrates and oligomers, and the remainder was colored organic acids less than 1000 molecular weight and similar to aquatic fulvic acids. The WSE had a higher DOC content, probably since the plant material we used was finely ground and passed through a 2 mm sieve, while the material used by Caine (1982) consisted of whole plant tissue. The variation in DOC distribution between the leachate isolated by Caine (1982) and the WSE may have been due to differences in the tissue parts used for each study, for example, straw versus leaf material.

Aquatic humic substances are related to plant litter leachate in that they originate from plant and soil systems, where they are leached by interstitial water of soil into rivers and streams (Thurman, 1985). Approximately 50% of the DOC in stream and river waters is comprised of humic substances, of which 80 to 95% are fulvic acids (Malcolm, 1990; Thurman, 1985). This is similar to the distribution of DOC of plant litter leachate reported by Caine (1982) and consistent with the predominance of fulvic acid in the hydrophobic fraction of the wheat straw extract.

Table 15. Percent of initial total water soluble wheat straw C separated into hydrophilic, fulvic, or humic acid fractions.

Fraction	Percent of initial DOC
Hydrophilic acids (nonadsorbing onto XAD-8)	75
Fulvic acid	16
Humic acid	9

The elemental chemical composition, carboxyl and phenolic functional group contents of the WSE-FA are listed in Table 16, along with the values for fulvic acids isolated from other sources (Thurman, 1985; Malcolm, 1990; and Stevenson, 1982). It should be noted that the soil fulvic acid fractions and soil fulvic acid listed in Table 16 were both extracted with NaOH, and represent the part of the alkaline soil extract which remains soluble at pH 1 (Malcolm, 1990). The difference between soil fulvic acid fractions and soil fulvic acids is that the former was not passed through a column containing XAD-8 resin (Malcolm, 1990). The WSE-FA had a C content of 55.5%, O content equal to 35.7%, and H equal to 5.2%. The chemical composition of the WSE-FA is more similar to aquatic than soil fulvic acids. Aquatic fulvic acids have a C content greater than 50% and an O content between 30 and 40%, while the C content for soil fulvic acids is generally less than 50% and the O content is usually higher than 40%. The atomic H/C ratio, related to the percent saturation of C atoms or aliphatic C content, was 1.12 for the WSE-FA. It is slightly lower than average values reported for fulvic acids from streams and rivers (1.16) and groundwater (1.19), but higher than fulvic acids from bogs and marshes (1.06) (Thurman, 1985). The C/N

Table 16. Elemental and functional group content of the wheat straw extract fulvic acid (WSE-FA) and fulvic acids from varying sources.

Source	C	H	O	N	Ash	−COOH	−Phenolic OH
			%			mmol charge g^{-1}	
WSE-FA	55.5	5.2	35.7	0.8	2.4	5.5	0.7
Groundwater							
Thurman (1985)	59.7	5.9	31.6	0.9	1.2	5.1-5.5	1.6
Malcolm (1990)	55.4	4.2	35.4	1.8	0.4	--	--
River Water							
Thurman (1985)	51.9	5.0	40.3	1.1	1.5	5.5-6.0	1.5
Malcolm (1990)	53.6-56.6	4.2-5.1	36.1-40.9	0.7-1.4	0.3-2.2	--	--
Lake Water							
Thurman (1985)	52.0	5.2	39.0	1.3	5.0	5.5-6.2	0.5
Marsh, Swamp & Bog Water							
Thurman (1985)	50.0	4.2	--	1.2	3.0	5.0-5.5	2.5
Soil Fulvic Acid							
Malcolm (1990)	49.8-50.5	4.0-4.4	42.6-43.2	2.7	0.8-14.7	--	--
Soil Fulvic Acid Fractions							
Malcolm (1990)	44.2-53.8	3.5-6.0	35.2-44.2	1.0-3.7	0.9-2.3	--	--
Stevenson (1982)	40.0-50.0	4.0-6.0	44.0-50.0	<1.0-3.0	--	5.2-11.2	0.3-5.7

ratio of the WSE-FA was 81:1, indicating that it was depleted in N compared to aquatic (C/N ratio ranging from 45 to 55:1) and soil fulvic acids (average C/N ratio 20:1) (Thurman, 1985). The higher N content in aquatic and soil fulvic acids may be related to N accumulation processes such as adsorption of N containing organic matter onto suspended organic matter (O.M.) and solid phase surfaces, precipitation of N containing O.M., and mineralization of N by heterotrophic bacteria (Thurman, 1985).

The carboxyl functional group content of the WSE-FA was 5.5 meq g^{-1}, which is within the range of values reported for other fulvic acids (Table 16). The phenolic functional group content was 0.7 meq g^{-1}. This is in the lower range of values listed in Table 2. The reported pK_as for phenolic groups is > 7.0 (Thurman, 1985). Since the pH of the wheat straw extract was 4.5, phenolic groups in the extract were protonated or in their associated form, and consequently had lower aqueous solubility. This may explain the lower phenolic content of the WSE-FA and also aquatic fulvic acids.

The molecular weight of the WSE-FA determined by vapor-pressure osmometry (VPO) was 620 daltons, which was close to the predicted molecular weight of 572 daltons measured by gel-filtration. The WSE-FA molecular weight is similar to the molecular weight of aquatic fulvic acids, ranging from 500 to 950 daltons, measured by VPO using tetrahydrafuran as a solvent (Aiken and Malcolm, 1987). When ultrafiltration was used as a measure of molecular weight, about 50% of the C in the WSE-FA fraction passed through the 500 MW filter. This indicates that the WSE-FA was polydisperse, characterized by particles of varying size in a disperse phase, having

components with MW < 500 and others > 500. However, this could also be due to limitations in the filter membrane. According to the manufacturer (Amicon technical services, personal communication), filters are tested on globular proteins, and they may pass materials of similar size differing from such a protein structure. Generally, some disagreement occurs between molecular weight measurements determined by VPO and measurements by ultrafiltration and gel-filtration. The latter methods are subject to solute-membrane and solute-gel interactions that yield higher molecular weights of fulvic acids (Aiken, 1984). We noticed that 3 to 20% of the WSE-FA was retained on the higher MW filters (1000 and 3000 MW filters), which would result in an overestimation of the WSE-FA molecular weight range.

The E_4/E_6 ratio for the WSE-FA was 14.8. Although this value is higher than values reported for soil fulvic acids (6 to 8.2) (Stevenson, 1982), it is within the range of values reported for aquatic fulvic acids (5.5 to 22) (Thurman, 1985). The magnitude of E_4/E_6 ratios for humic substances is regulated predominately by the particle sizes and molecular weights of humic materials (Chen et al., 1977). Generally, the smaller molecular weight materials such as fulvic acids have larger ratios than larger molecular weight humic acids (E_4/E_6 = 2 to 5) (Thurman, 1985). The higher E_4/E_6 ratio of the WSE-FA is consistent with a fulvic acid characterized by a relatively low MW.

The CPMAS ^{13}C-NMR spectrum of the WSE-FA exhibited more structural detail than usually observed for humic substances and provided the most information on the chemical structure of the WSE-FA (Figure 20). Peak areas were integrated to

determine the percent distribution of C in the WSE-FA and compared to CPMAS ^{13}C-NMR integrated peak areas for soil fulvic acid fractions (isolated soil fulvic acids that have not been passed through a XAD-8 column), soil fulvic acid, and aquatic fulvic acids from rivers and groundwater (Table 17) (Malcolm, 1990). The distribution of C was divided into three major regions. Region one contained 28% unsubstituted aliphatic C (0 to 50 ppm); region two contained 29% aromatic C (aromatic C fraction (*f*a) = peak area of ^{13}C-NMR spectrum from 110 to 160 ppm/total peak area of ^{13}C-NMR from 0 to 190 ppm); and region three contained about 24% C in the C-O region (60 to 110). A majority of the C in the 50 to 96 ppm region has been designated as saccharide moieties within the structure of the WSE-FA that cannot be removed by chromatographic separation using XAD-8 resins (Malcolm, 1990). The remaining C was comprised of 8% due to methoxy groups (50 to 60 ppm) and 11% C due to carboxyl C groups (160 to 190 ppm). The carboxyl functional group content measured by CPMAS ^{13}C-NMR was 5.1 meq g^{-1}, which was close to the value of 5.5 meq g^{-1} determined titrametrically.

The nature of the WSE-FA as determined from ^{13}C-NMR was intermediate between that of soil fulvic substances and aqueous fulvic acids. For example, the WSE-FA had the higher aromatic C content and saccharide content characteristic of soil fulvic substances, but also the greater aliphatic content characteristic of aqueous fulvic acids (Table 17). The soil fulvic acid fractions have a higher saccharide (60 to 110 ppm) content than the soil fulvic acid, since they have not been exposed to the XAD-8 resin and still contain free hydrophilic acids. The WSE-FA may be similar to

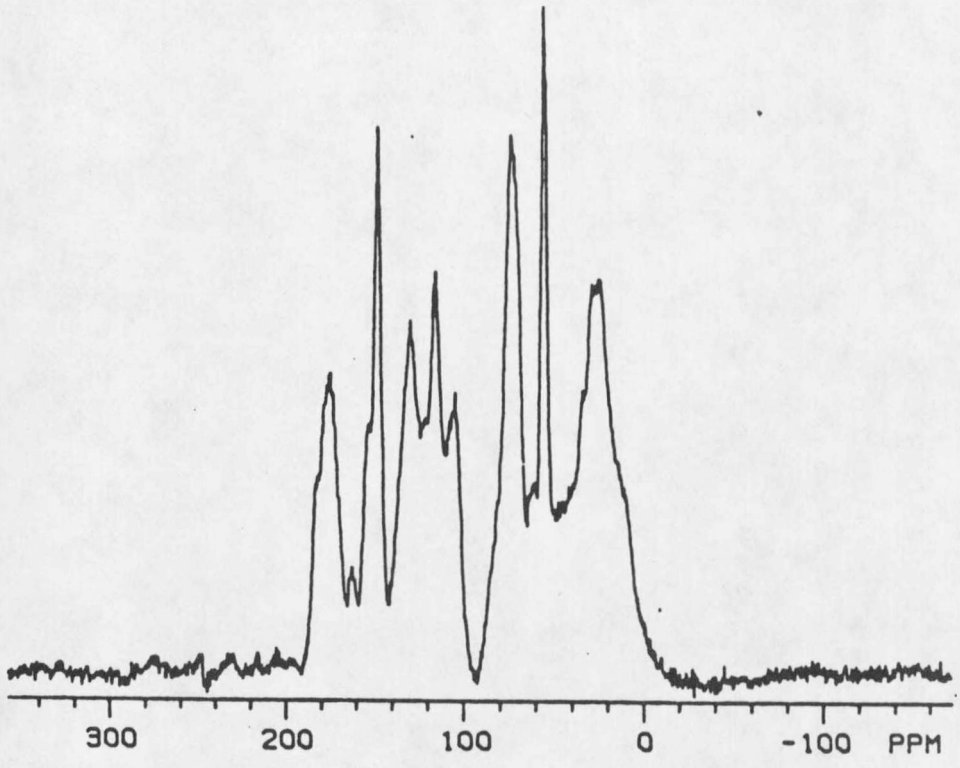


Figure 20. Solid state ^{13}C-NMR spectrum of the wheat straw extract fulvic acid (WSE-FA).

Table 17. Distribution of C in the wheat straw extract fulvic acid (WSE-FA), soil fulvic acid fractions, soil fulvic acid, and aquatic fulvic acid as determined by solid state CPMAS ^{13}C-NMR.

Sample	Percentage distribution of C within indicated ppm regions							Degree of aromaticity (%) fa
	0-50	50-61	61-95	95-110	110-142	142-160	160-190	
WSE-FA	28	8	18	6	19	10	11	29
River Water†	31-37	5-7	11-14	2-4	11-13	3-6	17-18	14-18
Biscayne Groundwater†	39	6	9	1	13	4	19	17
Soil Fulvic Acid Fractions†	13-23	3-6	17-45	5-10	5-20	2-6	10-22	6-28
Soil Fulvic Acid† (Elliot mollic epipedon)	22	5	16	4	21	5	24	26

† Malcolm (1990).

the soil fulvic acid fractions in that both are polydisperse (Stevenson, 1982). Specifically, the WSE-FA may be comprised of components varying in molecular weight which was also observed by MW determination by ultrafiltration, whereas aquatic fulvic acids are considered to be monodisperse (Aiken and Malcolm, 1987).

The FTIR spectrum for the WSE-FA (Figure 21) is typical of the spectra observed for aquatic and soil fulvic acids (Thurman, 1985). The spectrum provides little additional information about the chemical structure of the WSE-FA, but confirms the presence of hydroxyl, carboxyl, methyl, and aromatic C groups that had already been revealed through NMR spectroscopy and functional group characterization. The spectrum consisted of four strong bands at 3400 cm^{-1} (stretch of H-bonded OH), 2900 cm^{-1} (stretch of aliphatic C-H), 1725 cm^{-1} (stretch of C=O in CO_2H and ketonic C=O), and 1200 cm^{-1} (stretch of C-O in OH deformation in CO_2H). Bands at 1650 cm^{-1} and 1510 cm^{-1} indicate the stretch of aromatic C=C conjugated with C=O and/or COO− and stretch of aromatic C=C bonds, respectively.

We only compared the WSE-FA to other fulvic acids and not to humic acids. In general, fulvic acids are lower in molecular size and weight, lower in C and N content, higher in O, lower in aromaticity, and have different CPMAS ^{13}C-NMR spectra than humic acids from the same environment (Malcolm, 1990). Overall, the chemistry of the WSE-FA was most similar to aquatic fulvic acids. Therefore, the hydrophobic fulvic acid portion of the aqueous leachate of wheat straw residues may be a precursor to the formation of aquatic humic substances. Thurman (1985) reported that many aquatic humic substances are of plant origin.

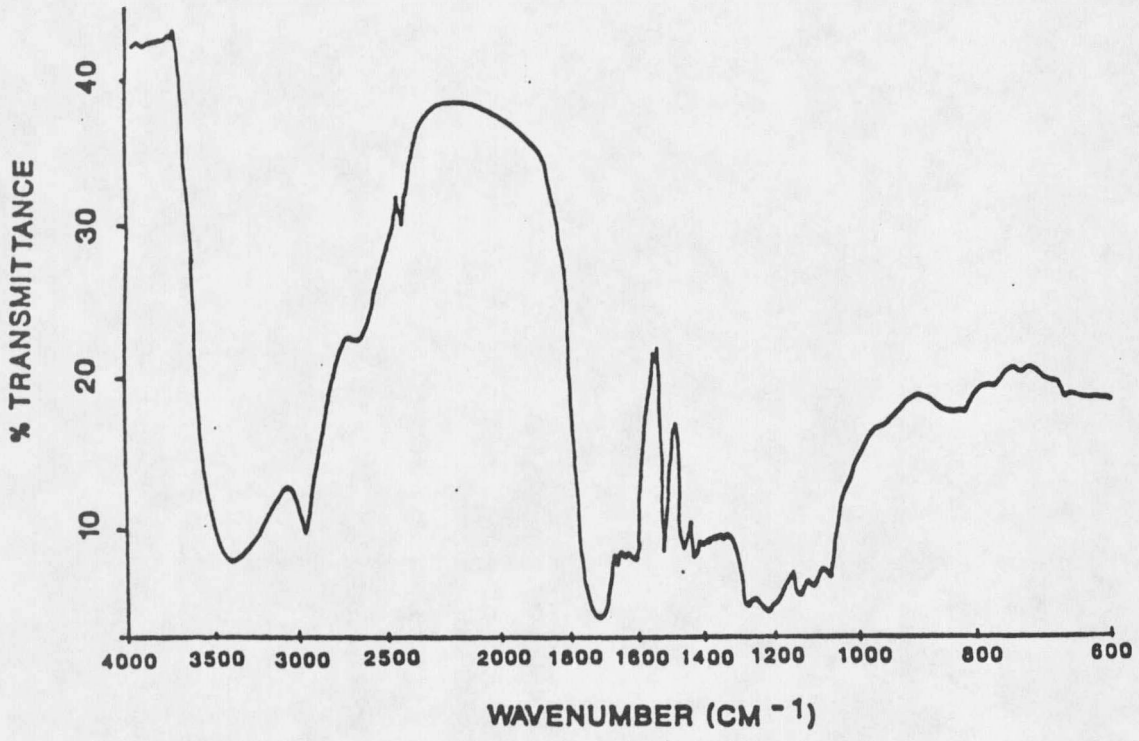


Figure 21. Fourier transform IR (FTIR) spectrum of the wheat straw extract fulvic acid (WSE-FA).

Operationally, the WSE-FA can be defined as a fulvic acid; however, in a strict sense, it is not a true fulvic acid because the source material has not undergone any secondary synthesis reactions (microbial degradation). Little information presently exists on the chemical nature of "humic" substances isolated from the DOC of plant leachates, although this material may comprise a significant portion of the DOC in the interstitial waters of soils and play an important role in soil chemical processes.

Conclusion

The fulvic acid isolated from a water soluble extract of wheat straw is representative of the hydrophobic, nonhumified fraction of the initial leachate of plant residues. The molecular weight of the WSE-FA was approximately 600 daltons. It was polydisperse consisting of particles with molecular weights greater and less than 500 daltons. Overall, the chemistry of the WSE-FA was most similar to fulvic acids isolated from aquatic environments. Characterization of the WSE-FA provides a better understanding of soil chemical processes occurring in environments associated with crop residues.

CHAPTER 6

SUMMARY

Humic, fulvic, tannic, and citric acids all inhibited the rates of DCPD and OCP precipitation. Inhibition of precipitation was due to adsorption of these organics onto DCPD and OCP crystal seed surfaces resulting in blockage of active crystal growth sites. The amount of C adsorbed as humic, fulvic, tannic, and citric acids onto DCPD and OCP, and their ability to inhibit DCPD and OCP formation is related to their functional group content, size, geometry, and orientation on the adsorbent. Precipitation of DCPD overgrew humic and fulvic acids adsorbed onto DCPD seed crystals after an induction period which was proportional to the amount of C adsorbed. Precipitation kinetics of OCP and HAP (Inskeep and Silvertooth, 1988a) in the presence of organic acids did not demonstrate overgrowth of adsorbed C onto their respective crystal surfaces. This phenomenon, and the fact that soluble organic C at levels often found in soil solutions is most effective at inhibiting HAP precipitation, may explain the metastability of DCPD and OCP with respect to HAP often observed in soil environments.

The presence of dissolved organic matter was shown to increase the solubility and availability of phosphate to spring wheat in calcareous soils. Organic acids may prevent the fixation of soluble P from solutions oversaturated with respect to DCPD

by inhibiting P retention processes. Such processes include precipitation of insoluble Ca phosphates and adsorption of phosphate onto oxide and clay surfaces.

In Chapters 2 and 3, precipitation experiments were conducted using pure Ca phosphate minerals under controlled laboratory conditions. These experiments clearly demonstrated that the presence of soluble organic C inhibited the precipitation of DCPD and OCP. However, such dramatic effects were not observed when similar organics were added to soil systems (Chapter 4). We hypothesize that this is because there are more variables to account for with soil systems than with pure mineral systems. For example, phosphate fixation in soils does not only involve precipitation reactions but also involves adsorption of phosphate onto clays, oxides, and organic matter. Phosphate can also be immobilized by the microbial biomass. Plus, in soil systems the presence of competitive foreign ions can influence phosphate precipitation and adsorption reactions. Similarly, the organic C that was added to soils may be preferentially attracted to surfaces not involved in phosphate fixation reactions, and may be altered by soil microorganisms.

Chemical characterization of a fulvic acid isolated from the water soluble extract of wheat straw suggested that it was representative of the hydrophobic, yet water soluble organic C leached from nonhumified wheat straw residues after a rainfall event. The nature of this substance was most similar to fulvic acids isolated from aquatic systems. Humic substances produced from the breakdown of plant residues will enhance the efficiency of P fertilizers by reducing the rate of formation of insoluble Ca phosphates, and potentially increase P bioavailability in calcareous soils.

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