MOLECULAR SPECIATION OF PHOSPHORUS IN ORGANIC AMENDMENTS AND AMENDED SOILS USING NUCLEAR MAGNETIC RESONANCE AND X-RAY ABSORPTION SPECTROSCOPIES

BY

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ABSTRACT

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Characterization of phosphorus (P) in organic amendments is essential for environmentally sustainable fertilization of agricultural soils. The sequential chemical extraction (SCE) technique commonly used for P characterization does not provide any direct molecular information about P species. Studies were conducted to characterize P species in organic amendments and amended soils at a molecular level. The SCE was used to fractionate P in organic amendments including biosolids, hog, dairy and beef cattle manures, and poultry litter. The extracts were analyzed for total P and P species using inductively coupled plasma - optical emission spectroscopy (ICP-OES) and solution ^{31}P nuclear magnetic resonance (NMR) spectroscopy, respectively. The relative proportions of P species in intact organic amendments and residues after each extraction, and calcareous soils amended with organic amendments and monoammonium phosphate (MAP) were estimated using the synchrotron-based P 1s X-ray absorption near edge structure (XANES) spectroscopy. The solution ^{31}P NMR provided a detailed characterization of organic P in the non-labile NaOH and HCl fractions of organic amendments, but was limited in characterizing the labile fractions of most of these

organic amendments due to their proneness to alkaline hydrolysis. The XANES analysis, however, identified the actual chemical species constituting the labile P that was only characterized as inorganic P or orthophosphates by sequential extraction and solution ^{31}P NMR. In the amended Vertisolic and Chernozemic soils, XANES analysis estimated 'soluble and adsorbed P' as the dominant P species. For the Vertisolic soil, both the unamended and soil amended with biosolids and MAP contained hydroxyapatite (HAP). In addition, soil amended with biosolids, hog and dairy manures contained β-tricalcium phosphate (TRICAL), a more soluble CaP than HAP. TRICAL was found in all amended soils except in that amended with hog manure, while HAP was present in appreciable amount only in the control. Overall, the combination of techniques used in these studies improved the understanding of P species in organic amendments and amended soils that would not have been possible with any individual technique. Technological advances in P analysis should therefore be combined with conventional chemical extraction techniques to determine the fate of P in the environment.

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INTRODUCTION

The overall objective of this dissertation was to characterize phosphorus species in organic amendments, such as biosolids and manures, and soils heavily amended with these amendments and fertilizer at a molecular level. Molecular identification of P species formed in soils with manure application will facilitate a better understanding of P release and prediction of potential loss of P from agricultural soils, which will be helpful in proposing effective mitigation strategies to reduce P loss from soil.

Repeated land application of organic amendments such as manures and biosolids can lead to accumulation of P in soils. This is because land application of these amendments to agricultural soils to supply crop requirements for nitrogen is the most economically feasible waste disposal option, which makes P balance on a farm basis difficult to achieve. Non-point source P pollution from accumulated P in agricultural soil has been linked to accelerated eutrophication in nearby surface waters (Sharpley et al. 1996; OECD 1982), which may pose potential risks to the overall aquatic organisms and humans. To address this problem, an improved understanding of P chemistry, in terms of P species in organic amendments and manure-amended soils and detailed molecular and structural characterization is essential.

Most of the studies on P forms in organic amendments and soils have been carried out in aqueous phases involving chemical extraction with variable recovery of total P. The procedure essentially involves the use of various chemical extractants that target

different forms of P in the organic amendments or soil. Sequential chemical fractionation has been widely used to characterize forms of P present in manures (Sharpley and Moyer 2000; Ajiboye et al. 2004) and manure-amended soils (Sui et al. 1999; Kashem et al. 2004). This technique involves the extraction of samples with a progressively stronger extractant which partitions P based on its bioavailability and association with cations in the samples. The orthophosphate extracted is then measured either colorimetrically or by atomic (optical) emission spectrometry. The colorimetric method for quantifying orthophosphate was originally developed for seawater (Murphy and Riley 1958, 1962) and is now routinely used in the field of soil science for P determination. This method involves the reaction of an acid molybdate with orthophosphate in the sample to form a phospho-molybdate complex. The complex is then reduced by ascorbic acid to form a relatively stable blue complex. The absorption of UV radiation by the blue complex is measured at the wavelength corresponding to its maximum absorbance (882 nm), which is directly related to P concentration according to Beer's Law. This 'molybdate-reactive P' is often assumed to be inorganic P. In the case of atomic emission spectrometry (usually involving an inductively coupled plasma), the extract containing the element of interest is nebulized into the hot plasma and converted into gaseous phase where it is atomized. The atom is then excited to a higher state and its relaxation to the ground state causes an emission of light with a characteristic wavelength, which is 213.618 nm for P. The intensity of emission is then recorded and considered to be directly proportional to the concentration of total P in the extract.

Because the characterization of manure P into labile and non-labile fractions based on sequential extraction does not give direct information on the structural environment of

the various P compounds, a molecular-scale understanding of P species in manures and manure-amended soils is therefore needed. This would provide a better approach for predicting the potential loss of P following manure application to agricultural lands.

Nuclear magnetic resonance (NMR) spectroscopy has been used in recent years for molecular-scale characterization of P in environmental samples. The basics of NMR spectroscopy can be found in various textbooks (Sanders and Hunters 1994; Canet 1996; Hornak 2006). For readers to appreciate the sensitivity and usefulness of NMR spectroscopy in speciating P in environmental samples, a description of an NMR spectrometer, and a brief discussion of the physical basics of NMR and some experimental considerations is necessary.

A typical NMR spectrometer contains a superconducting magnet with a bore. The magnet creates the magnetic field B_o and its strength is measured in Tesla (T). For example, the Buker AMX 500 MHz used for the NMR experiments reported in this thesis is 11.7 T. Next to the magnet within the bore are shims coil, which are used to homogenize the B_o field. Within the shim coil is the probe that contains an RF coil, which produces a B_1 magnetic field necessary to rotate the net magnetization vector from the spin systems about the axis of B_1. The sample (or NMR tube containing the solution) is placed within the RF coil of the probe. This coil also detects signal from the spins within the samples. Another aspect of NMR hardware is the field lock. This can be visualized as a separate NMR spectrometer within the spectrometer that corrects for any fluctuation in the B_o that may occur due to the aging of the magnet, movement of metal around the magnet and temperature changes (Hornak 2006). This lock is tuned to the resonance frequency of deuterium solvent (used to prepare the sample). It then

constantly monitors the deuterium signal and makes minor changes in the B_o magnetic field to keep the resonance frequency constant. Central to the NMR spectrometer is the computer, which controls a host of components within the machine, such as the frequency generator, pulse programmer, and RF amplifier that are connected to the RF coil. The RF source produces a sine wave of the desired frequency and the pulse programmer sets the width of the RF pulses. The computer also performs post-experiment processing of the time-domain signals recorded.

In NMR spectroscopy, unlike UV-Vis and IR absorption, nuclei, rather than outer electrons are involved. Molecules that are examined by NMR are made up of atoms. Inside each of these atoms, past the electron cloud, is a nucleus containing nucleons (neutron + proton). From the knowledge of the shell model for the nucleus, nucleons, just like electrons, fill orbitals and spin (a fundamental property of nature that occurs in unpaired protons, electrons and neutrons). Because not all nuclei possess spin, NMR spectroscopy can only be performed on any naturally abundant isotopes of elements in the periodic table with a non-zero nuclear spin, such as ^{1}H, ^{13}C, ^{14}N, ^{31}P, ^{19}F, etc. The spin can be thought of as a magnetic field with north and south poles. Therefore, when atomic nuclei that exhibit spin are placed inside a static magnetic field B_o, they form two energy states depending on the two possible orientation of the spin in the magnetic field. The low energy state occurs when the poles are aligned in N-S-N-S and a high energy state occurs at N-N-S-S. At room temperature, the lower energy state is more populated than the higher energy state as given by Boltzmann statistics [Equation 1.1].

$$N^-/N^+ = e^{-E/kT} \qquad [1.1]$$

where N^- and N^+ represent the number of spins in the higher and lower energy level, respectively. E is the energy difference between the spin states, k is Boltzmann's constant (1.3805×10^{-23} J/Kelvin), and T is the temperature in Kelvin. In an NMR experiment, absorption of photon in the radio frequency region occurs when the difference between the two energy levels is exactly equal to the energy of the photon (Carrington and McLachlan 1967). This energy (E) is related to its frequency (υ), which is called resonance frequency in this case, by Planck's constant ($h = 6.626 \times 10^{-34}$ J s) according to Equation 1.2.

$$E = h \upsilon \qquad [1.2]$$

The various spin systems in a given sample are partitioned into spin packets based on the similarity in the magnetic field they experience. The magnetic field due to these spin packets is represented by magnetization vectors, which result from the difference between the energy absorbed as the spin system transitions from lower to higher energy state and that emitted as the spin system relaxes back to the lower state. This size of this vector is proportional to the difference between N^+ and N^-. The vector sum of these magnetization vectors is referred to as the net magnetization and is important in describing pulsed NMR experiments. Before describing the common pulse sequence used in NMR experiments, it is worthwhile to describe the components of magnetization vectors and associated relaxation processes.

According to the NMR coordinate system, the external magnetic field and net magnetization at equilibrium (M_o) are both along the Z-axis. The Z component of this magnetization (M_z) is referred to as longitudinal magnetization. If the M_z is changed along the Z-axis by exposing the nuclear spin system to energy of a frequency equal to

the energy difference between the spin states, it returns back to equilibrium at a time constant called spin-lattice relaxation time (T_1). The T_1 process as a function of time t is governed by Equation 1.3.

$$M_z = M_o (1 - e^{-t/T1}) \quad [1.3]$$

It is also possible to move the M_z into the XY plane where it precesses (rotates) about the Z-axis at a frequency (Larmor frequency) equal to energy frequency of a photon that would cause a transition between the spin energy level. The resulting transverse magnetization M_{XY} begins to dephase over time due to differences in the magnetic field experienced by the each of the spin packets causing it to rotate at its own Larmor frequency. The net effect is the overlap of vectors from different spin packets, which fills the XY plane completely before returning to equilibrium along the +Z-axis. The time constant that described the return to equilibrium of M_{XY} is called spin-spin relaxation time T_2 and is governed by Equation 1.4

$$M_{XY} = M_{XYo}\, e^{-t/T2} \quad [1.4]$$

Both T_1 and T_2 processes occur simultaneously but T_2 is less than or equal to T_1 (Hornak 2006).

During the NMR experiments, the RF coils placed around the X-axis provide a pulse of alternating current, which creates a B_1 magnetic field along the X-axis. The spin system responded to this pulsed magnetic field by rotating the equilibrium magnetization vector (along the Z-axis) about the axis of B_1 at an angle θ (in degrees) according to Equation 1.5

$$\theta = \gamma B_1 \tau \quad [1.5]$$

where, γ is the magnetogyric ratio (17.25 MHz T^{-1} for ^{31}P), τ the duration of the pulse. The magnitude of the B_1 magnetic field can be calculated according to Equation 1.6

$$\upsilon_1 = \gamma B_1 \qquad [1.6]$$

where, υ_1 is the frequency of the pulsed radiation.

The rotation of the resulting transverse magnetization vector about the Z-axis dephases with time, as previously mentioned, and is accompanied by decay in the magnitude of the vector as it returns to equilibrium. This magnitude of this transverse magnetization vector as it precesses about the XY plane is detected by the RF coil and recorded as a time-domain signal (intensity as function of time) called free induction decay (FID). This time-domain signal is later converted into frequency- domain (signal intensity plotted as function of frequency) by Fourier Transformation (FT). The pulse sequence is usually repeated until an appropriate signal-to-noise (S/N) ratio is achieved. For example, a 90-FID pulse sequence involves the rotation of the net magnetization into the XY plane with a 90º pulse followed by its decay and return to equilibrium. Theoretically, the amplitude of the signal after FT (S) will depend on T_1 and delay time of the sequence (DT), as given in Equation 1.7.

$$S = k\,\rho(1 - e^{-DT/T_1}) \qquad [1.7]$$

Where *k* is a proportionality constant and ρ is the density of the spin in the sample

For quantitative analysis, complete relaxation of the excited nuclei is required as it affects the intensity of the peaks. This is usually verified by using an inversion-recovery pulse sequence. This is essentially a 180-90-FID pulse sequence in which the net magnetization is first rotated by 180º along the - Z-axis. The longitudinal magnetization is the allowed to undergo a spin-lattice relaxation (T_1) and return to

equilibrium along the +Z-axis. But before it reaches equilibrium, a 90º is applied at a certain time interval (TI) after the 180º to rotate the magnetization in the XY plane about the Z-axis. The magnitude of the magnetization then dephases over time to give an FID signal. The signal from a single pulse sequence (without repetition) is given by Equation 1.8.

$$S = k\,\rho(1 - 2e^{-TI/T_1}) \qquad [1.8]$$

If the FT signal is plotted for all TIs, the zero crossing (null point) of Equation 1.8 will occur at $TI = T_1 ln\ 2$.

Using a pulse angle less than 90º in NMR experiments reduces DT because the spin system will remain close to equilibrium. However, the smaller signal per pulse produced by employing smaller pulse angles requires more repetition of the pulse sequence to achieve the same S/N that would have been obtained using 90º and a longer DT.

The FT NMR spectrum that is the final product of one-dimensional NMR is usually expressed as chemical shift. The chemical shift phenomenon originates from the fact that electrons surrounding each nucleus in a molecule rotate about the direction of the applied magnetic field and opposes it. The effective field experienced by the nucleus is thus less than the applied field, and will vary depending on the types of the nuclei and bonds in the molecules. The resonance frequencies of various nuclei in a molecule will therefore differ depending on the strength of the applied magnetic field B_o. Therefore, to make a comparison of spectra obtained from spectrometers operating at different magnetic fields possible, the term chemical shift (δ) is used. The δ (in ppm) represents

the difference between the resonance frequency of a nucleus υ and a standard υ_{REF}, relative to the standard as given in Equation 1.9

$$\delta = (\upsilon - \upsilon_{REF}) \times 10^6 / \upsilon_{REF} \quad [1.9]$$

For solution ^{31}P NMR, the commonly used external standard is 85 % H_3PO_4. In the environmental science application of solution ^{31}P NMR, a library of chemical shifts of model organic P compounds is used to identify the compounds in the experimental spectrum.

Solution ^{31}P nuclear magnetic resonance (NMR) spectroscopy has been used successfully to characterize P in soil extracts (Dai et al. 1996; Cade-Menun et al. 2002; Turner et al. 2003), manures (Frossard et al. 2002; Turner 2004), and manure amended soils (Turner and McKelvie 2002; Turner and Richardson 2004; Koopmans et al. 2003). Solid-state ^{31}P NMR spectroscopy has also been used with limited success (Cade-Menun 2005). Solid-state ^{31}P NMR spectra of manures have a poor resolution due to the presence of poorly ordered minerals, paramagnetic impurities such as, Fe and Mn, and structural inhomogeneity of the ^{31}P nuclei in the manure samples (Hinedi et al. 1989; Frossard et al. 2002). Recent advances in the use of liquid-state ^{31}P NMR allow for identification of multiple P compounds in the complex matrices of manure extracts. These advances are in the form of improvement in the extraction procedure, signal identification, and understanding of compound degradation during extraction and analysis (Turner 2004). For example, the use of a chelator ethylenediaminetetraacetic acid (EDTA), together with alkaline extractants like NaOH has been reported to improve the recovery of total P in the sample and minimize line broadening by paramagnetic metals (Cade-Menun and Preston 1996; Turner et al. 2003; Turner 2004). Another recent

improvement is the use of appropriate signal delay time to allow for sufficient spin-lattice relaxation between scans for P compounds (Cade-Menun et al. 2002), making identification of many organic and inorganic P compounds possible.

Phosphorus speciation in the soil aqueous phase has been modelled in the past using the solubility equilibrium approach under different environmental conditions such as pH, dominant cations and solution P concentrations. This approach involves the use of mineral stability diagrams to describe the solubility of P compounds formed in the soil (Pierzynski et al. 1990). However, the complex composition of manure and the difficulty in attaining an equilibrium condition in the amended soil may complicate the use of solubility equilibra in inferring P mineralogical composition of soils (Shenker and Bloom 2005; Sharpley et al. 2005). Therefore, an *in situ* solid-state speciation technique is required.

The X-ray absorption spectroscopy (XAS) has been shown to be advantageous in studying P compounds in environmental samples due to its element specificity, *in situ* (non-destructive) investigation, determination of oxidation states, and the local chemical and structural environment of the element (Fendorf and Sparks 1996). XAS is one of the synchrotron radiation-based techniques available in only approximately 65 active synchrotron light sources around the world (Lightsources.org 2007). Synchrotron radiation is extremely bright electromagnetic radiation generated when electrons are accelerated at approximately the speed of light around a large circular path using powerful electro-magnets and radio frequency waves. When the bend magnets alter the course of the accelerated electrons, a natural phenomenon occurs in which a very brilliant and highly focused light is emitted tangentially to the circular orbit. The full spectrum of

emitted light is directed down the beamlines where the desired wavelengths are selected for different kinds of experiments. For XAS experiments, the desired wavelengths (λ) are in the X-ray region (0.01 - 10 nm) and are often expressed as energy, E (electron volt, eV) according to Equation 1.10,

$$E = \frac{hc}{\lambda} \qquad [1.10]$$

where h is Planck's constant and c, the speed of light.

The physical basis of XAS can be found in many textbooks and educational resources available on the websites of many synchrotron facilities (Fendorf and Sparks 1996; Newville 2003; Lightsources.org 2007; CLSI 2007). X-ray absorption spectroscopy generally involves the interaction of X-rays with atoms and molecules. From the knowledge of an atomic model, electrons surround a nucleus in an atom and move in quantized orbits with discrete energies. When an atom absorbs X-ray photons, a core level (K, L, or M) electron is knocked off, and the atom becomes ionized and is promoted to an excited state. The ejection of the core level electron into the continuum (unoccupied electronic states that are not localized on the absorbing atom), leaves behind an empty electronic level (core hole). The excitation process is usually followed by de-excitation, which involves the filling of the core hole by a higher level electron and subsequently, an emission of X-ray fluorescence or an Auger electron if the energy released during de-excitation knocks off another electron into the continuum. The energies of the photon absorbed or emitted photo-electron or the intensity of the emitted fluorescence is measured in a high vacuum absorption chamber. The recorded signal over a range of incident photon energies is then used to create an XAS spectrum. The

spectrum usually produces an 'absorption edge' at a photon energy that approximates the binding energy of the orbital (K, L, or M) electrons and gives information about the oxidation states, and the local chemical and structural environment of the element of interest. A typical XAS spectrum is made of the near edge-region: XANES - X-ray absorption near edge structure, making up of regions some electron volts (eV) before the edge and approximately 50 eV post edge, and EXAFS - extended X-ray absorption fine structure, ranging from 50 eV to some 1000 eV post-edge.

There are three detection modes commonly used in XAS, namely: transmission, fluorescence yield (FY), and total electron yield (TEY). In a transmission mode for example, the X-ray photon beam is fired through a sample of thickness *x* and the intensity of the beam is measured before and after passing through the sample. The absorbance (*A*) of the sample is expressed according to Beer's Law as given in Equation 1.11.

$$A = \mu x = \ln (I_0/I_f) \qquad [1.11]$$

where μ is the absorption coefficient (cm^{-1}) and is a product of absorption cross-section σ (cm^2g^{-1}) and mass density of the sample ρ (g cm^{-3}), *x* is the sample thickness, I_0 is the incident photon flux and I_f the transmitted photon flux. The transmission mode is best used at high photon energies (> 4000 eV) that can passed through the sample and for samples with a reasonably high concentration (3-5 wt-%). To avoid the distortion of the signal for experiments in the transmission mode, it is important to ensure that the sample is homogeneous and its thickness uniform over the entire area irradiated by the X-ray beam. The sample is usually ground, either pure or mixed with an inert compound of low atomic number (Z) such as boron nitride (BN), and packed into the slot of a sample holder of known thickness and covered front and back with an appropriate adhesive tape.

The second detection mode, FY, is best suited for less concentrated and thin samples. The intensity of the fluorescence signal (I_f) is proportional to absorption coefficient as a function of energy (Equation 1.12).

$$\mu(E) \alpha I_f/I_0 \quad [1.12]$$

The fluorescence signal can be recorded either as a total fluorescence yield or in energy dispersive mode in which only the fluorescence line of interest is collected. For example, the P K_α line that is emitted when a P L_3 electron drops into the K_1 hole. The minimum detectable limit of XAS fluorescence extends to about 1.0 ppm at best, depending on the beamline energy and detector. For experiments in the FY mode, the sample can be sprinkled on a tape but must be dilute and sufficiently finely powdered for a meaningful measurement to be made. Uniform sample thickness and small particle size are not required, but an homogenous distribution of the element of interest is still necessary because only a small area is used for the XAS measurement. At high concentration of the element of interest, self-absorption may be observed in the fluorescence spectrum. This effect is caused by the fact that as the energy is scanned across the absorption edge on the spectrum of the element of interest, its absorption cross section increases. As a result, the penetration depth of the incident X-rays decreases from ~ 5 μm before the absorption edge on the spectrum to ~ 1 μm above the edge, and a smaller amount of sample is analyzed. This decrease in signal intensity due to a smaller amount of sample analyzed partially compensates for the increase in the absorption due to the additional excitation path of the element of interest. However, the net result is a non-linear distortion and a suppression of the amplitude of the intense features in the measured XAS spectrum. The self-absorption effect can be minimized, in theory, by either decreasing the sample

thickness, or diluting the concentration of the samples. The former does not really work because no matter how thinly the sample is spread on the tape, the penetration depth would still decrease as we scan across the absorption edge. A sample of < 0.1μm thickness would have to be created and making such thin samples requires specialized tools. Sample dilution is a more promising option to minimizing self-absorption problem. Certain XAS data analysis programs, such as, ATHENA and SixPack now contain routines with which the self-absorption effect can be eliminated from the fluorescence spectra (Ravel and Newville 2005).

The other main detection method used in XAS is TEY, especially for light elements with low fluorescence yield. The total electron yield that is collected in this mode includes the initial photo-electron created by the excitation process and any Auger electron created by various decay processes of the core-hole excited state. This is achieved by applying a positive bias voltage to a collection wire located in front of the sample and measuring the drain current that flows back into the sample. Similar to FY detection, the intensity of the TEY (I_e) is proportional to $\mu(E)$ of the sample. TEY is a surface sensitive technique with the escape depth of the electron from the sample being less than 0.1 μm. Because the penetration depth of the incident X-rays is always greater than the escape depth of the electron, self-absorption effects is not observed in TEY spectra. A common problem with TEY measurement, however, is the charging of the sample with low concentration of the element of interest. This creates a strange irreproducible background features resulting in a low signal relative to the background. Whenever charging of the sample occurs, XAS measurement should be made in FY mode.

For all measurement modes in XAS, it is desirable to obtain a high signal-to-noise (S/N) in the spectrum. Since the random noise in the data decreases with √N, it is worthwhile to collect multiple scans to get better quality data. In addition, noise in FY is largely photon related and can be minimized by collecting for a longer period at each point.

Analysis of the XAS spectra is crucial in identifying the chemical species present in the sample. For the XANES spectra, qualitative and quantitative analyses are commonly used. The qualitative analysis involves the use of the spectral features of reference compounds as a fingerprint to identify their presence or absence in the spectra of the experimental samples. To obtain an estimate of the proportion of the species present in the sample, quantitative analysis is usually carried using statistical methods involving a combination of principal components analysis (PCA) and target transformation (TT), and least-squares linear combination fittings. The PCA uses a multivariate statistical procedure to identify the number of independent orthogonal components that constitute the sample spectra while TT identifies the actual chemical species (Beauchemin et al. 2002; Beauchemin et al. 2003). The success of these techniques largely depends on the quality and distinctiveness of the spectra of both reference compounds and samples.

The feasibility of applying XANES spectroscopy in P speciation of organic amendments and soils has been demonstrated (Hesterberg et al. 1999; Peak 2002; Beauchemin et al. 2003; Toor et al. 2005; Sato et al. 2005; Lombi et al. 2006). While this is a relatively new technique, many efforts are currently being devoted towards

unravelling P speciation and distribution in various environmental samples using this technique (Ingrid Pickering, Personal Communication)

All the studies carried out to achieve the objective of this dissertation will be presented in the subsequent chapters (Chapters 2 to 5). Beginning from Chapter 2, the modification of the NMR methodology to demonstrate that centrifugation is a required step in acquiring well-resolved NMR spectra is presented. This is followed by a detailed speciation of organic and inorganic P in sequential extracts of various organic amendments using ICP-OES and NMR and speciation of the intact (unextracted) and residues after each sequential extraction steps using XAS in Chapter 3. Chapter 4 deals with P speciation in calcareous soils heavily amended with some of the organic amendments characterized in Chapter 3, and fertilizer. Chapter 5 deals with an evaluation of quantitative data analysis used in XAS. Specifically, the least-squares linear combination fitting was explored further to get an estimate of accuracy of the proportions of P species obtained from the fitting. The final chapter presents the synthesis and summary of all the chapters with practical implications of the finding and recommendations for future studies.

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2. IS CENTRIFUGATION REQUIRED FOR ACQUIRING WELL-RESOLVED SPECTRA IN NMR SPECTROSCOPIC ANALYSIS OF MANURE P?

2.1 Abstract

In characterizing phosphorus (P) in manures using solution ^{31}P nuclear magnetic resonance (NMR) spectroscopy, it is necessary to maximize P recovery and minimize hydrolysis of the extracted P species to obtain a good spectrum. One of the ways of optimizing the NMR signal is to concentrate P in manure extract by lyophilizing and then re-dissolving the dried extract in smaller volume required for NMR analysis. This trial was carried out to obtain well-resolved spectra for hog manure extract re-dissolved in NaOH-EDTA solution, with varying concentrations of NaOH (0.0, 0.25, 0.5, and 1.0 M) and a fixed concentration of EDTA (0.1 M). Solutions were analyzed with and without centrifugation. Poorly resolved spectra were obtained when NaOH concentration was less than 1.0 M, and peaks in non-centrifuged extracts were broader than those in centrifuged extracts. The extract re-dissolved in 1.0 M NaOH-0.1 M EDTA, with centrifugation, produced the spectra with the best resolution - sharpness and separation of the peaks. This trial concluded that centrifugation of the re-dissolved extract is necessary to reduce the viscosity of the solution and slows down molecular tumbling of the ^{31}P nuclei, thereby minimizing line broadening. It is therefore recommended that

centrifugation of ample re-dissolved extract should be done prior to NMR analysis, especially when the total P concentration in such sample is low.

2.2 Introduction

Solution ^{31}P nuclear magnetic resonance (NMR) spectroscopy is a technique that has advanced the understanding of organic P in organic amendments like manures (Cade-Menun 2005; Turner et al. 2005). In order to characterize P using NMR technique, it is often desirable to extract organic phosphorus in a form suitable for subsequent speciation. An ideal extractant for the chemical characterization of organic P compounds should maximize recovery and minimizes alteration of chemical structure. Organic P compounds are usually stabilized by association with mineral components such as hydrous iron or aluminum oxides, either directly or through polyvalent bridging cations such as calcium or ferric ion (Turner et al. 2005; Celi and Barberis 2005).

A mixture of NaOH and EDTA is the most common single-step extractant used for the extraction of organic P in manure. The NaOH in this mixture creates electrostatic repulsion by increasing the negative charge of both organic and mineral components and replaces polyvalent bridging cations with less effective Na^+, while EDTA increases organic P recovery by chelating metal cations (Russell 1988; Bowman and Moir 1993; Cade-Menun and Preston 1996). Apart from the recovery of organic P in the sample, NaOH also raises the pH of the extract above 13, thereby ensuring a consistent NMR signal because the position of the signal of relative to the external reference used in solution ^{31}P NMR (85 % H_3PO_4) is highly dependent on pH. This is due to the degree of

protonation of the orthophosphate, since protons shield the ^{31}P nucleus from the magnetic field thereby changing its resonance frequency (Crouse et al. 2000). For example, at a pH greater than the pK_{a3} of the H_3PO_4 (12.67), orthophosphate exist more as PO_4^{3-} than as HPO_4^{2-}. This will cause less shielding of ^{31}P by protons, thereby resulting in a consistently large positive chemical shift relative to the reference compound (H_3PO_4).

Several studies have shown that the alkaline pH required for NMR analysis of manure extracts can result in the hydrolysis of labile organic P fractions like phospholipids and RNA, and polyphosphates in manures (Lienweber et al. 1997; Turner et al. 2003; Turner 2004). Turner (2004) reported that using a 1.0 M NaOH solution improved spectral resolution in extracts of broiler, swine, and dairy manures, but this improvement was compromised by the loss of resonances from phospholipids and polyphosphates. The author suggested that this hydrolysis may be minimized by using 0.25 M NaOH-EDTA (Turner 2004). However, in a trial NMR analysis of 0.25 M NaOH-50 mM EDTA extract of manures (Ajiboye 2004, unpublished data) using the method of Turner et al. (2003), only inorganic orthophosphate was identified in the NMR spectra of these extracts. This result suggested possible hydrolysis of organic P into inorganic P after re-dissolving the freeze-dried powder in NaOH prior to NMR analysis. For an alkaline-mediated hydrolysis, the concentration of the NaOH is an important factor. For example, Lienweber et al. (1997) attributed the greater peak intensities of orthophosphate and orthophosphate monoesters in 0.5 M NaOH solution than those in 0.1 M NaOH to alkaline hydrolysis of orthophosphate diester at higher NaOH concentration and preferential extraction of orthophosphate diester at lower NaOH concentration. Turner (2004) also reported the degradation of phospholipids and polyphosphates in 0.5

M NaOH-50 mM EDTA extracts of cattle and swine manure but these species were significant components of 0.15 M NaOH-50 mM EDTA extracts.

Optimization of the P concentration in the sample to be analyzed is also essential to obtain well-resolved NMR spectra. To achieve this, the practice is to concentrate the extract by lyophilizing and then re-dissolving the freeze-dried powder in the NMR tube prior to analysis (Turner et al. 2003; Turner and Leytem 2004; Turner et al. 2005). Sufficient samples need to be re-dissolved to obtain a good signal and this may result in a viscous solution, that increases line broadening due to a decreased rate of molecular tumbling of the spin system in the magnetic field (Turner et al. 2005). Earlier ^{31}P NMR studies of manure and soil extracts included centrifugation of the solution prior to signal acquisition in the sample preparation (Newman and Tate 1980; Cade-Menun and Preston 1996), but recent studies have excluded this step (Turner et al. 2003; Turner 2004; Leytem et al. 2004; Maguire et al. 2004). So far, the effect of centrifugation on the resolution of NMR spectra has never been investigated in detail.

The objective of this experiment was to examine if centrifugation is a required process for acquiring well-resolved ^{31}P NMR spectra of organic amendments and to test the optimum concentration of alkaline solvent that minimizes the hydrolysis of organic P.

2.3 Materials and Methods

A 2 g sample of freeze-dried hog manure was extracted with 40 mL of 0.25 M NaOH-50 mM EDTA solution in a 50 mL centrifuge tube for 4 hr on a horizontal shaker at room temperature. The extract was centrifuged at ~12 500 × g for 15 min. A 5 mL

aliquot of the supernatant was digested in H_2SO_4-H_2O_2 according to Akinremi et al. (2003) and analyzed for total P using inductively coupled plasma optical emission spectroscopy (ICP-OES). Phosphorus recovery by the NaOH-EDTA mixture was estimated by comparing the total P in the NaOH-EDTA extract with that in the freeze-dried manure. The remaining supernatant extracts were rapidly frozen at - 76 ˚C in a Fisher ultralow freezer and lyophilized in a Virtis Consol 25LL freeze dryer over a period of one week.

Four different concentrations of NaOH ranging from 0.0 M - 1.0 M in the mixture of NaOH-EDTA were used to re-dissolve the freeze-dried extracts prior to NMR analysis. These concentration used were 0.0 M NaOH-0.1 M EDTA, 0.25 M NaOH-0.1 M EDTA, 0.5 M NaOH-0.1 M EDTA, and 1.0 M NaOH-0.1 M EDTA. A 0.25 g of freeze-dried extract was re-dissolved in 4.5 mL of the solution + 0.5 mL D_2O. One set of the re-dissolved extracts was pipetted into a 10 mm NMR tube without centrifugation, while another set was pipetted into the tube after centrifugation at 12,500 × g. A model phytic acid (Ca-salt) dissolved in 1.0 M NaOH-0.1 M EDTA was also analyzed with and without the manure extract to enhance the understanding of this dominant organic P in the sample matrix. The NMR spectra were acquired on Bruker AMX 500 MHz spectrometer equipped with a 10 mm broadband probe, which was tuned to a resonance frequency of 202.456 MHz for ^{31}P. The higher sample volume in the 10 mm tube compared with the 5 mm tube used in the trial experiment (Ajiboye 2004, unpublished data) was expected to increase the signal-to-noise (S/N) (Cade-Menun 2005). A 45-FID pulse sequence with a relaxation delay of 2.0 s was used. This relaxation delay has been reported to be adequate for sufficient spin-lattice relaxation between scans for most of the

P species in manure extracts (Turner 2004; Turner and Leytem 2004). The NMR signal emanating after each pulse sequence was acquired for 0.4 s. A total of 10, 000 to 33, 000 scans (excitation-relaxation cycle) were acquired for amendments and 40-80 scans for pure compounds. During the experiment, the temperature of the probe ranged between 20-25 ºC. Chemical shifts of the peaks were determined in ppm relative to 85 % H_3PO_4 and assigned to P species as reported in the literature (Turner 2004; Turner and Leytem 2004). The resulting spectra of hog manure extracts in different concentrations of NaOH and the model organic P compound (phytic acid) were plotted with a 2.0 Hz line broadening. All the NMR spectra were analyzed using SpinWorks 2.5.4 (Marat 2006) and the chemical structures were drawn using ISIS^{TM}/Draw 2.5 (MDL 2002).

2.4 Results and Discussion

The amounts of P in hog manure sample extracted by the 0.25 NaOH-50 mM EDTA was 64 ± 2 % of the total P based on the ICP-OES measurement of H_2SO_4-H_2O_2 digest of the manure. This value is comparable with P recovery by alkaline extract of swine manure reported by Turner (2004). However, Leytem et al. (2004) reported P recovery of up to 97 % in the 0.5 NaOH-50mM EDTA extracts of manures from swine fed with low-phytate grains. Recovery of organic P in extracts depends on the dominant cation in the manure. For example, Turner et al. (2005) reported that calcium phytates are insoluble in alkaline solutions, whereas iron and aluminum phytates are insoluble in acids. The hog manure used in the current experiment has been reported to be dominated

by Ca (Ajiboye et al. 2004). This result suggests that the likely formation of Ca phytate in this sample might have contributed to the low recovery of P in the NaOH used.

The NMR spectra of hog manure extracts re-dissolved in different concentrations of NaOH are shown in Fig. 2.1 - 2.4. Re-dissolving the freeze-dried extracts in EDTA alone resulted in a very broad orthophosphate peak at approximately 6.2 ppm (Fig. 2.1) with poorly resolved peak in the monoester region and pyrophosphate (4.5 ppm). The monoester region appeared to be better resolved with centrifugation but not sufficient to identify the species. In the spectra of manure extracts re-dissolved in 0.25 M NaOH-0.1M EDTA, the orthophosphate peak was broader in the solution analyzed without centrifugation than the one with centrifugation (Fig. 2.2). A broad pyrophosphate peak was also present in the non-centrifuged sample but not in the centrifuged sample. Instead, two peaks were identified around -1.0 ppm and - 2.96 ppm, corresponding to deoxyribonucleic acid (DNA) and degradation products of pyrophosphate, respectively. DNA is an example of an orthophosphate diester with two C moieties per P atom and is easily hydrolysed in alkaline extract; hence it can be identified only when the extract is redissolved in a less concentrated NaOH solution. Lienweber et al. (1997) also observed that NaOH concentration affected the appearance of the peaks in the NMR spectra. Their spectrum of 0.1 M NaOH extract of liquid hog manure showed three distinctive peaks at 1.5 ppm, 0.4 ppm and – 0.3 ppm for the diester-P. However at the higher concentration of 0.5 M NaOH, the peak at 0.4 ppm disappeared and the one at 1.5 ppm was reduced, and a small pyrophosphate peak emerged at –5 ppm. This appearance of pyrophosphate was attributed to the hydrolysis of diesters (Condron et al. 1985; Lienweber et al. 1997).

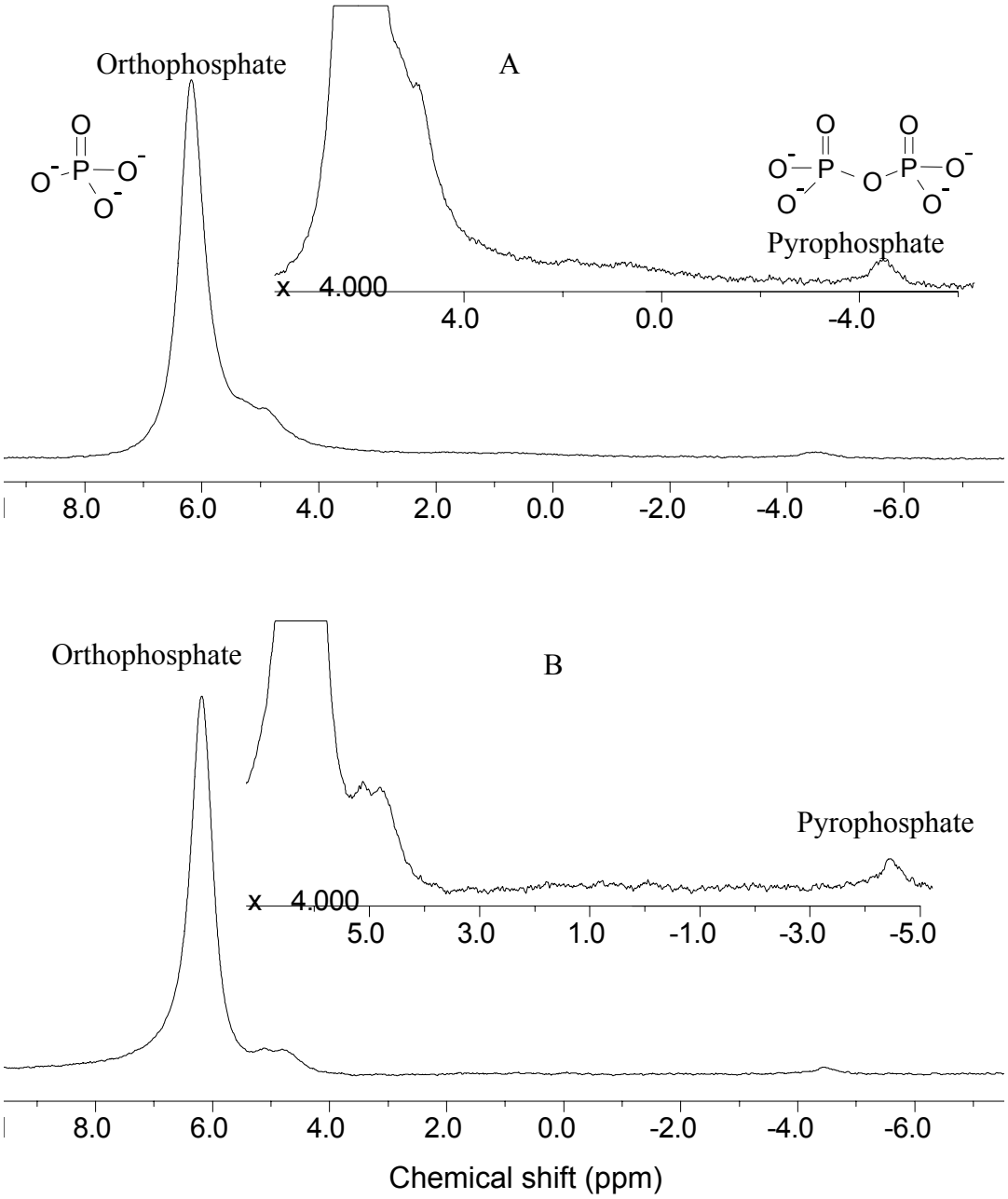

Figure 2.1 Freeze-dried hog manure extracts re-dissolved in 0.1M EDTA alone (no NaOH). (A) without centrifugation, and (B) with centrifugation at ca. 12500 × g. Orthophosphate chemical shift was approximately 0.5 ppm relative to 85% H_3PO_4 at 0 ppm prior to calibrating it to 6.2 ppm

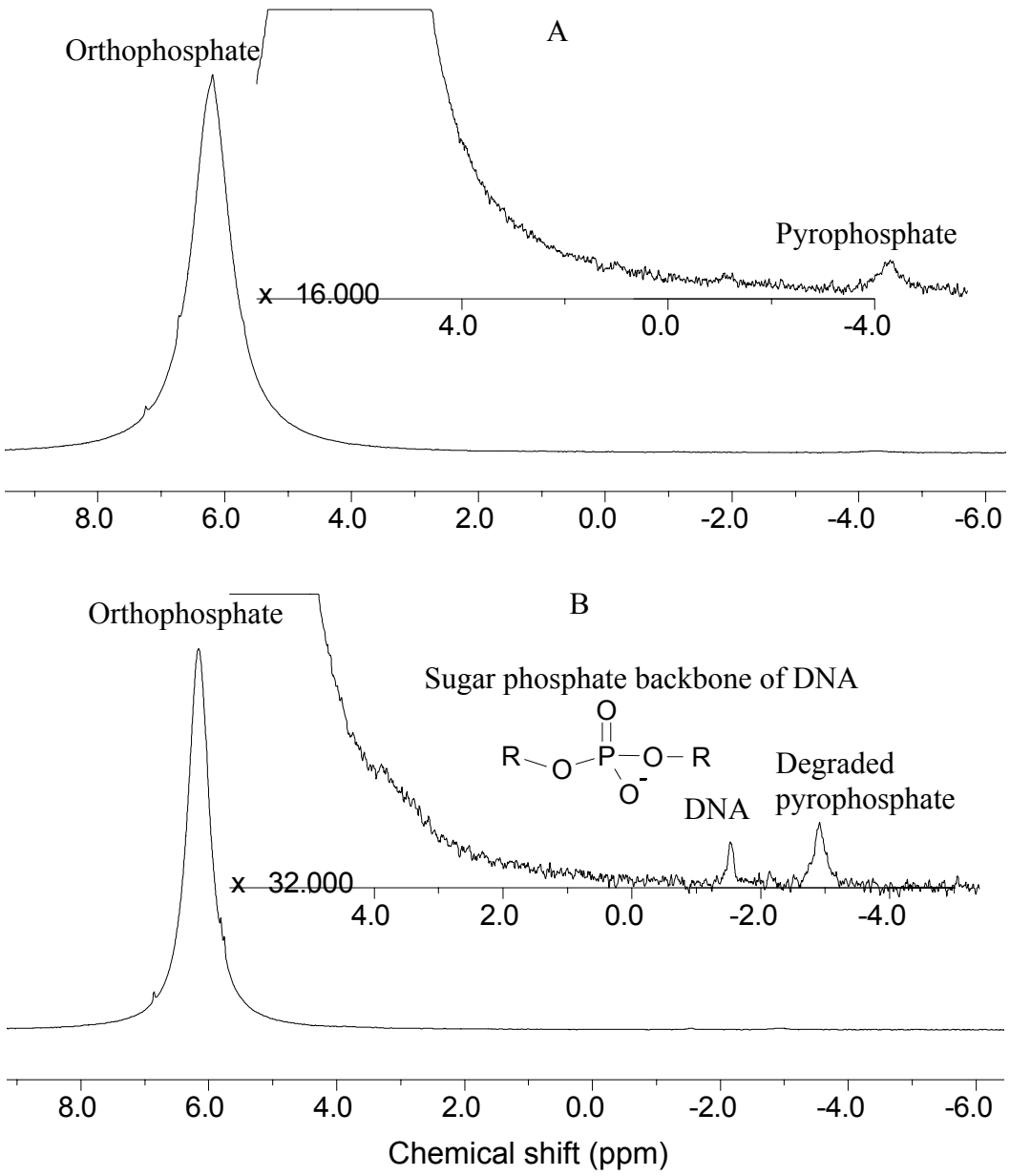

Figure 2.2 Freeze dried hog manure extracts re-dissolved in 0.25 M NaOH-0.1M EDTA (A) without centrifugation, and (B) with centrifugation at ca. 12500 × g.

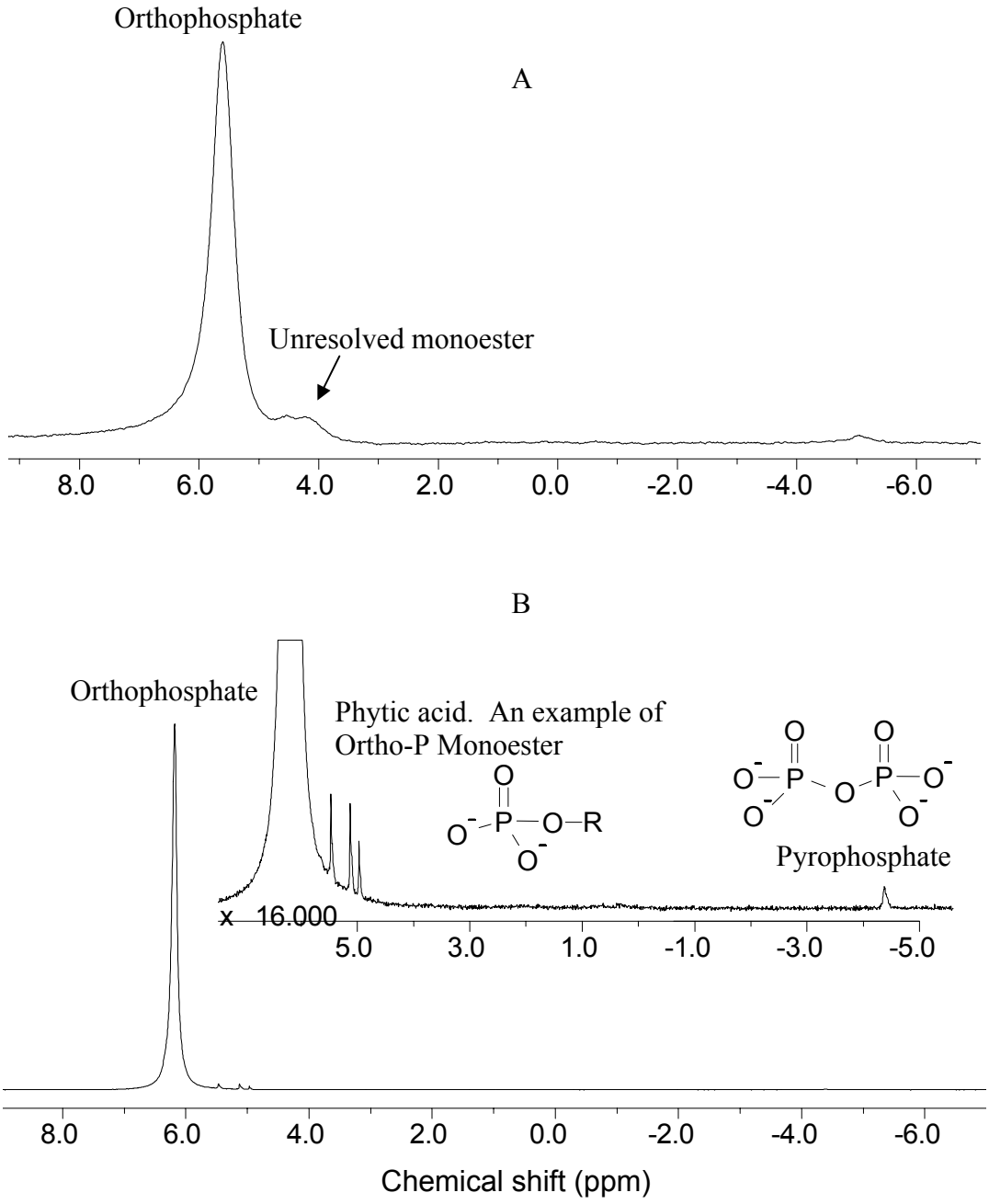

Figure 2.3 Freeze dried hog manure extracts re-dissolved in 0.5 M NaOH-0.1M EDTA with (A) without centrifugation, and (B) with centrifugation at ca. 12500 × g.

The ^{31}P NMR spectra of the re-dissolved hog manure extract in 0.5 M NaOH-0.1 M EDTA was not sufficiently resolved to identify other species apart from orthophosphate in the non-centrifuged sample (Fig. 2.3a). However, with centrifugation, the line width

of the orthophosphate peak became narrower and phytic acid was identified, however with an uneven baseline (Fig. 2.3b).

Using 1.0 M NaOH-0.1M EDTA to re-dissolve the freeze-dried extract produced the best resolved spectra (Fig. 2.4). Not only was the orthophosphate peak sharper compared with other solutions, the pyrophosphate peak was also well resolved with or without centrifugation. The phytic acid peaks were not well resolved from the orthophosphate peak without centrifugation but, with centrifugation, there was an increase in the signal height and separation of phytic acid peak from the orthophosphate. However, the P_2 nucleus associated with the C2 position of the inositol ring was not visible in the spectrum (Fig. 2.4b). The most well-resolved spectra obtained by re-dissolving the freeze dried extract in 1.0 M NaOH - 0.1 M EDTA compared with other solutions may be due to a pH effect. Turner (2004) and Turner and Leytem (2004) also reported that a strong 1.0 M NaOH solution and EDTA improved the ^{31}P NMR spectral resolution for extracts of swine manure and broiler litter.

Paramagnetic impurities are generally known to cause rapid relaxation of the ^{31}P nuclei in the extract thereby resulting in line broadening. The addition of EDTA to the extracts was meant to aid complexation with paramagnetics like Fe and Mn in the extract (Turner and Richardson 2004). In this experiment, rapid relaxation of the ^{31}P NMR signal was still observed in spite of the addition of the EDTA. Well resolved spectra were obtained only by using the supernatant obtained after centrifuging the re-dissolved extracts at 12 500 × g for 15 mins. Cade-Menun and Preston (1996) in investigating the effects of extractants on delay times and peak saturation of ^{31}P NMR spectrum used centrifugation as part of the sample preparation steps. The centrifugation step was

excluded from the Turner (2004) procedure possibly due to the initial micro-filtration (0.45 μm) of the extracts that was later lyophilized. However, the need to re-dissolve ample amount of freeze-dried extract in the NMR tube required to optimize the intensity of the peaks may have increased the viscosity of this solution. In this experiment, the lower viscosity of the centrifuged sample compared with non-centrifuged probably increased the rate of molecular tumbling and subsequently reduced the relaxation rate and corresponding peak width, hence well-resolved spectra. Turner (2004) also suggested that the viscosity of the extracts of dairy manure might have influenced spectral resolution more than the concentration of paramagnetics. This suggestion arose from the observed similar spectral resolution with different extracts having a wide range of paramagnetics concentration. The viscosity issue was recommended for further investigation because similar improvement in spectral resolution of solution with different concentration of paramagnetics would significantly enhance the NMR analysis of P in a range of environmental samples.

The NMR spectrum of the centrifuged sample acquired 72 hrs after preparation was similar to that acquired immediately after re-dissolving the dried extract (Fig. 2 4c) suggesting that the hydrolysis of the organic P species in the 1.0 M NaOH-0.1M EDTA solution did not occur at the later stage of NMR experimentation. The absence of other orthophosphates monoesters and diesters, even in the best-resolved spectra suggested that these species were not present in the solution and is supported by the low recovery of total P (64 %) in the initial extract.

The NMR spectra of phytic acid in NaOH-EDTA is shown in Fig. 2.5a. This model spectrum showed four peaks at approximately 1.70, 1.08, 0.54, and 0.07 ppm,

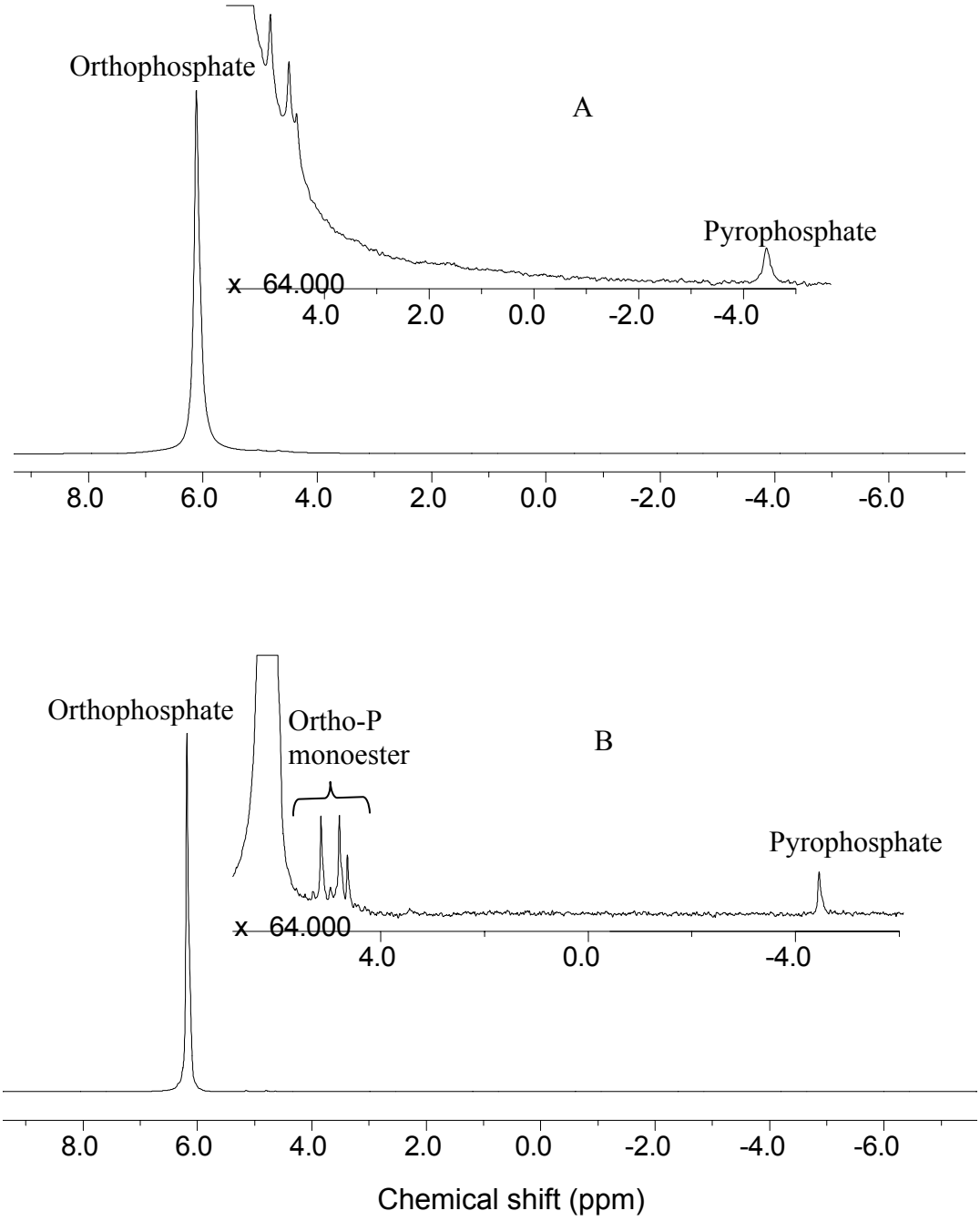

Figure 2.4 Freeze dried hog manure extracts redissolved in 1.0 M NaOH-0.1M EDTA (A) without centrifugation, and (B) with centrifugation at ca. 12500 × g.

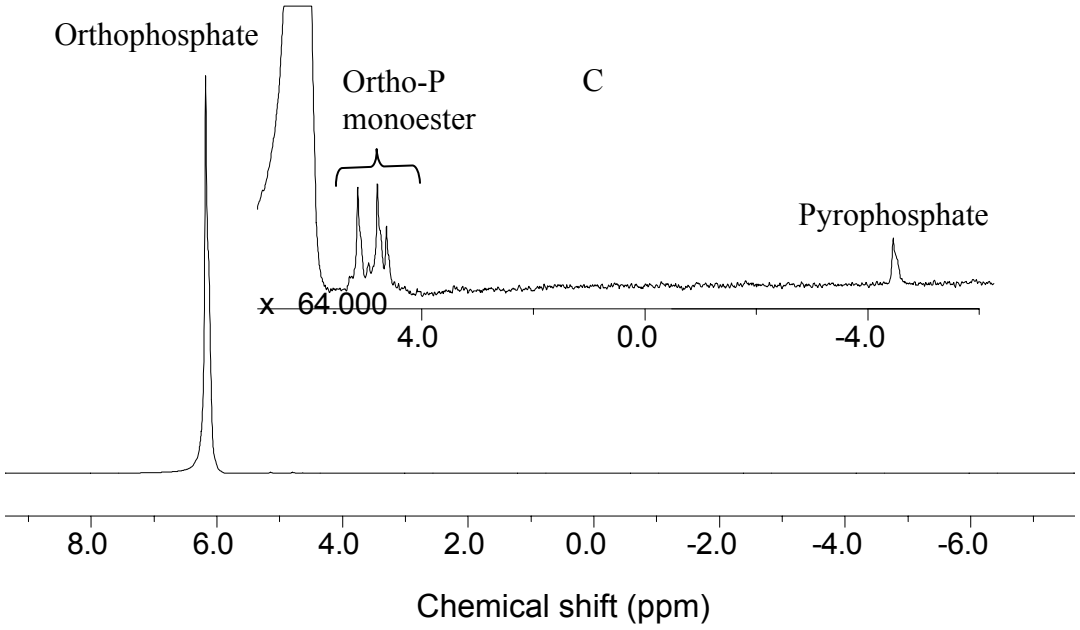

Figure 2.4 contd. (C) Freeze dried hog manure extracts redissolved in 1.0 M NaOH-0.1M EDTA with centrifugation after 72 hours.

respectively. These peaks were in the ratio of 1:2:2:1 corresponding to P_2:P_4+P_6:P_1+P_3:P_6 on the inositol ring (Fig. 2.5a). The spectrum of phytic acid in the matrix of hog manure extract in NaOH-EDTA showed a small orthophosphate peak at ~ 6.0 ppm while the four phytic acid peaks were at 5.7, 4.8, 4.4 and 4.3 ppm, respectively. The peak corresponding to P_2 on the inositol ring was closer to orthophosphate peaks than to other peaks originating from phytic acid (Fig. 2.5b). Given the position of the P_2 of the phytic acid in the presence of an orthophosphate, it is possible that the broad peak of the orthophosphate in Fig. 2.4b obscured the peak from P_2 of the phytic acid. The difference in the chemical shift of the phytic acid dissolved in NaOH-EDTA (Fig 2.5a) and that dissolved in the NaOH-EDTA with hog manure extract (Fig 2.5b) could be attributed to the pH effect.

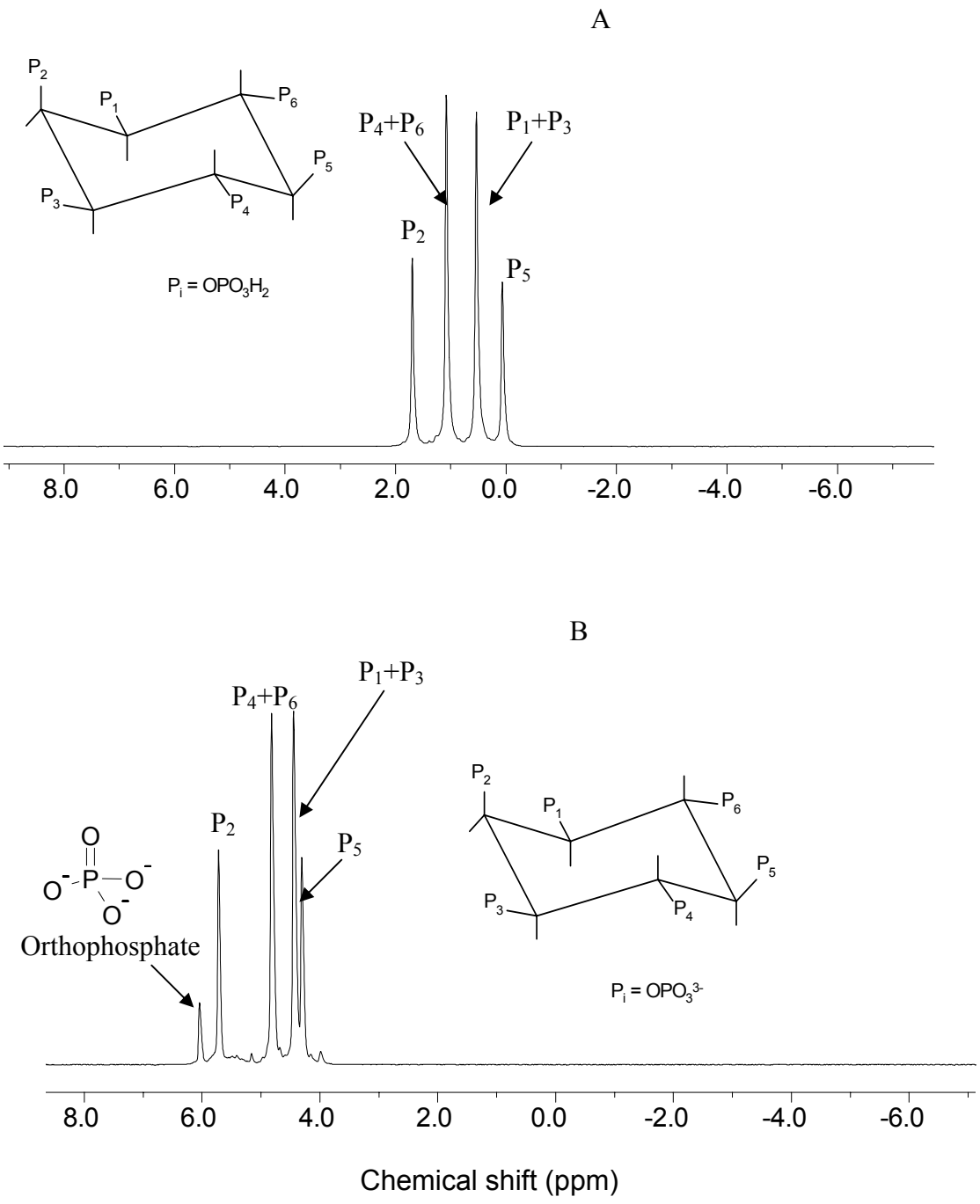

Figure 2.5 Phytic acid (Ca-salt) dissolved in 1.0 M NaOH-0.1 M EDTA and (B) Freeze dried hog manure extracts spiked with phytic acid in 1.0M NaOH-0.1 M EDTA.

The acidic pH (~2.0) of the resulting solution without manure extract might have favoured a more protonated form of orthophosphate resulting in more shielding by

protons and a small positive chemical shift relative to the external reference standard (H_3PO_4).

2.5 Conclusions

This study shows that a modification of the sample preparation for ^{31}P NMR experiment reported in the literature is necessary to obtain well-resolved spectra. This is very important given the cost of operating an NMR spectrometer. One of the key experimental considerations is how to maximize the extraction of P species while minimizing the hydrolysis of those easily degradable components. Using a mixture of 1.0M NaOH-0.1 M EDTA to redissolve the freeze dried extract proved to be best for NMR analysis of the selected manure samples. However, well resolved spectra that could be used for the chemical shift assignment of the identified peaks was only achieved with centrifugation of the re-dissolved extract. It is therefore recommended that centrifugation of ample re-dissolved extract should be done prior to NMR analysis, especially when the total P concentration in such sample is low.

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3. SPECIATION OF PHOSPHORUS IN SEQUENTIAL EXTRACTS OF MANURES BY NUCLEAR MAGNETIC RESONANCE AND X-RAY ABSORPTION SPECTROSCOPIES [*]

3.1 Abstract

The form of phosphorus in manure is an essential variable for proper management of manure for agro-environmental purposes. This study was carried out to elucidate the forms of phosphorus in various manures using state-of-the-art spectroscopic techniques. Organic amendments including anaerobically digested biosolids (BIO), hog (HOG), dairy (DAIRY), beef (BEEF) and poultry (POULTRY) litter were subjected to sequential extraction. The extracts and residues remaining after extraction were analyzed by solution ^{31}P nuclear magnetic resonance (NMR) and synchrotron-based P 1s X-ray absorption near-edge structure (XANES) spectroscopies, respectively. Most of the total P analysed by inductively coupled plasma- optical emission spectroscopy (ICP-OES) in the sequential extracts of organic amendments were orthophosphate, except POULTRY, which was dominated by organic P. While solution ^{31}P NMR provided a detailed characterization of organic P in the non-labile NaOH and HCl fractions of organic amendments P, it was limited in characterizing the H_2O and $NaHCO_3$ fractions of most

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organic amendments due the proneness of these labile fractions to hydrolysis. Furthermore, XANES analysis identified the actual chemical species of labile P as readily soluble calcium and some aluminum phosphates, which were characterized only as inorganic P or orthophosphates by sequential extraction and solution ^{31}P NMR. The combination of the three techniques used in this study provided molecular characterization of P in organic amendments that would not have been possible with any of the individual methods. This result suggests that *in situ* analysis of organic amendments P needs to be combined with conventional chemical extraction for detailed molecular characterization.

3.2 Introduction

The speciation of phosphorus (P) in organic amendments like manures and biosolids is important for understanding P solubility when these amendments are added to the soil. This characterization is essential in mitigating the adverse environmental impact of P on lake and surface water eutrophication. The simple characterization of phosphates in manure into labile and non-labile forms based on their solubility in extractants of increasing strengths according to sequential extraction technique does not provide direct information on the structure and chemical form of the various P compounds in the manure (Leytem et al. 2004). In addition, the reported discrepancies between the inductively-coupled plasma optical emission spectroscopy (ICP-OES) and colorimetry procedure commonly used for the measurement of soluble and total P in these extracts make subsequent interpretation of results and P management recommendation difficult

(Pierzynski et al. 2005). Furthermore, specific groups of compounds 'operationally' assigned to a particular fraction may be present in more than one fraction (Turner et al. 2005). There is now an increasing need to move away from this operationally-defined characterization of P to a more detailed molecular and structural characterization. For example, Maguire et al. (2004) argued that applying the sequential extraction method to manure without knowing the forms of P extracted at each step may create a potential problem in interpretation, due to the extraction of phytate P and organic P by extractants other than NaOH. Chen et al. (2002) also argued that characterization of organic P pool into bioavailable P based on solubility may be misleading because plants can obtain P from the supposedly stable organic P fractions.

Detailed studies of inorganic and organic P pool in manures have been carried out recently using solution ^{31}P nuclear magnetic resonance (NMR) on alkaline extracts of manures (Cade-Menun and Preston 1996; Turner 2004; Turner and McKelvie 2002; Toor et al. 2005a; Turner et al. 2005). This technique provided direct molecular and structural characterization of organic P in alkaline solution with environmental relevance. For example, stable organic P species like phytic acid can be easily quantified and distinguished from the more labile organic species like phospholipids and other orthophosphate monoesters in NMR spectra (Turner et al. 2005; Toor et al. 2005a). However, there are several caveats in the interpretation of solution ^{31}P NMR results. Firstly, solution NMR does not provide direct information about the mineral phase of P, which is identified only as orthophosphate due to solubilization by the extractants. In addition, hydrolysis of some labile organic P species into orthophosphate in the alkaline extract may lead to the underestimation of organic P pools in the manure (Turner 2004;

Turner and Leytem 2004). Finally, the recovery of polyvalent cations in the extract used for NMR analysis does not provide a direct evidence of their association with either the organic or inorganic P pools. On the other hand, the solid-phase NMR is more limited than solution NMR for studying P pools. This is due to poor spectral resolution (broad peaks) resulting from chemical shift anisotropy and the presence of paramagnetic ions (Cade-Menun 2005) in the latter. Therefore, solution NMR may need to be combined with other solid-phase spectroscopic methods to augment the understanding of P pools in manures.

The use of X-ray absorption spectroscopy (XAS) technique to characterize P pool in manure offers some advantages over other methods involving chemical extraction. This is because minimal sample preparation is required to isolate an element of interest and the possibility of transforming phosphate species with extractants is eliminated. In addition, the near edge region of this technique provides information on the local chemical and structural environment and oxidation states of the element, and if the probing is carried out at a wide energy range above the binding energy of the element of interest, allows for determination of co-ordination numbers and bond distances (Hesterberg et al. 1999). The capability of X-ray absorption near-edge structure (XANES) spectroscopy to identify P species in soils and manures has been recently demonstrated (Peak 2002; Beauchemin et al. 2003; Toor et al. 2005b; Sato et al. 2005). For example, Toor et al. (2005b) identified dicalcium phosphate (DCP) as the dominant P species in litter of broilers fed with normal diet or reduced non-phytate P (NPP) and a mixture of DCP and hydroxyapatite (HAP) in litters of turkey fed with NPP. These authors also reported that water removed a part of DCP in broiler litter while HCl was effective in removing phytic acid in broiler and a

mixture of phytic acid and HAP in the turkey litter with reduced NPP. Sato et al. (2005) reported that weakly adsorbed and relatively soluble P species like DCP dominated the P 1s XANES spectrum of poultry manure. These authors also observed that the application of this poultry manure to an acidic soil, in the short-term, resulted in dissolution of FeP from the soil, adsorption of P onto the surface of other minerals, and formation of DCP, while prolonged application resulted in the disappearance of FeP and formation of more stable tricalcium phosphate.

To date, a direct link between dissolved P species identified by NMR and those in unextracted manure or remaining in the residue has not been investigated. Our hypothesis is that identification of P species that are removed by a particular extractant or those remaining in the residues of the sequential extraction procedure by NMR and XAS will be a significant advancement of the knowledge of the sequential extraction technique and may improve the characterization of P into labile and non-labile fractions of environmental relevance. The objective of this study was to combine the sequential chemical extraction with nuclear magnetic resonance and X-ray absorption spectroscopies to provide a detailed molecular speciation of P in organic amendments.

3.3 Experimental Methods

3.3.1 Sample Preparation

Five types of organic amendments comprised of biosolids (anaerobically digested sewage sludge), hog, dairy cattle, and beef cattle manures and poultry litter collected from different locations around Manitoba, Canada. These amendments were lyophilized

and stored prior to analysis. The total P and cations - Fe, Al, Mg, and Ca in the freeze dried amendments were determined by inductively-coupled plasma optical emission spectroscopy (ICP-OES) after H_2SO_4-H_2O_2 digestion (Akinremi et al. 2003). The description and chemical properties of these manures are shown in Table 3.1. Six replicates of each amendment were then subjected to sequential extraction using progressively stronger extractants according to Ajiboye et al. (2004). One set of three replicates of each extractant was analyzed for total P, molybdate reactive P, organic P (by difference), and total cations and the other set of three replicates was rapidly frozen at -81ºC using a Fisher ultra-low temperature freezer and then lyophilized using a ModulyoD freeze dryer (Thermo Electron Corp. Milford, MA) (Fig. 3.1). The residue remaining after each extraction stage was also lyophilized. Prior to total P analysis and rapid freezing, dilute HCl was added to the $NaHCO_3$ extracts to dissolve the carbonates and 1 M or 10 M NaOH added to the HCl extract to neutralize the carbonic acid formed upon dissolution of the carbonates in the manure.

3.3.2 NMR Analysis

The solution ^{31}P NMR analysis was conducted using a modification of the Turner and Leytem (2004) method. Specifically, 0.25g of lyophilized powder was redissolved in 4.5 mL 1.0M NaOH-0.1M EDTA with 0.5 mL D_2O, centrifuged at ca. 12 000 × g for 15 min, and the supernatant pipetted into a 10 mm NMR tube. The NMR spectra of the reconstituted samples were acquired on Bruker AMX 500 MHz spectrometer with a 10 mm broadband probe operating at 202.456 Hz for ^{31}P and 500.134 for ^{1}H (for broadband proton decoupling). A 45-FID pulse sequence and a relaxation delay of 2.0 s were used.

Table 3.1 Description and chemical properties of organic amendments used

Type	Description	% Solids	Total P [z]	Al	Fe	Ca	Mg
				------ mg/kg (dry wt) -------			
Biosolids	Anaerobically digested biosolids with no phosphorus removal	32	12604	2042	1979	48396	22417
Hog	Manure from a dug pit of a sow - farrow to nursery operation	12	39833	nd [y]	nd	39542	15625
Dairy	Manure from a milking cow operation	24	13177	nd	nd	30646	7208
Beef	Manure from feedlot beef operation	31	2182	135	nd	28411	8714
Poultry	Fresh litter from the broiler/breeder pullet operation	29	14333	nd	nd	39083	4083

[z] H_2O_4-H_2O_2 digestion and ICP-OES
[y] not detected

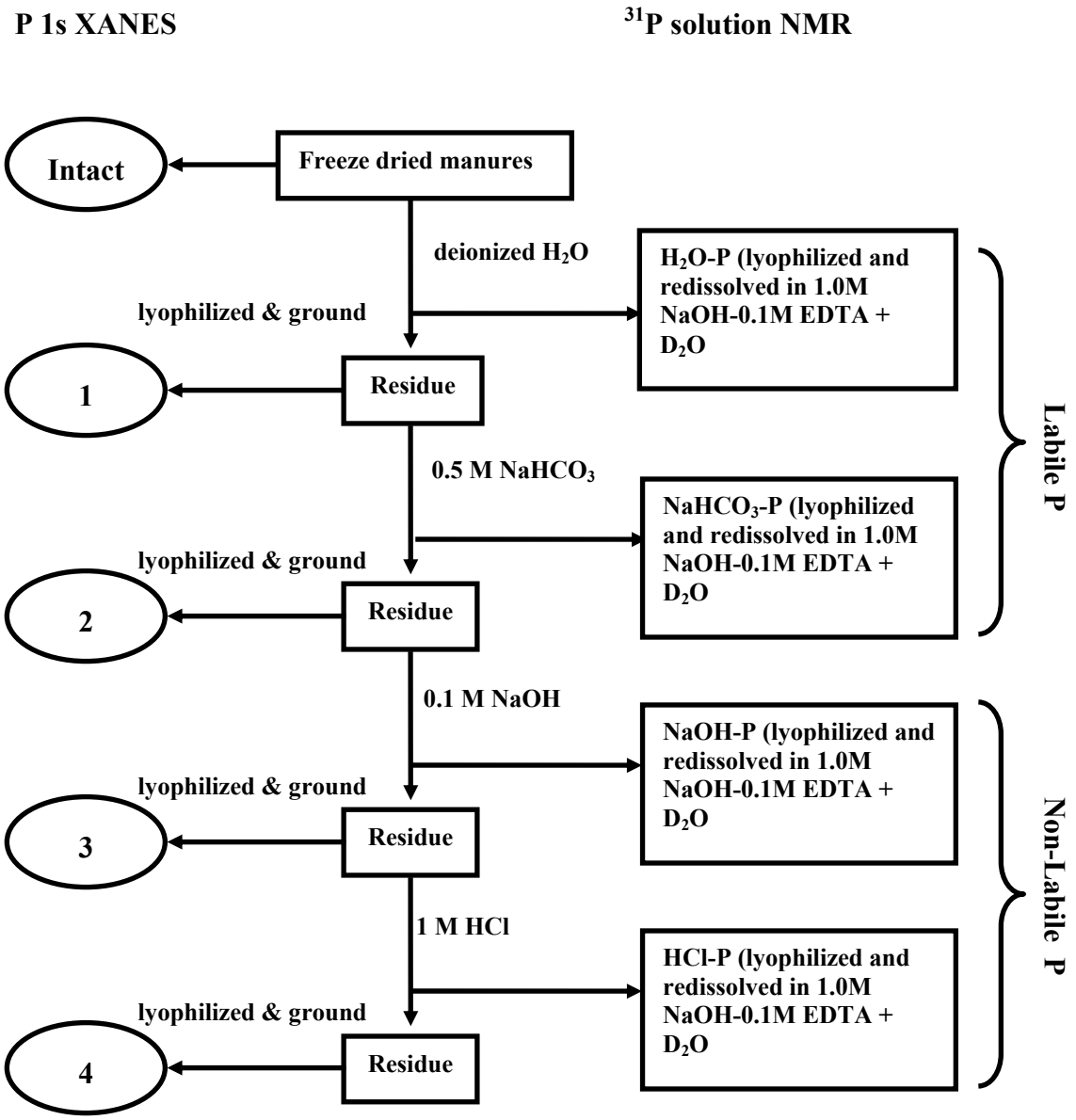

Figure 3.1 Sample preparation for NMR and XANES analyses

In order to verify that this relaxation delay was sufficient for quantitative analysis of the spectra, an inversion-recovery experiment was carried out according to McDowell et al. (2006) with a slight modification. This involved an inversion of the net magnetization

vector with 180º pulse and monitoring the recovery by waiting a variable time τ (0.001, 0.0625, 0.125, 0.25, 0.4, 0.6, 1.0, 2.0, 4.0, and 8.0 s) followed by a 90º pulse. A 20 s relaxation delay and acquisition time of 0.8 s were used. A total of 256 scans were collected at each τ. The T_1 was calculated for each of the identified peaks by regression analysis of Equation 3.1.

$$I[\tau] = I[0] + P\, e^{(-\tau/T1)} \qquad [3.1]$$

where I[τ] is the intensity of the signal at each τ, I[0] is the final intensity, and P magnitude of magnetization vector. The result showed that the spin-lattice (T_1) relaxation delay used in the current experiment was sufficient for most P species in the extracts, except for orthophosphate (Table 3.2). Further experiments using 2.0 s delay and 20 s delay (~ $3\times T_1$ for orthophosphate peak) for the two selected extracts with the highest and the least Fe concentration showed that the relaxation delay used in the current experiment did not interfere with peak identification and subsequent quantitation (Fig. 3.2). Similar studies have reported that the error associated with not meeting the T_1 for orthophosphate in manure extracts was less than 10 % (McDowell and Stewart, 2005a; McDowell et al., 2006) and did not interfere with quantitative analysis.

A total of 10, 000 to 33, 000 scans were acquired for the amendments with an acquisition time of 0.4 s per scan. The temperature of the probe ranged between 20 - 25º C. The chemical shifts of signals were expressed in ppm relative to an external standard (85 % H_3PO_4) and all spectra of manures were plotted with line broadening of ca. 2 Hz to show the relevant peaks. The inorganic orthophosphate peaks in the water, $NaHCO_3$, and NaOH fractions originally appeared upfield between 5-6 ppm compared with the literature values (Turner 2004; Turner and Leytem 2004), while the HCl fraction

appeared downfield at > 6.3 ppm. This was attributed to the difference between the transmitter frequency and the resonance frequency at 0 ppm (corresponding to the frequency of the 85 % H_3PO_4 during signal acquisition. In order to simplify peak identification, all the spectra were 'standardized' by setting the intense peak of the inorganic orthophosphate to 6.2 ppm. Experimental NMR spectra have been standardized in this way in the past so as to properly reference the identified peaks according to the literature values (Turner and Richardson 2004; Toor et al. 2005a). While this may not be a universal approach of referencing chemical shift, it however improved spectral assignment in the current study. Subsequently, the peaks in the standardized spectra were assigned to P species and organic P functional groups according to the literature values (Turner et al. 2003; Turner and Leytem 2004). The proportion of identified peaks relative to the total P in the solution was estimated by peak integration. Spinning sidebands, where present, were excluded from the peak integration. For the phytic acid, the peak area was calculated for well-resolved spectra by summing up the assigned four peaks as described in Chapter 2. In cases where the P_2 peak of the phytic acid overlapped with the orthophosphate peak, the peak was calculated by multiplying the area of the P_4+P_6 peak by 3 since this is equal to one-third of the total concentration of phytic acid. All spectra were processed using SpinWorks 2.5.4 software (Marat, 2006). Statistical analysis was not carried out because the high cost of running the NMR spectrometer prevented replication of measurements. However, in a previous study, the standard errors associated with estimating the proportion of P species by peak integration were approximately 5 % and 10 % for large and small peaks, respectively (Leinweber et al., 1997).

Table 3.2. Calculated spin-lattice (T_1) relaxation time of two sequential extracts representing the two extremes of Fe content. The T_1 was measured with an inversion-recovery pulse sequence according to McDowell et al. (2006), but using a relaxation delay of 20 s instead of 8 s and more variable times.

Extracts	Total Fe[z] (mg/mL)	T_1 (s)						
		Ortho-P	Phytic acid					Others [x]
			P_2	P_4+P_6	P_1+P_3	P_5	Mean	
HCl-HOG	0.07	7.406 (0.005)[y]	0.364 (0.13)	0.824 (0.04)	0.873 (0.02)	0.601 (0.1)	0.665	-
HCl-POULTRY	nd	7.310 (0.02)	1.288 (0.009)	1.223 (0.014)	1.226 (0.013)	1.169 (0.01)	1.226	1.411 (0.13)

[z] Concentration before freeze-drying
[y] ± standard deviation
[x] Unidentified peak

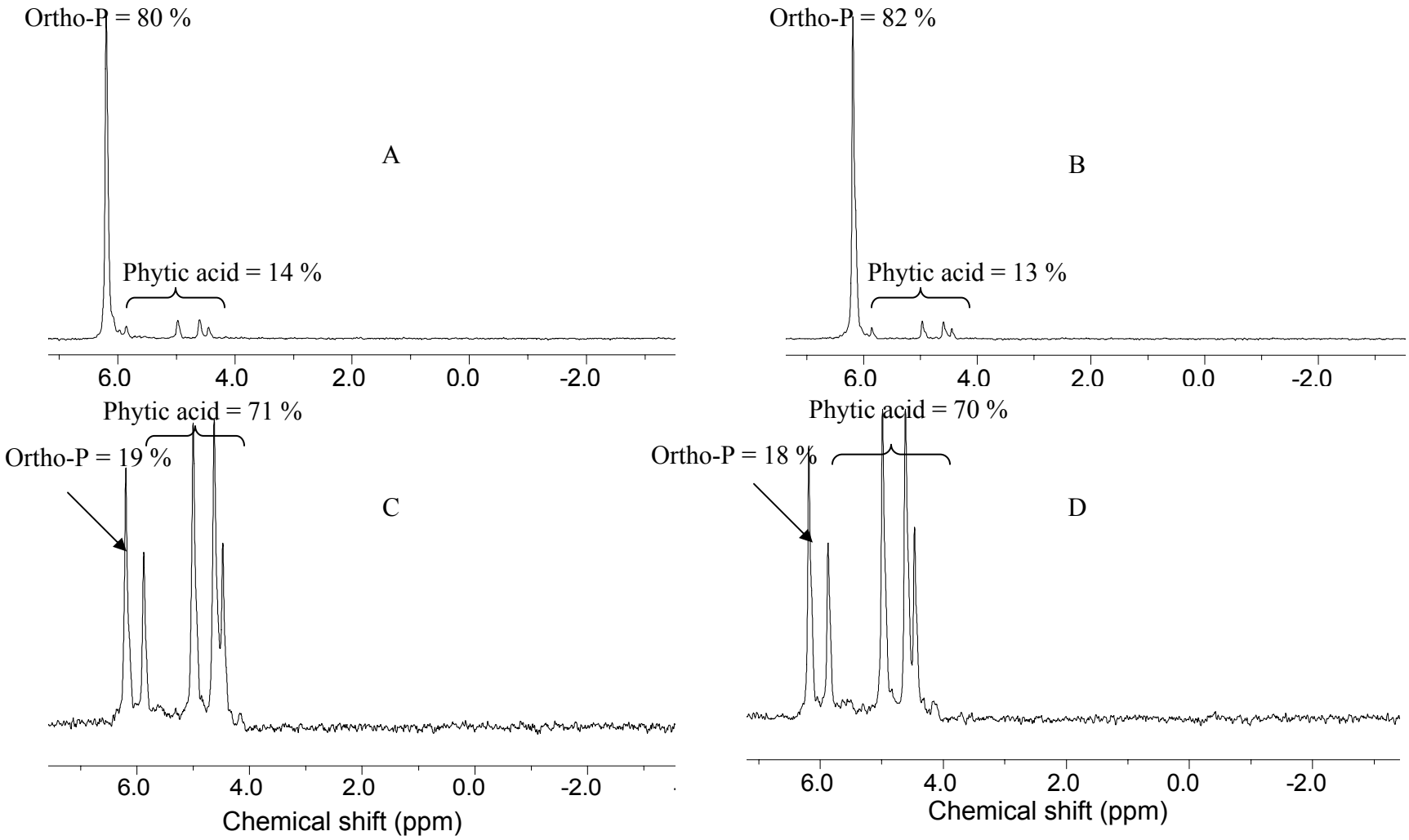

Fig 3.2. Solution ^{31}P NMR spectra of selected extracts with the highest Fe concentration acquired using (a) 2 s relaxation delay and (b) 20 s relaxation delay, (c) extract with the least Fe concentration acquired using 2 s relaxation delay and (c) 20 s relaxation delay.

3.3.3 XAS Analysis

The P 1s XAS experiment was carried out on the double crystal monochromator (DCM) beamline at the Synchrotron Radiation Centre, University of Wisconsin-Madison, housing the Aladdin storage ring operating at electron beam energy of 800 MeV or 1 GeV. The monochromator of this beamline is equipped with two InSb crystals with a photon resolution of ca. 0.9 eV. The beamline was calibrated for P K-edge by setting the white line (due to P 1s → 3p transition) of sodium pyrophosphate ($Na_4P_2O_7$) at 2152.4 eV on the energy scale (Lombi et al. 2006). The lyophilized residues and phosphate standards were ground to powder, thinly spread over double-sided conducting C tape and mounted on a stainless steel sample holder. The mounted sample was transferred via a load-lock system into the ultra-high vacuum, UHV (10^{-9} torr) absorption chamber equipped with a nine-element solid-state Ge detector for fluorescence yield (FY) measurements. Measurements were simultaneously taken in both FY and total electron yield (TEY) mode, but only the FY measurement, corresponding to P Kα fluorescence emission, are reported here. A wide range of phosphate standards were used in this study as recommended by Sato et al. (2005) who identified additional peaks in the spectra of their sample that were not found in any spectra of their FeP and CaP reference compounds. Reference phosphate compounds analyzed included FeP, CaP, AlP, MgP, NH_4P, NaP, and organic P (Table 3.3). These compounds represent prospective examples of P species that may have been present in our samples and were purchased directly from different chemical manufacturers. However, adsorbed P on $CaCO_3$, iron hydroxides and gibbsites were not included as part of reference compounds analyzed due

to the lack of distinguishing features in their XANES spectra (Sato et al., 2005). A total of 2-3 scans of P 1s XANES spectra of samples and reference standards were acquired from 2140 - 2200 eV with a step size of 0.25 eV and a dwell time of 3.0 s/pt.

The energy scale of the P 1s XANES spectrum was re-calibrated by using the edge energy offset between the energy of the white line of $Na_4P_2O_7$ analyzed with the sample and that recorded during the calibration of the beamline. This offset value was used as pre-processing parameter for all other spectra. This re-calibration step is very crucial as the absolute position of the features in the XANES spectra of samples and reference compounds is needed for reliable fingerprinting and optimization of the quantitative analysis. The FY data were averaged, background corrected by a linear regression fit through the pre-edge region and a cubic spline through post edge region. The spectra were then normalized to a unit edge jump. All the data reduction was performed using ATHENA ver 0.8.049 (Ravel and Newville 2005). Qualitative P 1s XANES analysis was carried out using fingerprinting approach as a first step in identifying the P species in the intact organic amendments. Quantitative XANES analysis was performed using linear combination (LC) fitting alone, as justified later in the chapter. The P species removed by each extractant was identified by using the point-by-point difference between the XANES spectrum of any given residue and that of the next residue in the extraction sequence as depicted in Fig. 3.1. For example, the difference between XANES spectra of unextracted (intact) and that of residue after water extraction represents the P species extracted by water (Fig. 3.1). However, the noisiness of the resulting spectra precluded reliable identification of features of interest for each sequential fraction. Therefore, only the spectra of the labile P fractions, calculated as the

Table 3.3 Summary of inorganic and organic reference P compounds used in the experiment.

Reference P compounds		Chemical formula
	FeP	
Strengite	STRENG	Orthorhombic $FePO_4 \cdot 2H_2O$
Phosphosiderite	PSIDER	Monoclinic $FePO_4 \cdot 2H_2O$
	CaP	
Dicalcium phosphate	DCP	$CaHPO_4$
Dicalcium phosphate dihydrate	DCPD	$CaHPO_4 \cdot 2H_2O$
β-tricalcium phosphate	β-TRICAL	$Ca_3(PO_4)_2$
Hydroxyapatite	HAP	$Ca_{10}(PO_4)_6(OH)_2$
	AlP	
Variscite	VAR	$AlPO_4 \cdot 2H_2O$
Wavellite	WAVE	$Al_3(PO_4)_2(OH,F)_3 \cdot 5H_2O$
	MgP & NH_4^+P	
Newberryite	NEWBER	$MgHPO_4 \cdot 3H_2O$
Struvite	STRUV	$MgNH_4PO_4 \cdot 6H_2O$
Ammonium phosphate monobasic	APM	$NH_4H_2PO_4$
Ammonium phosphate dibasic	APD	$(NH_4)_2HPO_4$
	NaP	
Sodium phosphate dibasic	SPD	Na_2HPO_4
Sodium pyrophosphate	PYRO	$Na_4P_2O_7$
	Organic P	
Phytic acid, Ca salt	PA	$C_6H_6Ca_6O_{24}P_6$
Adenosine 5'-monophosphate	AMP	$C_{10}H_{14}N_5O_7P$
Adenosine 5'-triphosphate	ATP	$C_{10}H_{16}N_5O_{13}P_3$
Deoxyribonucleic acid	DNA	
Phosphatidyl ethanolamine	PEA	
Phosphatidyl choline	PCHOLINE	

difference between spectra of unextracted organic amendments (intact) and spectra of residues after $NaHCO_3$ extraction (residue 2), were used.

3.4 Results

3.4.1 Phosphorus in Sequential Extracts of Manures by ICP and Colorimetry

Total P in the organic amendments ranged from 2.2 g kg^{-1} in BEEF to 39.8 g kg^{-1} in HOG (Table 3.1). There were differences in the amounts of P recovered in the sequential fractions among the organic amendments. In BIO, most of the P was recovered in NaOH (40 % of total P) and HCl (42 %) fractions, with lesser amount in the H_2O (2 %), $NaHCO_3$ (12 %), and residual (3 %) fractions (Table 3.4). In HOG, most of the P was found in the H_2O (25 %), $NaHCO_3$ (22 %), and HCl (28 %) extracts, with lesser amounts in NaOH (7 %) and residual (18 %) fractions. Similar to HOG, most of the P in DAIRY was in the $NaHCO_3$ (35 %) and residual (29 %) fractions, with lesser amounts in H_2O (18 %), NaOH (10 %) and HCl (8 %) fractions. In contrast to HOG and DAIRY, most of the P in BEEF was in the H_2O (53 %) and $NaHCO_3$ (35 %) fractions with lesser amounts in NaOH (8 %), HCl (4 %), and residual fractions. Most of the P in POULTRY was partitioned between H_2O (36 %) and HCl (36 %) fractions and lesser amount in NaOH (12 %), $NaHCO_3$ (9 %), and residual fractions. By summing up the P extracted in the sequential fractions from H_2O to HCl, the percent P recovery was 97 % in BIO, 82 % in HOG, 71 % in DAIRY, 99 % in BEEF, and 94 % in POULTRY. Residual P was not included in calculating the percent recovery because it was estimated indirectly as the

difference between total P (ICP-P) and summation of sequential extracts from H_2O to HCl.

The molybdate-reactive P, considered as inorganic P, was greater than ICP-P in the H_2O fractions of BIO and HOG, and HCl fractions of BIO, HOG and BEEF, resulting in a negative value for organic P estimated as the difference between ICP-P and molybdate P in these fractions (Table 3.4). The magnitude of this over-estimation of inorganic P was highest in the HCl fraction of BIO, and if all the sequentially extracted P fractions were summed up (excluding the residual), the over-estimation will be at least 11% in BIO. This is more than the reported co-efficient of variation or relative standard deviation (RSD) for ICP-P in each of the sequential extracts of BIO (Table 3.4). For those extracts where over-estimation of molybdate P occurred, the RSD was comparable between the two methods (ICP and molybdate-blue) except for HCl fraction of BEEF (Table 3.4). The highest % RSD values for both ICP-P and molybdate-reactive P in HOG indicates that this sample is more heterogeneous than other organic amendments.

3.4.2 Accompanying Cations in Sequential Extracts of Manure

A quantifiable amount of Fe was detected only in BIO, whereas Al was detected in BIO and BEEF. Total Fe and Al were not detected in other manures at similar dilutions used for total P analysis, but all the manures contained considerable amounts of total Ca and Mg (Table 3.1). The cations in the organic amendments were partitioned among the different fractions. In the BIO, the H_2O fraction was dominated by Ca and Mg, which were about 3.5 % and 4.5 % of the total Ca and Mg, respectively (Table 3.4). Furthermore, the $NaHCO_3$ fraction contained more Ca than Mg and Fe, in absolute terms.

Table 3.4 Phosphorus fractions (g kg^{-1}) and accompanying cations in sequential extracts of manures. Values in parentheses are % RSD

Fractions	Total P	P_i [z]	P_o [y]	Ortho-P	Esters		Pyro-P	Other [v]	Al	Fe	Ca	Mg	Ca:P [t]
					mono- [x]	di- [w]							
										mg kg^{-1}			
Biosolids													
H_2O	0.3 (7)	0.4 (9)	-0.1	0.3	-	-	-	-	nd [u]	nd	1.7	1.0	4:1
$NaHCO_3$	1.5 (4)	1.4 (2)	0.1	1.5	-	-	-	-	nd	0.2	1.2	0.6	1:1
NaOH	5.1 (3)	4.1 (3)	1.0	4.8	-	0.1	0.04	0.16	4.1	0.2	0.4	nd	1:16
HCl	5.3 (3)	7.3 (1)	-2.0	5.2	-	-	0.03	0.05	nd	nd	36	nd	5:1
Residual	0.4												
Hog manure													
H_2O	9.9 (8)	10 (8)	-0.1	9.9	-	-	-	-	nd	nd	1.1	5.5	1:11
$NaHCO_3$	8.6 (19)	7.9 (18)	0.7	8.6	-	-	-	-	nd	nd	2.2	4.7	1:5
NaOH	2.8 (28)	2.4 (34)	0.4	2.2	0.3	0.08	-	0.22	0.2	nd	0.3	nd	1:14
HCl	11.2 (7)	11.4 (8)	-0.2	10.7	0.5	-	-	-	0.6	1.3	28	2.6	2:1
Residual	7.3												

[z] Molybdate reactive P
[y] Total P minus molybdate reactive P
[x] Inositol P (phytic acid) estimated by peak integration of the ^{31}P NMR spectra
[w] Phospholipids and DNA or RNA estimated by peak integration of the ^{31}P NMR spectra
[v] Unassigned peaks in the ^{31}P NMR spectra
[u] below detection limit or less than 0.01 g kg^{-1}
[t] mmol kg^{-1}

Table 3.4 cont'd.

Fractions	Total P	P_i[z]	P_o[y]	Ortho-P	Esters		Pyro-P	Other[v]	Al	Fe	Ca	Mg	Ca:P[t]
					mono-[x]	di-[w]				mg kg^{-1}			
Dairy Cattle manure													
H_2O	2.4 (2)	2.2 (5)	0.2	2.3	-	0.05	-	-	nd	nd	7.0	5.4	2:1
$NaHCO_3$	4.6 (10)	4.3 (8)	0.3	4.6	-	-	-	-	nd	0.02	1.4	1.2	1:4
NaOH	1.4 (11)	0.8 (9)	0.6	0.9	0.3	0.1	0.01	0.09	0.06	0.02	0.4	nd	1:4
HCl	1.0 (0.4)	0.7 (0.3)	0.3	0.7	297	29	-	-	nd	nd	9.0	0.7	7:1
Residual	4.0												
Beef Cattle manure													
H_2O	1.1 (2)	1.0 (2)	0.1	1.1	-	-	-	-	0.01	0.2	0.9	0.8	1:1
$NaHCO_3$	0.8 (6)	0.8 (4)	nd	0.8	-	-	-	-	nd	nd	0.3	1.0	1:3
NaOH	0.2 (8)	0.1 (6)	0.1	0.12	0.04	0.03	-	0.01	nd	nd	0.3	nd	1:1
HCl	0.08 (11)	0.2 (2)	-0.1	0.08	-	-	-	-	0.1	0.5	44	14	434:1
Residual	0.03												
Poultry litters													
H_2O	5.2 (3)	5.2 (7)	nd	4.9	-	0.03	0.01	0.26	nd	nd	4.9	2.7	1:1
$NaHCO_3$	1.3 (8)	1.0 (0.4)	0.3	0.8	0.4	0.04	-	0.06	nd	nd	2.6	0.9	2:1
NaOH	1.7 (4)	0.3 (10)	1.4	0.5	1.0	0.13	0.02	0.05	0.1	nd	0.6	nd	1:4
HCl	5.2 (18)	2.2 (13)	3.0	2.1	3.1	-	-	-	nd	nd	86	1.7	13:1
Residual	1.0												

However, the amount of Fe extracted by $NaHCO_3$, relative to the total, was higher (10 %) than those of Ca and Mg (~ 3%). In the NaOH fraction, Al was extracted in higher proportion than Fe and Ca, whereas, in the HCl fraction, Ca was the only cation identified and it constituted about 74 % of total Ca in BIO. Similar to BIO, the H_2O and $NaHCO_3$ fractions of HOG were dominated by Ca (3 - 6 % of total) and Mg (30 - 35 % of total); whereas Al and Ca were the dominant cations in the NaOH fraction, but they constitute less than 1 % of total Al and Ca. Substantial concentrations of all polyvalent cations were detected in the HCl fraction of HOG, even though Al and Fe were not quantified at the dilution used for the analyses of total P and cations (Table 3.4). In the DAIRY, Ca and Mg dominated the H_2O and $NaHCO_3$ fractions with a cumulative amount equal to 28 % of total Ca and 94 % of total Mg. Some trace amount of Fe was also found in the $NaHCO_3$ fraction. In the NaOH fraction of DAIRY, Al, Fe and Ca were extracted in quantifiable amounts, but only Ca and Mg were found in the HCl fraction.

The higher amount of Ca extracted in the HCl fractions of BEEF and POULTRY, and Mg in BEEF compared with the total Ca and Mg makes the expression of these cations relative to total P confounding. However, it will be sufficient to say that Ca and Mg dominated the H_2O and $NaHCO_3$ fractions of BEEF, although Al and Fe were still present in quantifiable amounts in the H_2O fraction. Calcium and trace amounts of Al were also found in the NaOH fraction, whereas all the cations were detected in the HCl fraction with Ca and Mg more than others (Table 3.4). The POULTRY contained predominantly Ca and Mg in the H_2O, $NaHCO_3$ and HCl fractions, but Al and Ca in the NaOH fraction (Table 3.4).

The presence and solubility of calcium phosphate in manures, as indicated by the Ca:P molar ratio, varied among the sequential extracts (Table 3.4). A Ca:P ratio greater than 1:1 indicated an extraction of sparingly soluble calcium phosphate by a particular extractant. For example, Ben-Nissan et al. (1995) reported when Ca:P ratio is 1, dicalcium phosphate is the dominant Ca-P, at 1.5, it is tricalcium phosphate, and at 1.6, hydroxyapatite, for a pure system. Although the Ca:P ratios in the sequentially extracted fractions of manures in the current study were higher than the range reported by Ben-Nissan et al. (1995), our study shows that the abundance or lack of Ca relative to other cations in the extract does not necessarily prove the presence or absence of calcium phosphates. For example, the Ca:P ratio in BIO suggested that calcium phosphate predominated in the H_2O and HCl fractions, even though $NaHCO_3$ and NaOH extracted quantifiable amount of Ca. In HOG, the Ca:P ratio suggested that calcium phosphate may not be present in the labile and NaOH fraction, but more likely in the HCl fractions. These results also suggested that calcium phosphate was present in only the H_2O and HCl fractions of DAIRY, HCl fraction of BEEF, and both $NaHCO_3$ and HCl fractions of POULTRY.

3.4.3 Identification of Phosphorus Species in Sequential Extracts by NMR

The NMR spectra of BIO are shown in Fig 3.3. Chemical shift (ppm) of the peak at ~ 6.2 ppm was assigned to orthophosphate. In Fig. 3.3c, peaks at 5.25 and 4.9 ppm were assigned to phosphatidic acid and β-glycerophosphate, respectively, and the one at -4.43 ppm assigned to pyrophosphate. In Fig. 3.3d, the peak at -3.74 ppm was assigned to pyrophosphate. Other peaks could not be assigned to any specific P species. Inorganic

orthophosphate was the dominant P species identified in all the sequential extracts of BIO, accounting for 100 % of the extracted P in both H_2O and $NaHCO_3$ fraction, and approximately 95 % and 98 % in the NaOH and HCl fractions, respectively. Phytic acid, which is a major orthophosphate monoester, was not identified in any of the sequential fractions. The phosphatidic acid and β-glycerophosphate identified in the NaOH fraction are considered to be hydrolytic products of phospholipids such as phosphatidic choline in alkaline extracts (Turner and Leytem, 2004). Pyrophosphate, a short-chained inorganic polyphosphate, was identified in the both NaOH and HCl fractions.

For the HOG, the assignments of the peak in the NMR spectra (Fig 3.4) were as follows. Peaks at approximately 6.2 - 6.28 ppm were assigned to orthophosphate P. In Fig 3.4c, peaks at 6.12, 5.13, 4.74, and 4.64 ppm in the ratio 1:2:2:1 were assigned to P_2: $P_4 + P_6$: $P_1 + P_3$: P_5 of the ring structure of phytic acid. Peaks at 5.37 and 4.98 ppm, were assigned to phosphatidic acid and β-glycerophosphate, respectively. Peaks at 4.89, 4.54, and 4.47 ppm were unidentifiable. In Fig 3.4d, peaks at 5.75, 4.86, 4.44, and 4.3 ppm in the ratio of 1:2:2:1 were assigned to phytic acid. Similar to the result for biosolids, Fig 3.4 shows that the sequential extracts of hog manure was dominated by inorganic orthophosphate, making up 100 % of extracted P in the H_2O and $NaHCO_3$ fractions, and approximately 79 % in NaOH fractions and 96 % in HCl fractions. In the NaOH fraction, phytic acid was the main form of organic P identified, making up about 11 % of the total P while phosphatidic acid and β-glycerophosphate were in trace amounts (Fig. 3.4c; Table 3.4). In the HCl fraction of hog manure, phytic acid accounted for approximately 4 % of the total P.

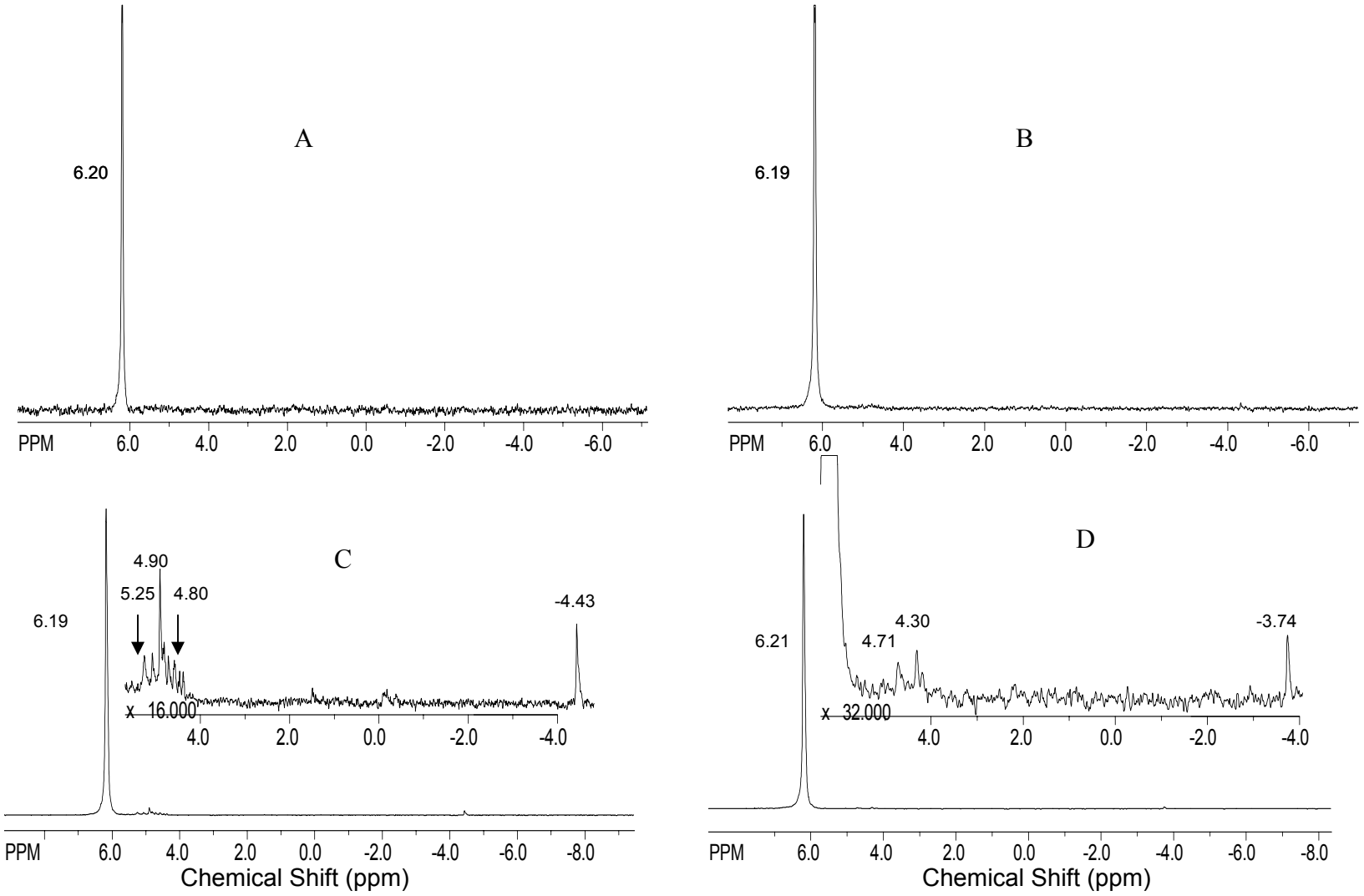

Figure 3.3. ^{31}P NMR spectra of biosolids sequentially extracted with (A) Water, (B) $NaHCO_3$, (C) NaOH and (D) HCl

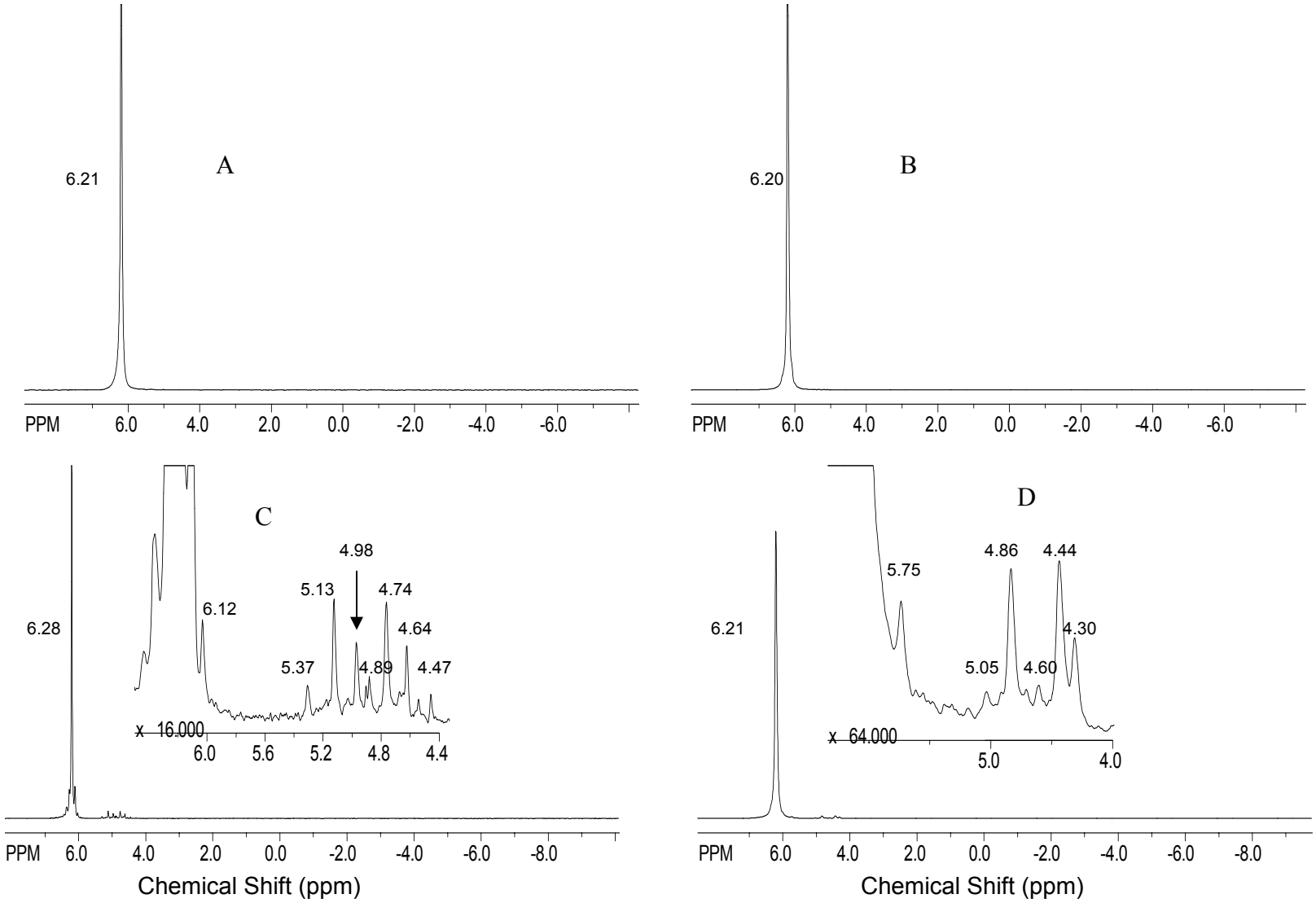

Figure 3.4. ^{31}P NMR spectra of hog manure sequentially extracted with (A) Water, (B) $NaHCO_3$, (C) NaOH and (D) HCl

The ^{31}P NMR spectra of DAIRY is shown in Fig 3.5. Peaks at ~ 6.2 - 6.28 ppm were assigned to orthophosphate P. In Fig. 3.5c, peaks at 6.0, 5.10, 4.74, and 4.59 ppm in the ratio 1:2:2:1 were assigned to P_2: P4 + P_6: P_1 + P_3: P_5 of phytic acid, while those at 5.28 and 4.94 ppm were assigned to phosphatidic acid and β-glycerophosphate, respectively. However, peaks at 4.89, 4.54, and 4.47 ppm were unidentifiable. In Fig 3.5d, peaks at 5.81, 4.90, 4.51, and 4.39 ppm in the ratio of 1:2:2:1 were assigned to phytic acid. Following spectral integration, inorganic orthophosphate was 98 % of the total P in the H_2O fractions while phosphatidic acid and phospholipids was identified in trace amounts (Fig. 3.5a, Table 3.4). In the $NaHCO_3$ extracts, only inorganic orthophosphate was identified (Fig. 3.5b). In comparison with BIO and HOG, DAIRY had a lower inorganic orthophosphate and higher orthophosphate monoesters in the NaOH and HCl fractions. In the NaOH fraction, orthophosphate and phytic acid constituted approximately 64 % and 21 % of the total P, respectively (Fig. 3.5c, Table 3.4). Similarly, in the HCl fraction, inorganic orthophosphate and phytic acid were 70 % and 30 % of the total P, respectively. Hydrolytic products of phosphatidyl choline were identified in substantial amounts in the NaOH fraction (7 % of total P), but not in the HCl extracts.

In the BEEF, inorganic orthophosphate was the only species identified in the H_2O, $NaHCO_3$, and HCl fractions (Fig. 3.6) as depicted by the peaks at ~ 6.2 ppm. In Fig. 3.6c, the peaks at 6.03, 5.31, 4.76, and 4.62 ppm assigned to phytic acid, while those at 5.31 and 5.97 ppm were assigned to phosphatidic acid and β-glycerophosphate, respectively. The peak at 4.89 ppm was unidentified. Spectra integration showed that the NaOH fraction contained 61 % inorganic orthophosphate, 31 % phytic acid, and 8 % phosphatidic acid plus β-glycerophosphate (Table 3.4).

The NMR spectra of POULTRY are clearly different from those of other organic amendments, in that they are the only one that shows significantly amount of other species apart from orthophosphates in the labile fractions - H_2O and $NaHCO_3$ (Fig. 3.7). The peaks at ~ 6.2 ppm were assigned to orthophosphate. In Fig. 3.7a, peaks at 5.92 and 5.60 ppm were unassigned, but the one at 5.36 ppm was assigned to phosphatidic acid. In Fig. 3.7b, peaks at 5.89, 4.98, 4.61, and 4.47 ppm were assigned to phytic acid, the peak at 5.69 ppm was unidentified, and the one at 5.42 ppm assigned to phosphatidic acid. In Fig. 3.7c, the peaks at 6.03, 5.13, 4.77, and 4.62 ppm were assigned to phytic acid. The peaks at 5.23 and 4.97 ppm, were assigned to phosphatidic acid and β-glycerophosphate, respectively. Other peaks were unassigned. In Fig. 3.7d, peaks at 5.75, 4.85, 4.45, and 4.33 ppm were assigned to phytic acid. Following spectra integration, Fig 3.7 shows that orthophosphate made up 94 % the total P in the H_2O fractions, 61 % in the $NaHCO_3$ fractions, 29 % in the NaOH fractions, and 40 % in the HCl fraction (Table 3.4). The H_2O fraction contained phosphatidic acid and other peaks in the monoester regions that have not been previously assigned (Fig. 3.7a). In the $NaHCO_3$ fraction, however, phytic acid made up about 31 % of total P and phosphatidic acid was also identified in quantifiable amount (3%). In the NaOH fraction, phytic acid constituted 59 % of total P while phosphatidic acid and β-glycerophosphate both made up approximately 8 % of the total P. In the HCl fraction, phytic acid was the only organic P present, making up 60 % of total P.

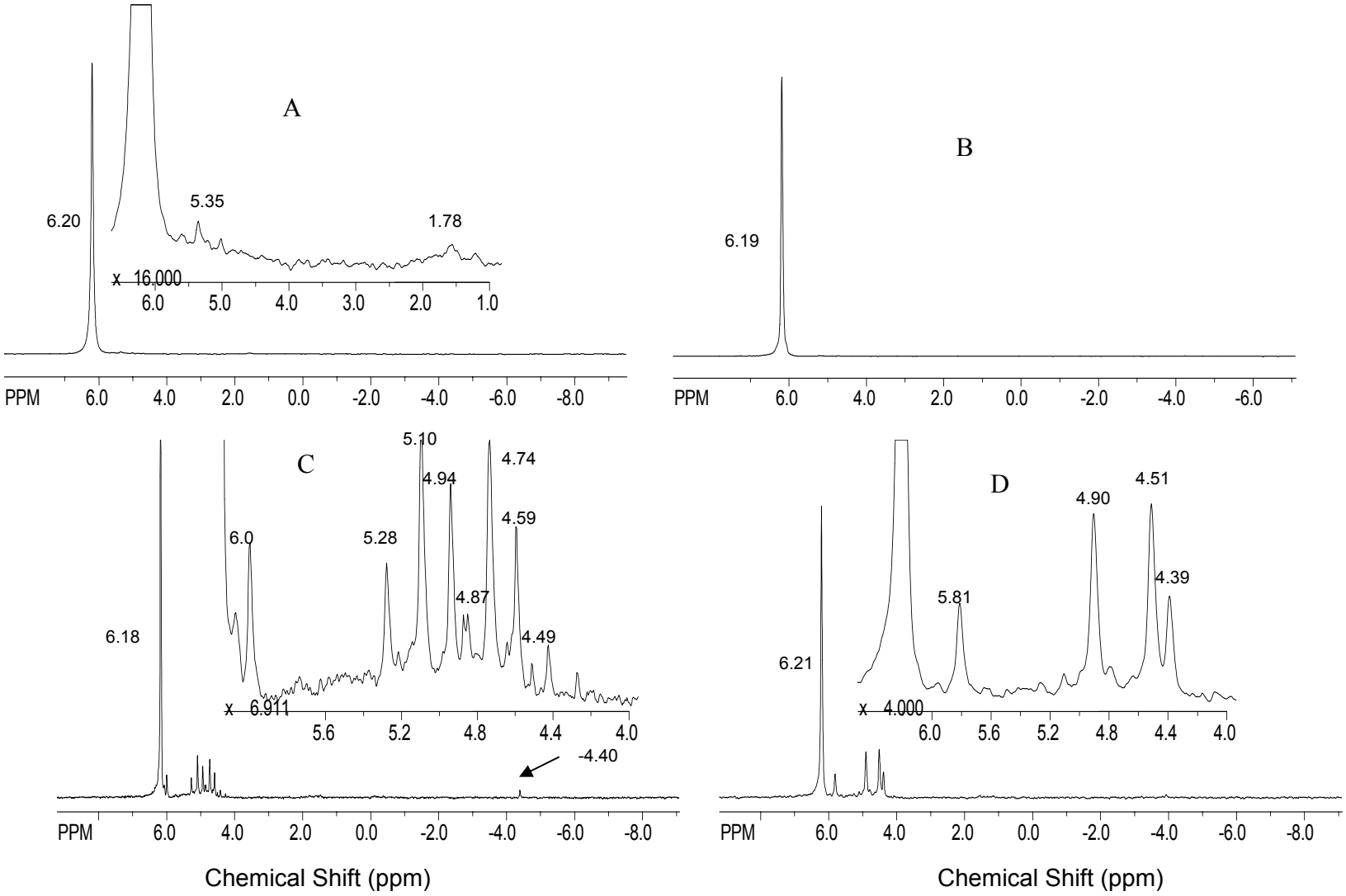

Figure 3.5. ^{31}P NMR spectra of dairy cattle manure sequentially extracted with (A) Water, (B) $NaHCO_3$, (C) NaOH and (D) HCl

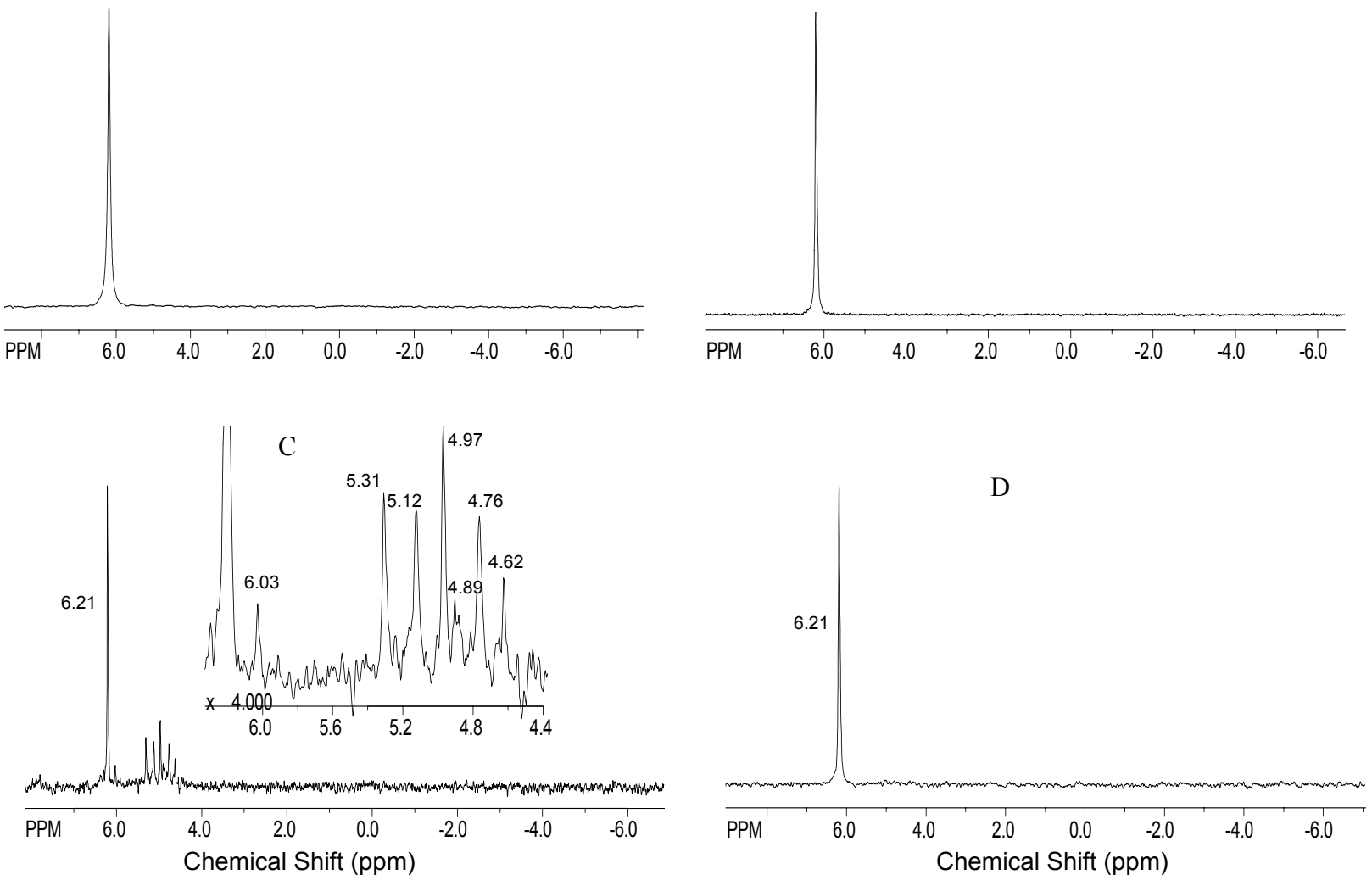

Figure 3.6. ^{31}P NMR spectra of beef cattle manure sequentially extracted with (A) Water, (B) $NaHCO_3$, (C) NaOH and (D) HCl

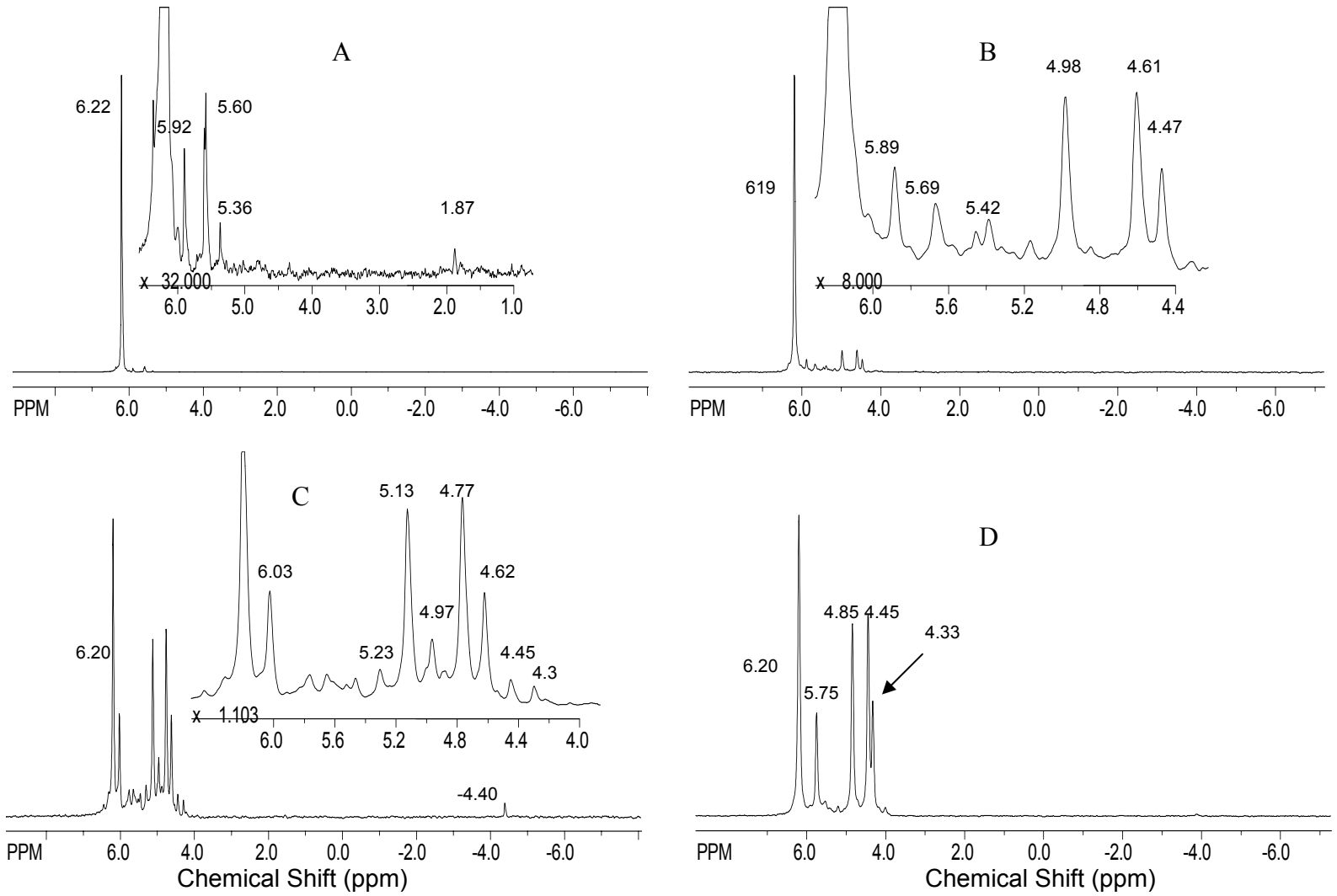

Figure 3.7. ^{31}P NMR spectra of poultry litter (manure + beddings) sequentially extracted with (A) Water, (B) $NaHCO_3$, (C) NaOH and (D) HCl.

3.4.4 Identification of Phosphorus Species in Sequential Residues by XANES

The XANES spectra of the reference P compounds are shown in Fig. 3.8. The spectra were displayed as normalized absorption, in arbitrary units, plotted against the photon energy (in eV). The spectra of each group of reference compounds were plotted with a slight vertical displacement to allow for easy comparison of the features. Each class of the reference compounds exhibited characteristics features that were used as fingerprints in identifying phosphates species in the spectra of the organic amendments. For example, the two iron phosphates (PSIDER and STRENG) exhibited pre-edge features around 2148 eV (Fig 3.8a). This feature originates from a quadrupole-allowed transition of 1s electron to the partially filled 3d level of the FeP molecule. The presence of the feature around 2162 eV distinguishes the monoclinic (PSIDER) from orthorhombic (STRENG) forms of $FePO_4 \cdot 2H_2O$. The shape of the peak feature around 2170 eV that represents the oxygen oscillation was broad for the two FeP standards. Calcium phosphate standards had a shoulder around 2154 - 2158 eV (Fig. 3.8a). This feature was sharper in β-TRICAL and HAP than in DCPD, but slightly obscured in the spectrum of DCP, similar to those of Hesterberg et al. (1999) and Sato et al. (2005) who found that the shoulder feature became more well-defined with decreasing solubility and increasing thermodynamic stability. Furthermore, the lack of this shoulder has been used to distinguish between crystalline and amorphous HAP, as amorphous HAP lacked a shoulder (Peak 2002). A post-edge feature occurred in DCPD around 2162 eV and shifted to approximately 2163 eV in the spectra of DCP and HAP, but was absent in the spectra of β-TRICAL. The oxygen oscillation occurring at 2170 eV was generally broad for the entire CaP standards except for DCPD.

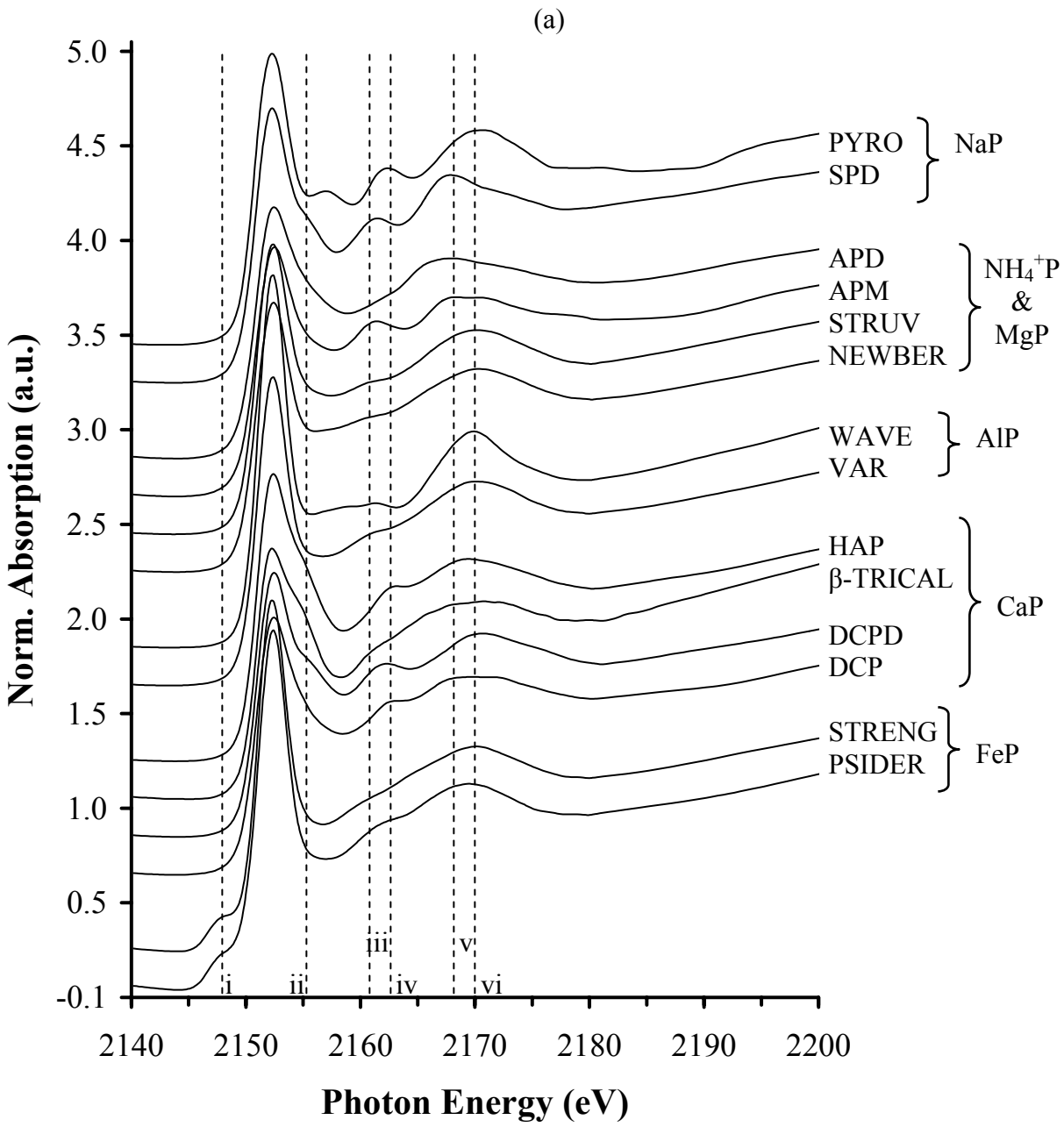

Figure 3.8 (a) Normalized P 1s XANES spectra of reference inorganic P compounds. The spectra were plotted with slight vertical displacement to aid comparison of the features. (i) the pre-edge of FeP, (ii) shoulder of CaP, (iii) 2161 eV peak (iv) 2163 peak, and (v and vi) various oxygen resonances.

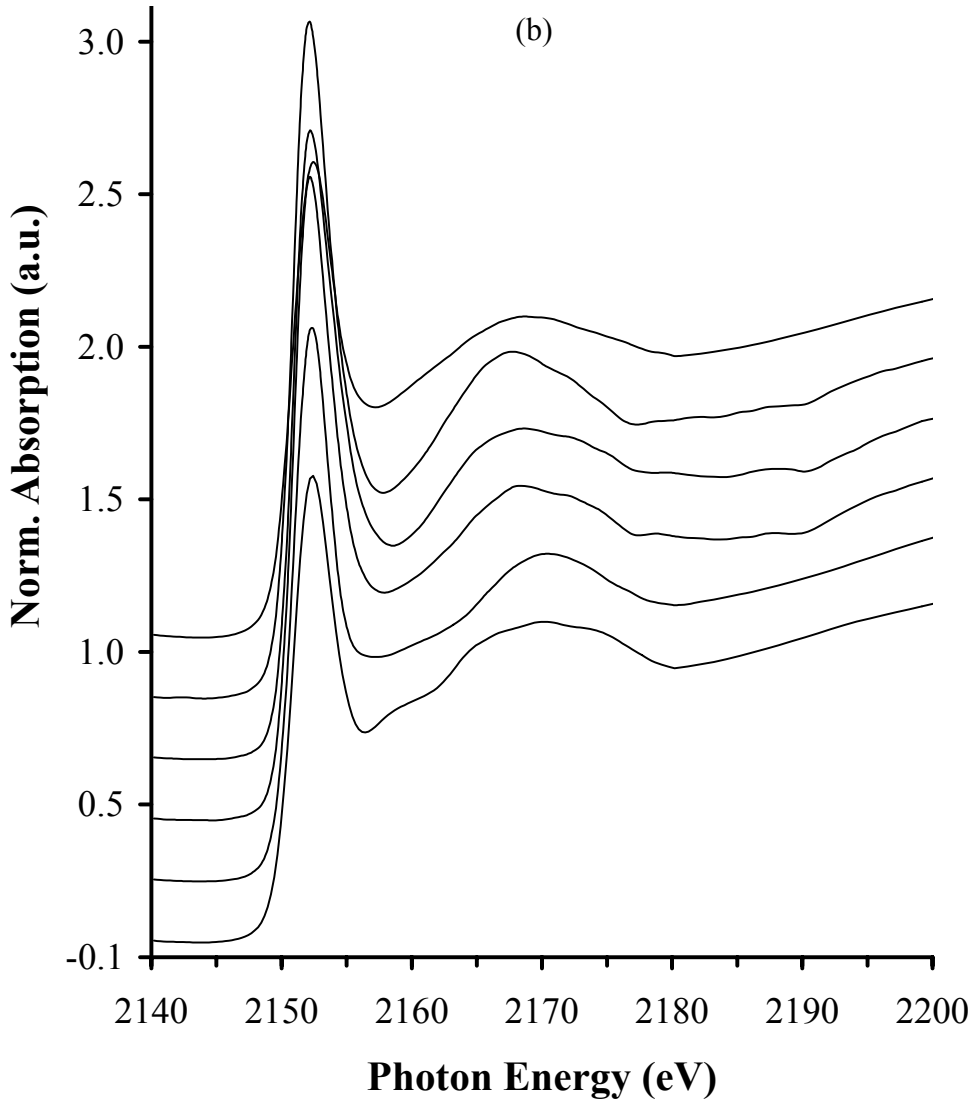

Figure 3.8 (b). Normalized P 1s XANES spectra of reference organic P with no resonances. The spectra were plotted with slight vertical displacement to aid comparison of the features

For the reference aluminum phosphate standards, WAVE exhibited a long valley between the absorption edge at 2154 eV and the oxygen oscillation at 2170 eV, while VAR had a subtle feature at approximately 2162 eV similar to PSIDER (Fig. 3.8a). This subtle feature was also observed by Franke and Hormes (1995). The spectrum of WAVE clearly resembles that of amorphous $AlPO_4$ reported by Peak (2002). The shape of the oxygen oscillation peak was conical in the spectrum of WAVE but broad in that of VAR.

The spectra of all the magnesium and ammonium phosphates are also shown in Fig. 3.8a. The presence of a subtle feature around 2160 eV in STRUV can be used to distinguish between STRUV and NEWBER given the similarity in the shape of their oxygen oscillations around 2170 eV. Similarly, the presence of the peak at 2161 eV distinguished APM from APD, but their oxygen oscillation occurred at 2168 eV compared with 2170 eV in STRUV and NEWBER. The P 1s XANES spectra of NaP have distinct features that could be used to distinguish between the two types used in this study. For example, SPD has a shoulder similar to the CaP but this shoulder was absent in PYRO, which has a unique feature at 2157 eV that was not found in all other reference compounds. In addition, the peak at 2162 eV and the oxygen resonance at 2170 eV in PYRO occurred at lower energies in SPD; ca. 2161 eV and 2168, respectively.

In the organic phosphates group, the orthophosphate diesters - PEA, PCHOLINE, and DNA had no post edge features and similar oxygen oscillation occurring at 2166 eV and therefore was indistinguishable from one another (Fig. 3.8b). However, the spectra of the orthophosphate monoesters - ATP, AMP, and PHYTIC were different from one another and from other organic phosphates (Fig. 3.8b). The ATP had a pronounced valley ranging from 2156 - 2164 eV and a conical oxygen peak at 2170 eV, while AMP

had a feature around 2160 eV and a broad oxygen peak covering 2166 - 2174 eV similar to PHYTIC. In general, all organic P standards have sharp absorption edges but no resonance features and thus, are very different from the inorganic P compounds. This lack of distinguishing features may complicate the identification of these organic P compounds in the XANES spectra of organic amendments.

The XANES spectra of organic amendments (intact) are shown in Fig 3.9. Initial qualitative XANES analysis using 'fingerprinting' approach indicated that BIO spectrum lacks a shoulder post-edge at approximately 2156 eV but has a subtle post-edge feature at 2162 eV and an oxygen peak at 2170 eV, indicating the predominance of VAR (Fig. 3.9). This spectrum may also indicate the presence of adsorbed P on either amorphous or crystalline $Al(OH)_3$ (gibbsite) if the Al content of the BIO is considered. Peak (2002) showed that the spectra of P sorbed on amorphous and crystalline $Al(OH)_3$ had no resonance feature and were similar to those of organic orthophosphate monoesters shown in the current study.

The spectra of BEEF, DAIRY and HOG all have shoulders around 2156 eV, post-edge features around 2161 eV, and a broad oxygen oscillation around 2170 eV (Fig. 3.9). The sharpness of the shoulders and post-edge features indicates that DCPD predominated in DAIRY while DCP was the dominant species in BEEF and HOG (Fig. 3.9). The spectrum of POULTRY had a very subtle post-edge feature and an oxygen resonance at 2170 eV, suggesting that PHYTIC and perhaps some DCP were the dominant P species in this sample (Fig. 3.9).

Different approaches have been reported in the literature for performing a quantitative XANES analysis of dominant P species in environmental samples. For

example, the number of components used in LC fitting has been determined by using principal component analysis (PCA) and target transformation (TT) (Beauchemin et al. 2002, 2003; Ressler et al. 2005; Toor et al. 2005b). In another instance, it was determined as the maximum number of components whose successive inclusion decreased the residual factor of the fit by 20 % (Shober et al. 2006). Another approach is a combinatorial fitting, in which the program's default order of combinations of all the standards is used, and the order is increased or decreased based on the fit results (Sato et el. 2005). In the current study, the presence of different dominant P species in the organic amendments precluded the use of PCA and TT for quantitative analysis. In addition, using PCA and TT requires that twice as many spectra as dominant components present in the set of spectra is used to confidently determine the number of 'target species' (Ressler 2004). Consequently, a maximum of four standards was used for the LC fitting in the current study because it is unlikely that PCA would have identified more than four orthogonal components as sufficient to reconstruct the XANES spectra of five organic amendments. LC of binary to quaternary combinations of all reference P compounds, excluding iron phosphates and Na pyrophosphate that have been shown to be absent in the samples by fingerprinting, was then carried out. The fitting was performed over the relative energy range of -11 to 49 eV. The weights of the components were constrained to be between 0 and 1 and forced to sum to 1 during the fit. The threshold energy, E_0, was allowed to float to a maximum of ± 2.5 eV energy shift, which is equivalent to the step size used during data acquisition. The goodness-of-fit was judged by the residual factor (R-factor) and chi square (χ^2) values. The fit with the least R-factor

and χ^2, and the least standard deviation (uncertainties) of the estimated proportion was chosen as the best fit.

The results of the LC fit of P standards to the organic amendments are shown in Fig. 3.10. The proportions of the identified components in the fit represent the best estimate available given the constraints used in the LC analysis. The goodness-of-fit statistics reported along with the result is not a measure of the accuracy of the fit. Thus, a measure of relative error associated with this fit, as with any other LC fitting of XANES data, warrants further investigation and is expected to be a significant contribution to quantitative XANES data analysis.

The XANES spectrum of BIO was shown to be dominated by VAR and HAP, amounting to approximately 86 % and 14 % of the total phosphates, respectively (Fig. 3.10a). This result is complementary to qualitative XANES analysis (fingerprinting) earlier reported where VAR was identified. The LC fitting of P 1s XANES of other organic amendments indicated that DCPD was a dominant species with proportions as high as 50 and 60 % of total phosphate in HOG and DAIRY, respectively, and between 18 % and 20 % in BEEF and POULTRY (Fig. 3.10b - e). Qualitative XANES analysis identified this Ca-P to be DCP in these organic amendments, except DAIRY. This result is likely due to the fact that spectral features are particularly useful for distinguishing among classes of compounds and not necessarily for identifying compounds within classes (O'Day et al. 2004). Given that the least-squares LC fitting works best for chemical components whose spectra are substantially different, this technique may not be able to distinguish between DCP and DCPD because the spectra of these CaP were similar with the intensity of the shoulder being the main difference.

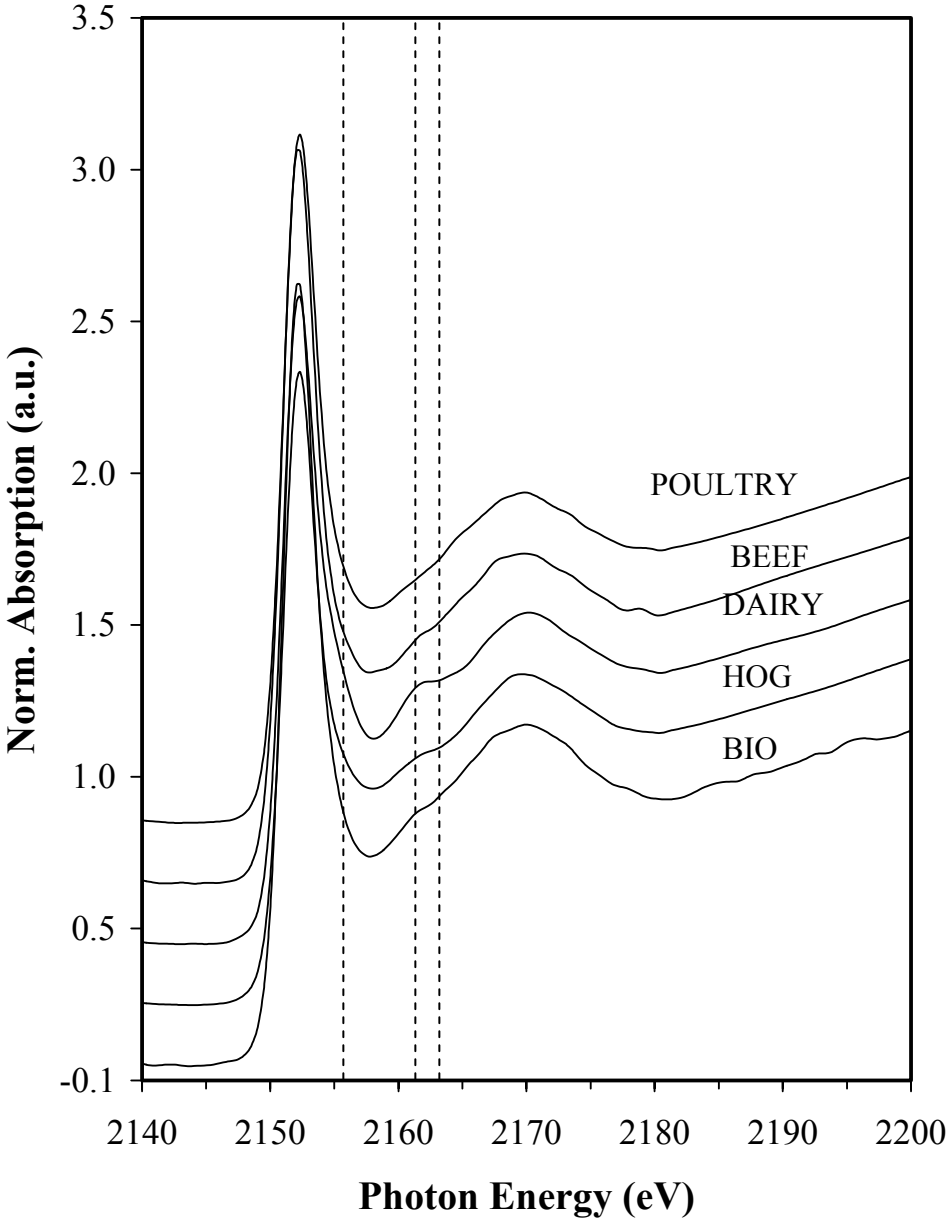

Figure 3.9. Normalized P 1s XANES spectra of intact (unextracted) organic amendments. The spectra were plotted with slight vertical displacement to aid comparison of the features

In addition to DCPD, PHYTIC, the most ubiquitous form of organic P in the environment, was identified as a dominant constituent of XANES spectra of the manures, ranging from 20 % in BEEF, 32 % in HOG, 35 % in DAIRY, to as high as 70 % in POULTRY (Figure 3.10 b - e). Toor et al. (2005b), in using LC fitting of XANES spectra of litters of broilers fed with different combinations of 'normal' and 'high available P' corn, also identified phytic acid with values ranging from 7 - 20 % of total P and in litters of turkey fed with normal and P-deficient corn with values ranging from 16 - 32 % of total P.

Earlier study on quantitative P speciation using PCA and LC fitting could not fit phytic acid to spectra of soils with long-term history of manure application due to the lack of any distinct features in the near-edge region (Beauchemin et al. 2003). Toor et al. (2005b) also observed a good LC fit with or without phytic acid in a particular treatment with 'high available P corn' and considered the LC fit as inconclusive for that sample. Other notable P species identified as dominant in the manures using the LC fitting technique were: VAR, making up 18 % of total P species in HOG; and STRUV, constituting 68 % and 12 % of total phosphates in BEEF and POULTRY, respectively. These species were not identified by the fingerprinting technique.

The labile P fractions (estimated by the difference spectra method) of some of the organic amendments were similar to those in the intact sample (Fig. 3.10). For example, the labile P fractions in BIO appeared to be dominated by VAR as given by the absence of a shoulder and presence of a post-edge feature at 2162 eV. Similarly, the spectrum of labile P in HOG lacks a shoulder but has a subtle feature at 2162 that resembles that of STRUV. The spectra of labile P in BEEF and DAIRY indicated that DCPD

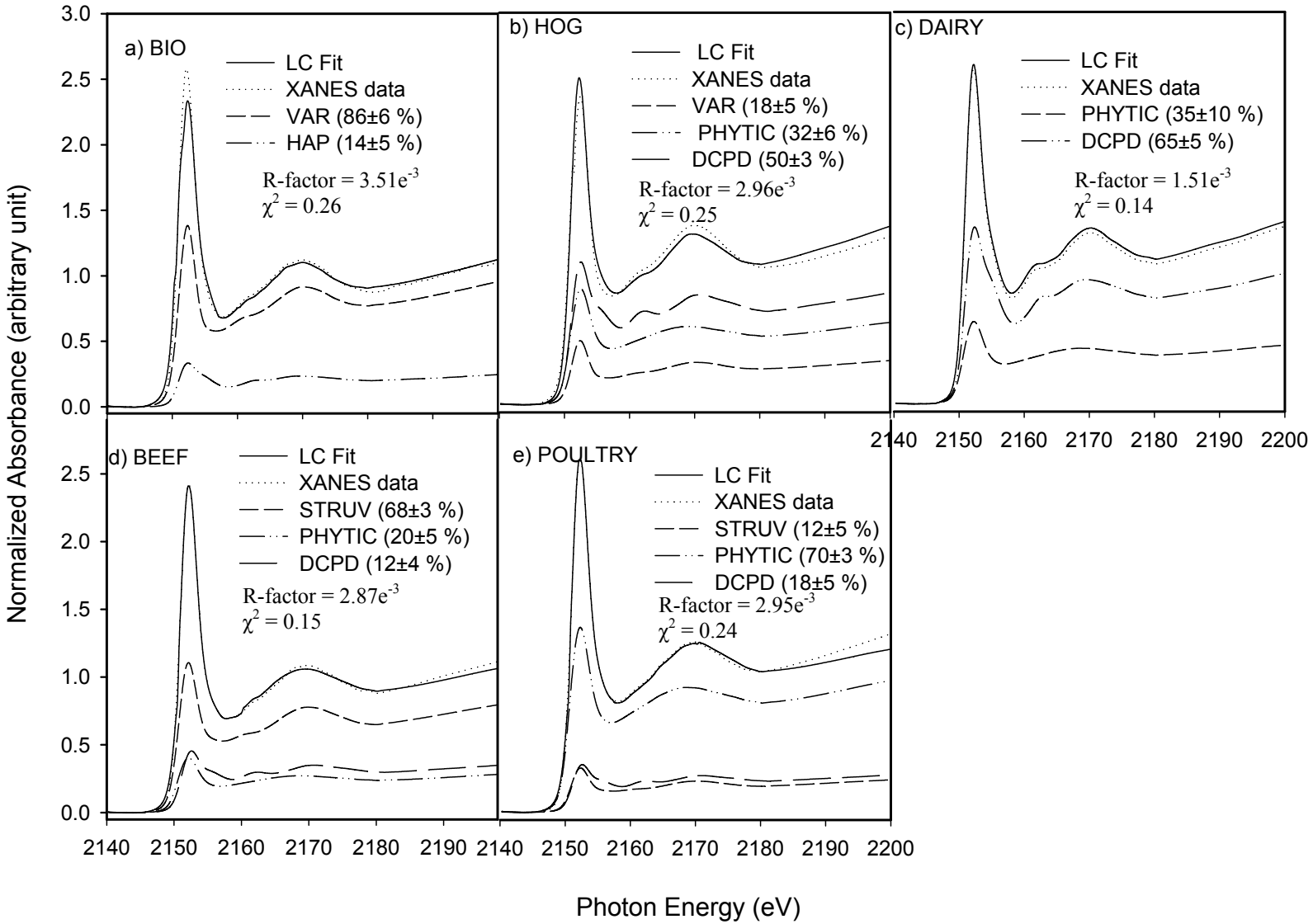

Figure 3.10 Linear combination (LC) fit showing proportion of identified P compounds in organic amendments; (A) biosolids, (B) hog manure, (C) dairy cattle manure, (D) beef cattle manure and (E) poultry litters.

predominated, similar to the intact (unamended) sample. The labile P fraction in POULTRY show a more distinct shoulder and an intense peak at ca. 2162 eV, which were absent in that of the intact sample (Fig 3.8, 3.10). This indicates the dominance of DCPD, which could not be identified by 'fingerprinting' in the spectrum of the intact manure, and therefore points to the usefulness of the difference spectra.

3.5 Discussion

3.5.1 Phosphorus fractions and cations in manures

The results in this paper provide a detailed molecular characterization of P species in organic amendments. The amount of the total P, as measured by ICP (ICP-P), in the sequential extracts, indicated that BIO contained a smaller quantity of labile P and more non-labile P compared with other organic amendments. These results corroborate those reported by Ajiboye et al. (2004), in which biosolids contained a significantly lower H_2O + $NaHCO_3$-P (24 % of total extractable P) compared with livestock manures (63 %, 70 %, and 60 % in hog, dairy cattle, and beef cattle manures, respectively). The higher total Al and Fe in the intact and NaOH extracts of BIO relative to other amendments indicated that part of this non-labile P fraction was likely associated with Al and Fe.

The extraction of the labile P fractions with Ca in all organic amendments suggested the presence of readily soluble calcium phosphates. The fact that HCl extracted the greatest amount of P in all organic amendments compared with other fractions suggests the presence of less soluble calcium phosphates like HAP that could not be extracted with the weaker extractants. Similar to the result of the current study,

Cooperband and Good (2002) have also reported that Ca and Mg are the dominant cations controlling P solubility in manures.

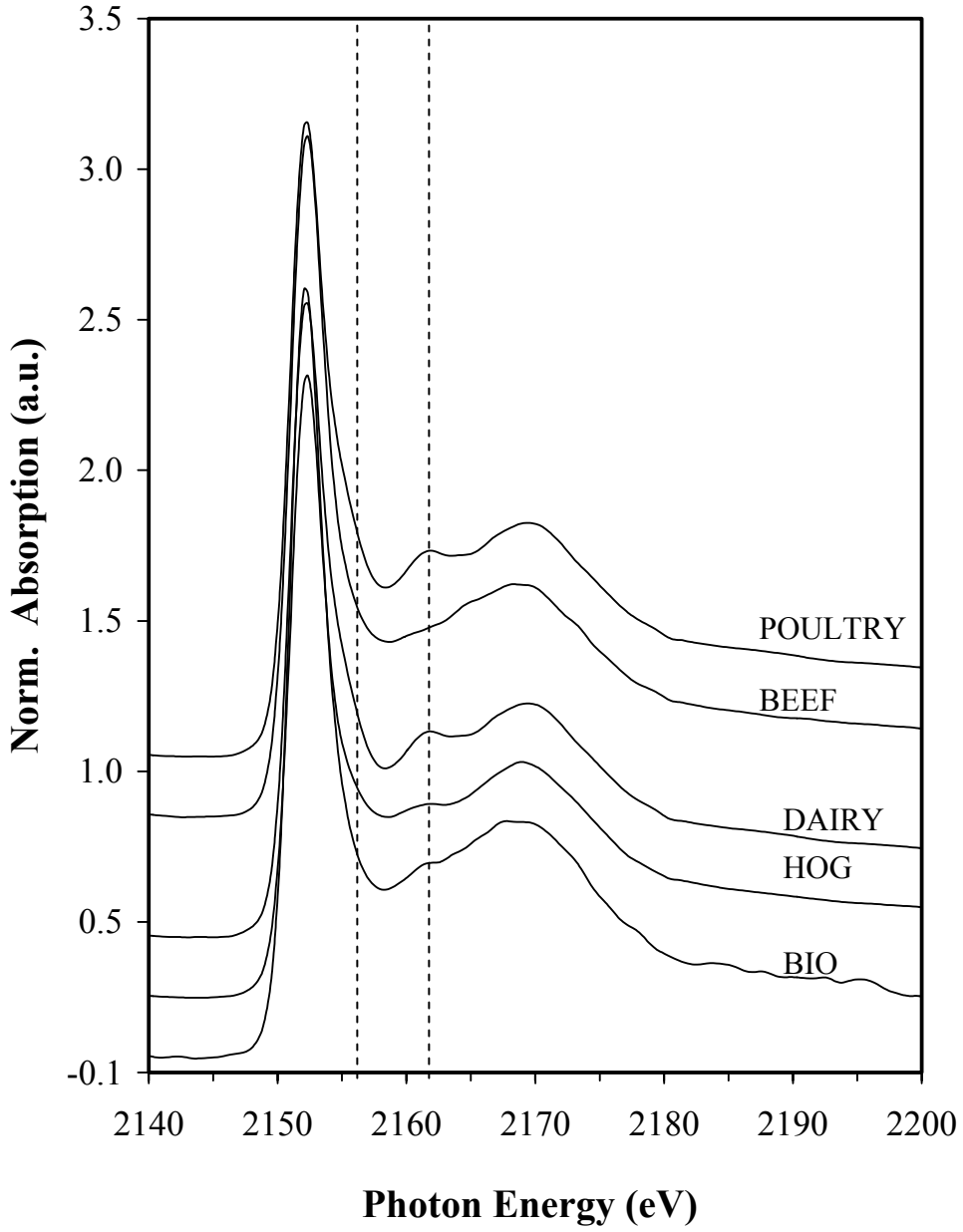

Figure 3.11 P 1s XANES spectra of labile P - as spectral difference between spectra of intact amendments and residues after $NaHCO_3$ extraction. The spectra were plotted with slight vertical displacement to aid comparison of the features.

The apparent over-estimation of inorganic P (relative to total P) by the colorimetric procedure in the H_2O fractions of BIO and HOG, and HCl fraction of BIO, HOG, and BEEF could not be attributed to heterogeneity of the samples alone, as suggested by the similarity in the % RSD of the analytical methods. Furthermore, this over-estimation may also be due to the hydrolysis of some organic and condensed P as observed in the two-step extraction with $NaHCO_3$ and HCl (Turner and Leytem 2004). However, it is possible that neutralization of the HCl extracts prior to ICP analysis caused a re-precipitation of orthophosphate as Ca-P, hence an under-estimation of total P. An over-estimation of inorganic phosphorus in water and 0.01 M $CaCl_2$ extracts of manure had been reported in other studies (Choate 2004; Ajiboye and Akinremi, 2005; Wolf et al. 2005; Pierzynski et al. 2005). The magnitude of this over-estimation averaged at least 7 % in water extracts of swine, dairy and poultry manures (Wolf et al. 2005), and 13 % in the $CaCl_2$ extract of oven-dried biosolids (Choate 2004). In most cases, little insight was provided on what might have caused the colorimetric analysis of P to be greater than ICP for water extract of organic amendments. However, Ajiboye and Akinremi (2005) attributed it to likely matrix interference due to the absorption of UV radiation at the wavelength used for measurement by other substances extracted along with P in the organic amendments. This matrix effect might have resulted in a positive bias in the colorimetric analyses compared with ICP. Matrix interference, however, has not been shown to be a problem with ICP measurement. For example, Pierzynski et al. (2005a) reported that no evidence of spectral enhancement of the P signal in ICP was present in a scan of spectral emission intensity across the P wavelength in P standards and unknowns. Clearly, further investigation on the cause of the inconsistency in colorimetric and ICP

measurements of P is urgently needed. The magnitude and cause of the discrepancy as well as factors contributing to higher ICP than colorimetry were recently identified as an important area of research necessary for generating correction factor and inter-conversion from ICP-P to molybdate reactive P (Pierzynski et al. 2005b).

3.5.2 Phosphorus Speciation by NMR

The dominance of orthophosphates in H_2O and $NaHCO_3$ fractions of all organic amendments, except POULTRY, suggest that the P was more bioavailable in these samples compared with that of POULTRY whose $NaHCO_3$ fraction contained phytic acid. Celi et al. (1999) reported that phytic acid is generally capable of forming stable complexes and insoluble Ca salt precipitates in alkaline soil and thus protected from microbial degradation, due to its higher charge density compared with other orthophosphate monoesters. Also, this higher charge density may lead to the preferential binding of phytic acid relative to other monoesters, making the latter more susceptible to mineralization or hydrolysis (Celi et al. 1999). Orthophosphate diesters and orthophosphate monoesters occurring from either enzymatic or alkaline hydrolysis of diesters, are weakly sorbed in soil and are therefore mobile (Turner et al. 2005). Furthermore, orthophosphate diesters mineralize more rapidly than monoesters and will therefore constitute the labile fractions of P in the manure and manure-amended soil (Condron et al. 1990; Condron et al. 2005). The results from the current study further suggest that inorganic P in a particular sequential fraction may be bioavailable, but the organic P may not be.

The absence of phytic acid in all fractions of BIO compared with other organic amendments as found in this study corroborates the results from another study where the total P of anaerobically digested municipal biosolids was almost entirely orthophosphate (Hinedi et al. 1989). Conversely, the association of cations with organic P extracted by NaOH and HCl fractions in the organic amendments except in BIO and HCl fractions of BEEF suggests a complexation of polyvalent cations with phytic acid in the sample. Phosphorus species including orthophosphate, orthophosphate monoesters, and pyrophosphate are thought to be bound with polyvalent cations such as Al, Fe and Mn (Miltner et al. 1998; Celi et al. 1999; Leytem et al. 2002; Toor et al. 2005a). These cations form bridges, thereby enabling organic P to take on condensed formations which may reduce the efficacy of the extractants (Swift, 1996). Therefore, the low concentration of Al and Fe in POULTRY (undetected, except for Al in NaOH extract) despite its higher concentration of organic P relative to other organic amendments may be due to condensation of these organic P forms because organic P held as humic-metal phosphate complexes are less labile in the environment. The higher proportion of the residual P in HOG and DAIRY relative to other manures suggests the presence of some recalcitrant form of P that could not be recovered during digestion and subsequent ICP analysis. Residual P has often been considered to be occluded P or P bound to stable humic substances (Cross and Schlesinger 1995). Celi and Barberis (2005) also pointed out that Ca phytates are insoluble in alkaline whereas Al and Fe phytates are insoluble in acid, hence the poor extractability of the latter in HCl.

The identification of phytic acid in the HCl extracts of HOG, DAIRY and POULTRY confirmed that it is erroneous to assume that this less labile fraction is only

composed of inorganic P in the form of CaP. Some studies that used modifications of the Hedley fractionation scheme to characterize manure P have omitted the HCl extraction step (He et al. 2004) or assumed that HCl-P is mainly Ca-P, similar to what is expected in soil (Ajiboye and Akinremi 2005; McDowell and Stewart 2005b).

The relatively higher proportion of other orthophosphate monoesters and diesters like phospholipids in POULTRY suggests that microbial activity was greater than in other organic amendments since these P species are usually associated with microbes (Turner et al. 2003). The presence of substantial labile monoesters and diesters in POULTRY indicates that organic P in this manure is present in a form that is bioavailable to aquatic organisms. Toor et al. (2005a) reported that diesters are generally less strongly sorbed in the soil making them bioavailable, although DNA may penetrate the interlayer space of clay minerals under acidic conditions.

The estimation of organic P as the difference between ICP-P and molybdate-P did not agree with the estimates by ^{31}P NMR. The solution ^{31}P NMR did not detect organic P in the H_2O and $NaHCO_3$ fractions of all organic amendments except in POULTRY and H_2O fraction of DAIRY. The organic P estimated by colorimetry was negative in the H_2O fraction of BIO, HOG and HCl fractions of BIO, HOG, and BEEF where an over-estimation of inorganic P occurred. In contrast to our results, McDowell and Stewart (2005b) reported a good agreement between organic P estimated by colorimetry and NMR for grazing dairy cattle, sheep, and deer even though phytic acid and other diesters were not well resolved in the NMR spectra and were only estimated by spectral deconvolution. As mentioned previously, the estimation of organic P in manure as the

difference between ICP-P and molybdate reactive P may not hold true for all manures and such results should be interpreted with caution.

3.5.3 Linking P Species in Sequential Extracts by NMR with Residues by XAS

The presence of HAP in BIO may be responsible for the least amount of labile P in BIO relative to other organic amendments. Ajiboye et al. (2004) had earlier reported that BIO contained a proportionately smaller amount of P in the water extract than in HCl extract, suggesting that biosolids P is more in the non-labile fraction and less in the labile fraction compared with manures. Although HAP and VAR were the dominant P species in BIO, VAR is readily soluble in neutral to alkaline solutions that is used to extract the labile P pool, and its presence in the XANES spectra for labile P suggests that the non-labile P in BIO was in the form of HAP. Because HAP is the most thermodynamically stable form of CaP, its presence infers less solubility, and lower risk of BIO P loss to the environment compared with other amendments. The absence of HAP in the HOG, BEEF, and POULTRY may be due to the inhibition of precipitation of P by organic acids from these amendments as indicated by their phytic acid content. Inskeep and Silvertooth (1988) reported that the presence of organic acid decreases precipitation of P due to sorption of organic anions onto the minerals and coating of the crystal growth sites. The absence of pre-edge in the spectra of organic amendments indicated that these amendments contain no FeP within the detection limit of the XAS technique.

The high amount of labile P in other organic amendments, especially in HOG and DAIRY may be attributed to the predominance of DCPD in these samples because it is more labile than the thermodynamically favoured HAP. The near edge features of the

difference spectra, which indicated that DCPD and DCP constituted the labile P pool in all the manures expect POULTRY, supported this result. The calcium phosphates (DCP and/or DCPD) identified in the labile P XANES spectra could also be interpreted to be P adsorbed onto $CaCO_3$. According to Peak (2002), the spectra of adsorbed P on $CaCO_3$ showed resonance features similar to, but not as sharp as, DCP and HAP. The identification of calcium phosphates in the labile fractions on these amendments further reinforces that Ca:P in the extracts cannot be conclusively used to infer the presence or absence of calcium phosphates.

The predominance of STRUV in BEEF and in substantial amounts in POULTRY is of particular agro-environmentally importance. Struvite is important in specialty agriculture as a source of slow-release P fertilizer and the recovery of STRUV in livestock manure is one of the technological options currently being pursed to control P release from livestock into the environment (Zeng and Li 2006; Huang et al. 2006). Neither the sequential chemical extraction nor NMR spectroscopy could be used to identify STRUV in these samples. Only the Mg:P ratio could have been used indirectly to infer the presence of STRUV had XAS not been used, indicating the importance of combining chemical extraction with solid phase speciation.

The detection of DCPD and PHYTIC in the XANES spectra of other manures further corroborates the results from NMR analyses, where POULTRY had the highest amount of PHYTIC. This result is similar to that of Peak (2002) who observed that poultry manure unamended with alum ($Al_2(SO_4)_3$) contained DCP and some weakly bound organic P, but no HAP. It is worth mentioning at this point that the spectra of various reference compounds, such as aqueous phosphate (Sato et al. 2005), adsorbed P

on amorphous $Al(OH)_3$ and gibbsite (Peak 2002), and organic orthophosphate monoesters like phytic acid in the current study, were all similar, lacking any resonance features. While Sato et al. (2005) did not detect phytic acid in their poultry manure, the combined analyses of NMR and LC of P XANES of organic amendments in our study showed that PHYTIC was present in considerable amounts in all manures and more so in POULTRY than in HOG, DAIRY and BEEF. Although XANES is a powerful technique in identifying inorganic P species in environmental samples (Peak 2002; Beauchemin et al. 2003), the lack of distinguishing XANES features in organic P compounds is a major limitation to determining accurately their proportion in samples. As such, the high amount of phytic acid obtained in the LC fit of organic amendment in our study should be interpreted with caution. The presence of high amount of phytic acid may be a surrogate for other organic P species in the amendments.

The intense features of DCPD in the labile P spectrum of POULTRY, which was absent in that of the intact sample may be due to higher amount of organic P (as measured by NMR of sequential extracts) in the latter. This suggests that high amount of organic P in the intact POULTRY masked the features of DCPD in the XANES spectrum, which were noticeable only in the spectrum of labile P that contained a lesser amount of organic P. Organic constituents generally inhibit crystallization of P minerals and the spectra of amorphous CaP have been reported to lack resonances (Peak, 2002). The type of P species present in the labile fraction of the organic amendments is of particular environmental relevance. For example, the dominance of AlP (VAR) in the labile P fraction of BIO suggest that this fraction will be readily bioavailable under neutral to alkaline soil pH but not under acidic soil condition. However, studies have shown that

where this biosolids was added to high pH soil, labile inorganic P increased at the expense of organic P with time, indicating mineralization and the presence of organic P (Kashem et al. 2004a,b), which was not detected in the XANES spectra of our study. The presence of DCPD in the labile P fraction of DAIRY and BEEF also has an environmental significance. These species are less soluble in alkaline pH; therefore, their availability in soil environment will depend largely on the change in soil solution pH.

3.6 Conclusions

This study provides a detailed molecular characterization of P in different organic amendments using a combination of sequential chemical extraction, solution ^{31}P NMR and XANES spectroscopies. Overall, the P speciation results from these three techniques were complementary to one another; they provided molecular characterization of P in organic amendments that would not have been possible with any of the individual or combination of any two of these techniques. While solution ^{31}P NMR provided a detailed characterization of organic P in the NaOH and HCl fractions of organic amendments P, it was limited in characterizing the H_2O and $NaHCO_3$ fractions of most organic amendments probably due the proneness of these labile fractions to hydrolysis. However, ICP and molybdate-blue colorimetry indicated the presence of organic P in these sequential extracts. Furthermore, XANES analysis of the sequential extraction residues identified the actual chemical species of labile P as readily soluble calcium and some aluminum phosphates, which was characterized only as inorganic P by the molybdate-blue colorimetry of sequential extracts and as orthophosphates by solution ^{31}P NMR.

XANES appeared to be limited in quantitating organic P species in the organic amendments. Hence, the high amount of phytic acid, especially in poultry litter and hog manure, may be a surrogate for other organic P compounds. Although an attempt was made to identify P species in each step of the sequential extraction procedure using 'difference spectra', the noisiness of the spectra of the residues in the later stage of the extraction sequence, due to low concentrations of P, precluded this identification. While a direct comparison could not be made between the operationally-defined P forms in all stages of sequential extraction and P species identified by XANES due to the concentration issue, further advances in optimizing the detection of low concentration species may make this possible in the nearest future.

3.7 Acknowledgement

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4. XANES SPECIATION OF PHOSPHORUS IN ORGANICALLY-AMENDED AND FERTILIZED CALCAREOUS SOILS [*]

4.1 Abstract

The dominant phosphorus (P) species present in the soil following the application of organic amendments and fertilizer is important in understanding the fate of P in the environment. This study was carried out to apply an X-ray absorption spectroscopy (XAS) technique to provide insight on P species in two calcareous soils (Gleysolic Humic Vertisol and Gleyed Rego Black Chernozem) treated with organic amendments (biosolids, hog, and dairy cattle manure) and fertilizer (monoammonium phosphate, MAP). Phosphorus 1s X-ray absorption near edge structure (XANES) spectra were analyzed quantitatively by fitting the spectra of reference compounds to those of amended soils. The result showed that aqueous phosphate, representing 'soluble and adsorbedP' was the dominant P species in both soils. For the Vertisolic soil, the unamended soil and those amended with biosolids and MAP contained appreciable amount of hydroxyapatite, HAP; the most thermodynamically favoured CaP. In addition, soil amended with biosolids, hog and dairy manures contains β-tricalcium phosphate, TRICAL, a more soluble CaP than HAP. The amended Chernozemic soil contained a

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variety of species in addition to the dominant aqueous P. While TRICAL was found in all amended soil except that amended with hog manure, HAP was present in appreciable amount only in the unamended soil. Overall, this result shows the feasibility of P 1s XANES spectroscopy in identifying P species in organically amended and fertilized soil which can be applied in other investigations of P reactions in the soil.

4.2 Introduction

The dominant phosphorus species present in the soil following the application of organic amendments and fertilizer to the soil is important in understanding the potential loss of P to water bodies where it constitutes a serious environmental problem. It is well known that soil can retain most of the P applied in excess of crop requirement through various transformation processes such as, adsorption and precipitation as well as immobilization (Pierzynski et al. 2005). Studies have shown that the bioavailability of P in manures and manure-amended soils determined using wet chemistry approaches varies, depending on the types of manures and soil (Dormaar and Chang 1995; Sui et al. 1999; Ajiboye et al. 2004; Kashem et al. 2004a,b). This difference in availability was generally attributed to the presence of P compounds of differing solubility in manures and the type of reaction products formed when these amendments are applied to soil.

For example, Kashem et al. (2004b) in investigating the P fractions in soil amended with organic and inorganic P sources using sequential extraction observed higher inorganic P in the NaOH fraction of biosolids-amended soil than other amended soils at different rates of P addition. This NaOH fraction has been 'operationally-

defined' to extract P associated with organically-complexed Fe- and Al-oxides. This P pool represents a 'sink' for P and may be responsible for the lower water-extractable P and P increment as a function of application rate with biosolids than with other amendments. Furthermore, Ajiboye (2003) in studying the P retention characteristics of two calcareous soils with different levels of exchangeable Ca^{2+} reported that P sorption mechanisms in the two soils were different from each other when amended with monoammonium phosphate (MAP). The P retention in the soil with higher exchangeable Ca^{2+} was dominated by precipitation while the lower Ca in the other soil favoured a combination of precipitation and surface retention (adsorption) of P. The author concluded that further studies to distinguish between the adsorbed and precipitated phases of P in the amended soil were needed. A molecular-scale speciation using X-ray absorption spectroscopy (XAS) technique that is able to distinguish between adsorbed and precipitated species was proposed for such investigation, according to Hesterberg et al. (1999) and Beauchemin et al. (2003).

The XAS technique is fast becoming an ideal tool for solid-phase speciation of element such as P in many environmental samples. This technique is advantageous in studying soil due to its element specificity and the capability to probe the element of interest *in situ*, and determination of oxidation states and the local chemical and structural environment of the an element (Fendorf and Sparks 1996). Beauchemin et al. (2003), in using the near edge of XAS spectra (XANES) to speciate P in a range of acid to slightly alkaline soils, reported that P adsorbed on Al- and Fe oxides was present in all the soils while a poorly crystalline FeP was present in the acid soil. These authors also found that the thermodynamically favoured CaP (hydroxyapatite, HAP) was present in all soils

regardless of pH, but octacalcium phosphate (OCP) was found in the slightly alkaline soil. Sato et al. (2005) reported a predominance of FeP (strengite) in the XANES spectra of an unamended forest soil, but a combination of dicalcium phosphate (DCP) and FeP when the soil was amended with poultry manure in the short-term, and a more stable form of CaP (β-tricalcium phosphate) in the long-term. Furthermore, Lombi et al. (2006) in using XAS and isotopic dilution technique to determine the reaction products within a fertilizer reaction zone of calcareous soil reported that granular monoammonium phosphate (MAP) precipitated as HAP in the vicinity of application making it less labile compared with liquid MAP.

An *in situ* identification of P species formed in soils with manure application will ultimately provide a better understanding of P release and prediction of potential loss of P following organic amendments application to agricultural soils. The objective of this study therefore was to identify P species and/or P reaction products in organically amended and fertilized soils using quantitative XANES analysis.

4.3 Experimental Methods

4.3.1 Organic Amendments and Soil Samples

Organic amendments including biosolids, hog, and dairy cattle manures, and fertilizer (MAP) were used in this study. The description and chemical properties of these amendments have been reported earlier (Chapter 3 of this thesis). Two calcareous soils; Osborne (Gleysolic Humic Vertisol) and Lakeland (Gleyed Rego Black Chernozem) were selected for this study. The pertinent physio-chemical properties of these soils are

shown in Table 5.1. The treatment of the soils with organic amendments and subsequent incubation has been reported in details by Kashem et al. (2004a). Briefly, 100 g air-dry Osborne soil was mixed with organic amendment at the rate equivalent to 920 mg P kg^{-1} soil (based on the amendment P) with biosolids (O-BIO), hog manure (O-HOG), dairy cattle (O-DAIRY), and MAP in granular form (O-MAP). The treated soils were incubated at field capacity for 16 weeks at 20º C in a 500 mL glass jar with perforated lids to allow for air exchange. The Lakeland soil was also amended with biosolids (L-BIO), hog manure (L-HOG), dairy cattle manure (L-DAIRY), and MAP (L-MAP) in the same way, but at a slightly higher rate of 1228 mg P kg^{-1} soil. These application rates were chosen to bring the total phosphorus levels close to those found in soils with long history of manure application (Beauchemin et al. 2003; Dormaar and Chang 1995). The concentrations of ammonium oxalate extractable P, representing amorphous and crystalline P, ranged from 27 - 31 mmol P kg^{-1} in all the amended Osborne soil and 31 - 46 mmol P kg^{-1} in all the amended Lakeland soil (Ajiboye 2004, unpublished).

4.3.2 Quantitative XANES Analyses

The various P reference compounds used in this study included CaP, AlP, MgP, NH_4^+P, NaP, and organic phosphates (as previously reported in Chapter 3). In addition, aqueous phosphate (AQ-P) was included as a reference compound. The dominant aqueous P in the pH range of the calcareous soil used in this study was HPO_4^{2-}. A 50 mM P of this solution was prepared by dissolving Na_2HPO_4 in 0.1M KCl background electrolyte.

Table 4.1 Selected properties of the unamended Lakeland (L-CONTROL) and Osborne (O-CONTROL) soils

Soil	pH [z]	Texture [y]			Extractable P		PSI [v]	CO_3- C [u]	Total C [t]
		Sand	Silt	Clay	Olsen-P [x]	P_{ox} [w]			
		------- (g kg^{-1}) -------			---- mmol kg^{-1} ----			----- mg kg^{-1} ----	
L-CONTROL	8.0	79	624	297	1.9	24	10.2	6.0	9.22
O-CONTROL	7.6	84	326	590	0.4	18	21	3.4	5.12

[z] In 1:2 soil-water suspension (Hendershot et al. 1993)
[y] Using pipette method (Sheldrick and Wang 1993)
[x] Olsen-P (buffered 0.5 M $NaHCO_3$) (Kuo 1996).
[w] Ammonium oxalate extractable P (Jackson et al. 1986).
[v] P Saturation Index = Olsen-P/P_{sorb75} (single point sorption capacity for unamended non-incubated soils
[u] Volumetric calcimeter method (Loeppert and Suarez 1996)
[t] Dry combustion according to (Schepers et al. 1989).
Source: (Ajiboye 2003).

Studies have shown that it is impossible to distinguish between the XANES spectra of aqueous and adsorbed P on Al oxides, due to their lack of distinctive features (Toor et al. 2005; Sato et al. 2005), therefore, AQ-P was used to represent 'soluble and adsorbed' phosphates in this study. The near edge spectra of P 1s XAS (XANES) of the reference P compounds and ground amended soil samples were acquired in duplicates as previously described in Chapter 3 of this thesis. Aliquots of the aqueous P (AQ-P) was transferred into a specialized stainless steel cylindrical sample holder covered with 6 μm Lexan® film that allows for passage of both incident and fluorescence X-ray photons and then transferred into the absorption chamber of the beamline. All the data reduction and quantitative analysis of P 1s XANES spectra were carried out using ATHENA software (Ravel and Newville, 2005). The spectrum of PYRO used for the monochromator calibration was compared with the one acquired alongside reference compounds to correct for any edge energy shift in the XANES spectra of standards and samples. Correcting for edge energy shift is very crucial in optimizing the curve fitting procedure used in the quantitative analysis. The XANES data were averaged and deglitched to remove high-frequency noise while retaining distinguishing features and then background-corrected by a linear regression fit through the pre-edge region and a cubic spline through the post edge region. The spectra were then normalized to a unit edge jump. The XANES spectra of all reference compounds, except DNA, PEA, and PC, were corrected for self-absorption prior to LC fitting since these compounds were not diluted. The self-absorption algorithm parameters were set according to the beamline configuration. Specifically, the angle of the incident photon relative to the sample was set to 60º and the angle of fluorescence emission (i.e. orientation of the detector to the

incident photon) set to 30º. LC fitting was performed using binary to quaternary combinations of all standards compounds over the relative energy range of -11 to 49 eV. The weights of the components were constrained to be between 0 and 1 and forced to sum to 1 during the fit. The threshold energy, E_0, was allowed to float to a maximum energy shift of ± 0.25 eV, which is equivalent to the step size used during data acquisition. The goodness-of-fit was judged by the R-factor and chi square values (χ^2). The fit with the least R-factor and χ^2 was chosen as the best fit after careful inspection of the fit and associated uncertainties.

4.4 Results and Discussions

4.4.1 Linking Phosphorus Species with Extractable P and P Fractions in Amended Soils

The linear combination fitting of the P 1s XANES spectra of dominant P species to those of amended soils are shown in Figs. 4.1 and 4.2. The noisiness of the spectra was due to the low concentration of P in these amended soils. For example, the total P in the unamended Lakeland soil was 0.13 % while that of amended soils except L-DAIRY ranged from 0.23 - 0.26 % (Akinremi et al. 2003). More research effort is clearly needed to improve the detection of P in low concentration samples such as those used in this study. Beauchemin et al. (2003) suggested that using particle-size separation to obtain clay + silt fraction for XAS analysis as a means of concentrating the sample may be a method to address the low concentration issue. However, this sample pre-treatment was not used in the study because there was no way of controlling the P loss and change in the

chemical form that may occur. Despite the noisiness of the spectra, the proportions of the dominant species in the spectra were reasonably estimated with goodness-of-fit criteria (R-factor), ranging from 0.42 - 0.54 % and from 0.28 - 0.64 % in the amended Osborne and Lakeland soils, respectively (Table 4.2). These goodness-of-fit criteria are simply a statistical measure of variation in the fit and not a measure of relative error associated with the fit. A more robust measure of accuracy of the LC fitting than the statistical uncertainty in the fit, though not carried out in this study, is worth considering in future studies. Furthermore, the uniqueness of the fit may be subject to interpretation due to the noisiness of the sample spectra. Therefore, the composition of the species reported here is considered only as the best estimate available for the XANES data with the constraints used in the LC fitting and may not necessarily hold true. Subsequent interpretation of the results should be done with caution.

The result suggest that unamended Osborne soil (O-CONTROL) was dominated by AQ-P, representing 'soluble and adsorbed P' and HAP, the most thermodynamically favoured CaP, which were estimated to be approximately 62 % and 38 %, respectively. Similar to O-CONTROL was O-MAP, which was dominated by 'soluble and adsorbed P' (65 %), and HAP (35 %). However, O-BIO contains TRICAL (a more soluble CaP than HAP) in addition to these two P species present in the unamended soil (Fig 4.1b; Table 4.2). The other amended Osborne soil; O-HOG and O-DAIRY, were also dominated by the weakly bound P (AQ-P) and less soluble CaP (TRICAL). The O-DAIRY also contained some FeP in form of STRENG (Table 4.2). The presence of significant proportions of HAP in O-BIO and O-MAP was further supported by the fingerprinting approach (Fig. 4.2), and together with TRICAL in O-HOG and O-DAIRY corroborate the

results of Kashem et al. (2004a). These authors reported that the amount of readily soluble P (H_2O-P and NH_4Cl-P) and plant available P (Olsen P, Kelowna P, and Mehlich-3 P) after 16-wk incubation period was smaller in O-BIO and O-MAP than in O-HOG and O-DAIRY.

Similar to the amended Osborne soil, 'soluble and adsorbed' P dominated the amended Lakeland soil, though with slightly higher proportion except for L-DAIRY (Table 4.1; Fig. 4.3). While L-CONTROL and L-HOG contained thermodynamically stable HAP and PHYTIC, along with the 'soluble and adsorbed' P, L-BIO contained secondary AlP mineral, VAR and less soluble CaP (TRICAL) (Fig. 4.3). The L-MAP and L-DAIRY also contained TRICAL along with 'soluble and adsorbed' P, while L-DAIRY contained FeP in the form of STRENG in addition to these species, similar to its Osborne soil counterpart (O-DAIRY) (Fig. 4.3).

These results from XANES analysis did not completely support the trend in the extractable P, which was highest in L-MAP and lowest in L-BIO, with L-HOG and L-DAIRY being intermediate (Kashem et al. 2004a). Considering that the labile P fractions in the amended Lakeland soil were linearly correlated with the cumulative addition of P from these amendments (Kashem et al. 2004b), it is therefore reasonable to assume that the amended soil used in the current study, although at a higher rate that those used by Kashem et al. (2004b), will follow the same trend for the labile P; L-BIO < L-DAIRY < L-HOG < L-MAP. The XANES results could only partially explain this trend. The proportion of 'soluble and adsorbed P' was highest in L-MAP and L-HOG followed by L-BIO and L-DAIRY (Table 4.2). However, the presence of VAR in L-BIO, which was absent in L-DAIRY, may be responsible for the least labile P in L-BIO as reported by

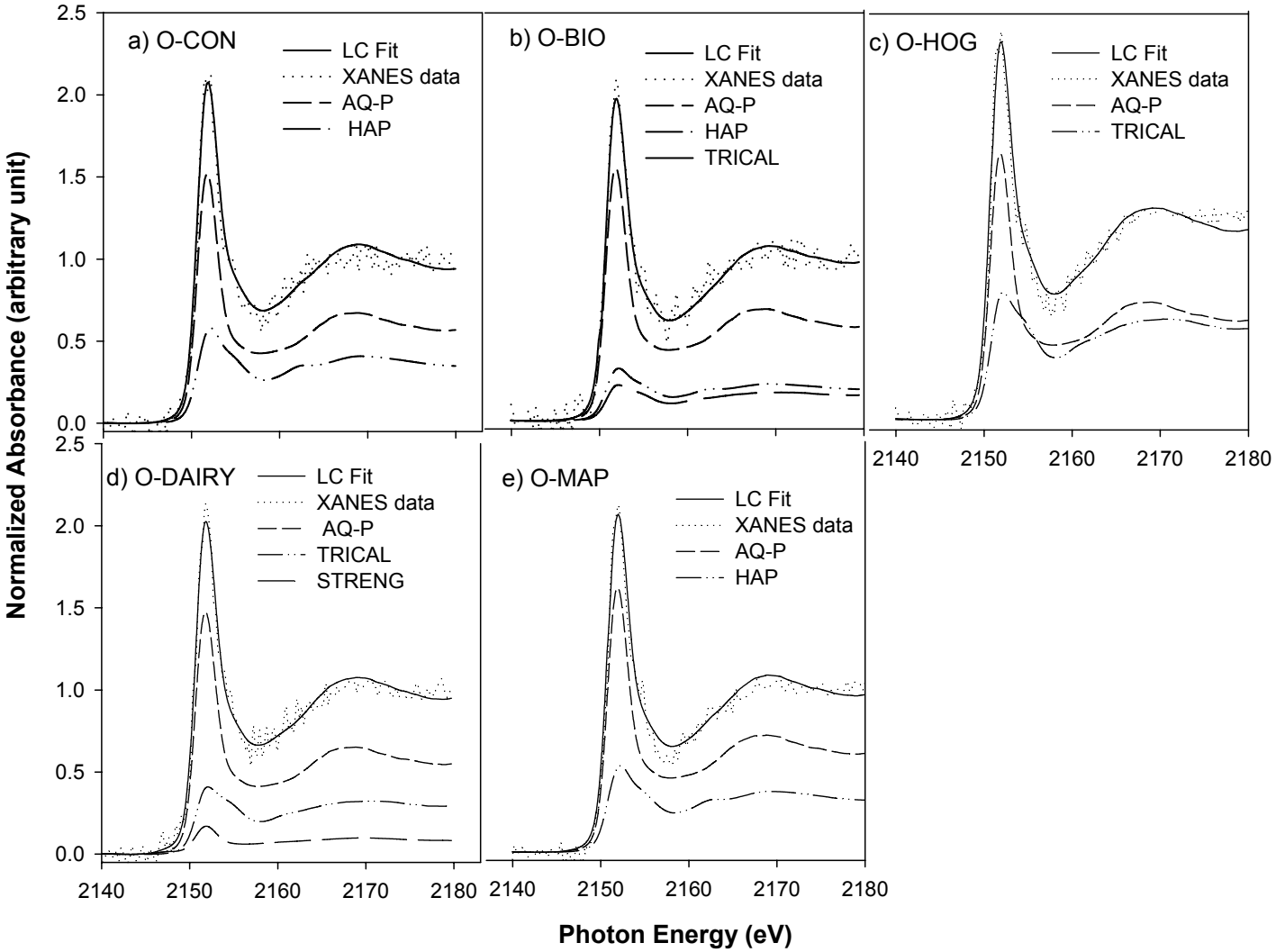

Figure 4.1. Linear combination fitting of amended Osborne soil.

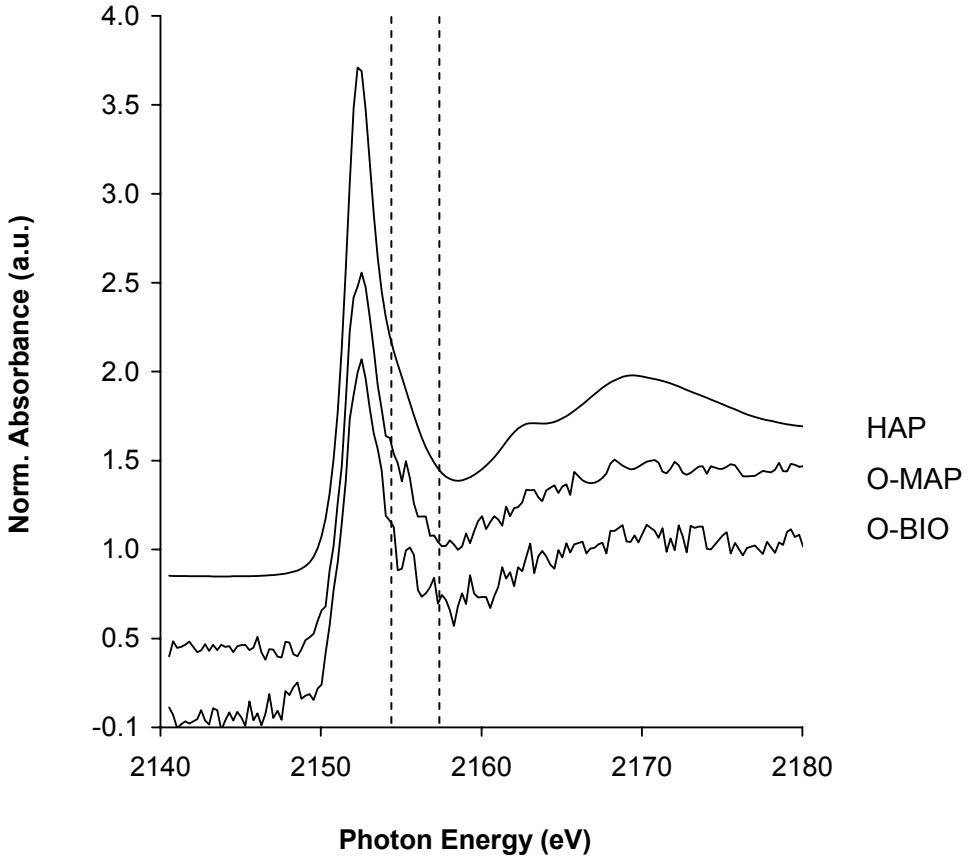

Figure 4.2. P 1s XANES spectra of Osborne soil amended with biosolids (O-BIO) and monoammonium phosphate, MAP (O-MAP) showing the shoulder feature (dashed line) of hydroxyapatite (HAP).

Kashem et al. (2004b).

The difference in the retention of P in MAP-amended Osborne and Lakeland soil had been earlier pointed out to require further investigation (Ajiboye 2003; Kashem et al. 2004b). It was initially hypothesized that precipitation was the dominant P retention mechanism in the amended Osborne soil while a combination of adsorption and precipitation dominated in the amended Lakeland soil. However, the dominance of 'soluble and adsorbed P' in both amended soils with proportions ranging from

approximately 53 % - 65 % of the P species in the amended Osborne soil and 53 - 82 % in the Lakeland amended soil, suggests that adsorption may be a more important retention mechanism of P than precipitation in these soils especially for organic amendments. It is interesting to note that 'soluble and adsorbed P' dominated the unamended soils with proportions even greater than some of the amended soil, especially O-HOG and L-DAIRY (Table 4.2). Sato et al. (2005) reported that 'soluble and adsorbed P' was the dominant P species in poultry manure. However, it was not clear what the sorbent in the manure matrix was. Hunger et al. (2004) speculated that CaP may be surface precipitated on $CaCO_3$ in the poultry manure. Given the calcareous nature (CO_3-C content ranging from 3.4 - 6 %) of the soils in our study, the dominance of AQ-P may indeed be indicative of weakly bound P on $CaCO_3$. This is further supported by the higher amount of AQ-P in the amended Lakeland (with higher CO_3-C) compared with amended Osborne soils.

Previous studies have revealed that the organic amendments used in this study contained appreciable amounts of Al and Fe, especially biosolids, which contained up to 0.2 % of Al and Fe (Ajiboye et al. 2004; Chapter 3 of this thesis). However, the fact that FeP was not detected in either O-BIO or L-BIO suggest that the alkaline pH of the soil promoted the dissolution of FeP that was probably applied with the biosolids, although not detected in the XANES spectra. This dissolution of FeP in alkaline soil may favour the secondary precipitation of P as CaP, and this may explain the formation of β-TRICAL in these soils. Sato et al. (2005) also reported that an increase in the pH of acid forest soil upon manure application resulted in the dissolution of strengite from the soil. These authors also attributed the lower proportion of CaP species in amended soils than those in

manure to the dissolution of CaP from manure due to the likely reduction in pH of manure-amended soil relative to the manure.

Conversely, the presence of FeP (STRENG) in the O-DAIRY and L-DAIRY may be linked to the organically complexed Fe and Al P in the dairy manure, which was next to that of biosolids (Chapter 3 of this thesis). Mineralization may have released this organo-complexed FeP, making it available to undergo secondary precipitation as FeP in the presence of soluble Fe at the alkaline pH of the soils. This mechanism may also explain the presence of AlP (VAR) in L-BIO out of all the amended soils despite the alkaline pH of the soil; mineralization of organo-complexed AlP from the manure undergoing secondary precipitation as VAR.

The absence of phytic acid in the amended Osborne soil and its presence in trace amount in the amended Lakeland soil corroborates other results in the literature where phytic acid was not detected by the XANES technique (Beauchemin et al., 2003; Sato et al., 2005). One explanation for this is that organic P was probably not present in sufficient amount as phytic acid to allow for detection by XANES in these amended soils. Another reason may be the absence of strong and distinguishing features in the spectrum of phytic acid. The spectrum of phytic acid and those of adsorbed P on $CaCO_3$ and iron hydroxide and gibbsite have been reported to be similar, lacking any distinguishing features. This remains as one of the challenges with interpreting P K-edge XANES data for heterogeneous systems like amended soil. Clearly, the wide acceptance of XANES spectroscopy in speciation P in soil in the future will depend on the possibility of generating spectra with distinguishing features, especially for the reference compounds.

Table 4.2 Proportion of P species in the amended Osborne (O) and Lakeland (L) soil from linear combination (LC) fitting.

Treatment [z]	Goodness-of-fit [y]		LC Fit (%)					
	R-factor	χ^2	AQUEOUS P		TRICAL	HAP		STRENG
O-CONTROL	0.00542	0.6589	61.7 ± 4.8 [x]		-	38.3 ± 4.8		-
O-MAP	0.00427	0.4549	65.3 ± 3.7		-	34.7 ± 3.7		-
O-BIO	0.00513	0.5960	62.4 ± 5.0		16.5 ± 0.3	21.0 ± 0.3		-
O-HOG	0.00461	0.4316	52.8 ± 3.5		47.2 ± 3.5	-		-
O-DAIRY	0.00420	0.4594	59.8 ± 5.2		31.0 ± 9.7	-		9.2 ± 1.1
			AQUEOUS P	VAR	TRICAL	HAP	PHYTIC	STRENG
L-CONTROL	0.00375	0.4484	79.1 ± 4.6	-	-	17.9 ± 6.1	1.7 ± 0.7	-
L-HOG	0.00400	0.4263	81.8 ± 3.0	-	-	5.6 ± 1.2	12.6 ± 3.2	-
L-BIO	0.00281	0.2801	64.3 ± 3.4	21.4 ± 0.9	14.4 ± 8.2	-	-	-
L-MAP	0.00311	0.3888	82.2 ± 2.8	-	17.8 ± 2.8	-	-	-
L-DAIRY	0.00641	0.7530	53.0 ± 6.8	-	19.4 ± 9.4	-	-	27.5 ± 11.6

[z] Treatments with prefixes 'O' and 'L' represent Osborne and Lakeland soils, respectively.

[y] $$R = \frac{\sum_{i=1}^{N}\left[y_{measured}(i) - y_{fitted}(i)\right]^2}{\sum_{i=1}^{N}\left[y_{measured}(i)\right]^2}; \quad \chi^2 = \frac{1}{\sigma^2}\sum_{i=1}^{N}\left[y_{measured}(i) - y_{fitted}(i)\right]^2$$

where $y_{measured}(i)$ = *ith* normalized absorbance data point measured for the sample, $y_{fitted}(i)$ = *ith* normalized absorbance data point fitted for the sample, σ = uncertainty (or experimental error) estimate, and N = number of data points.

[x] Uncertainties estimated as ± standard deviation

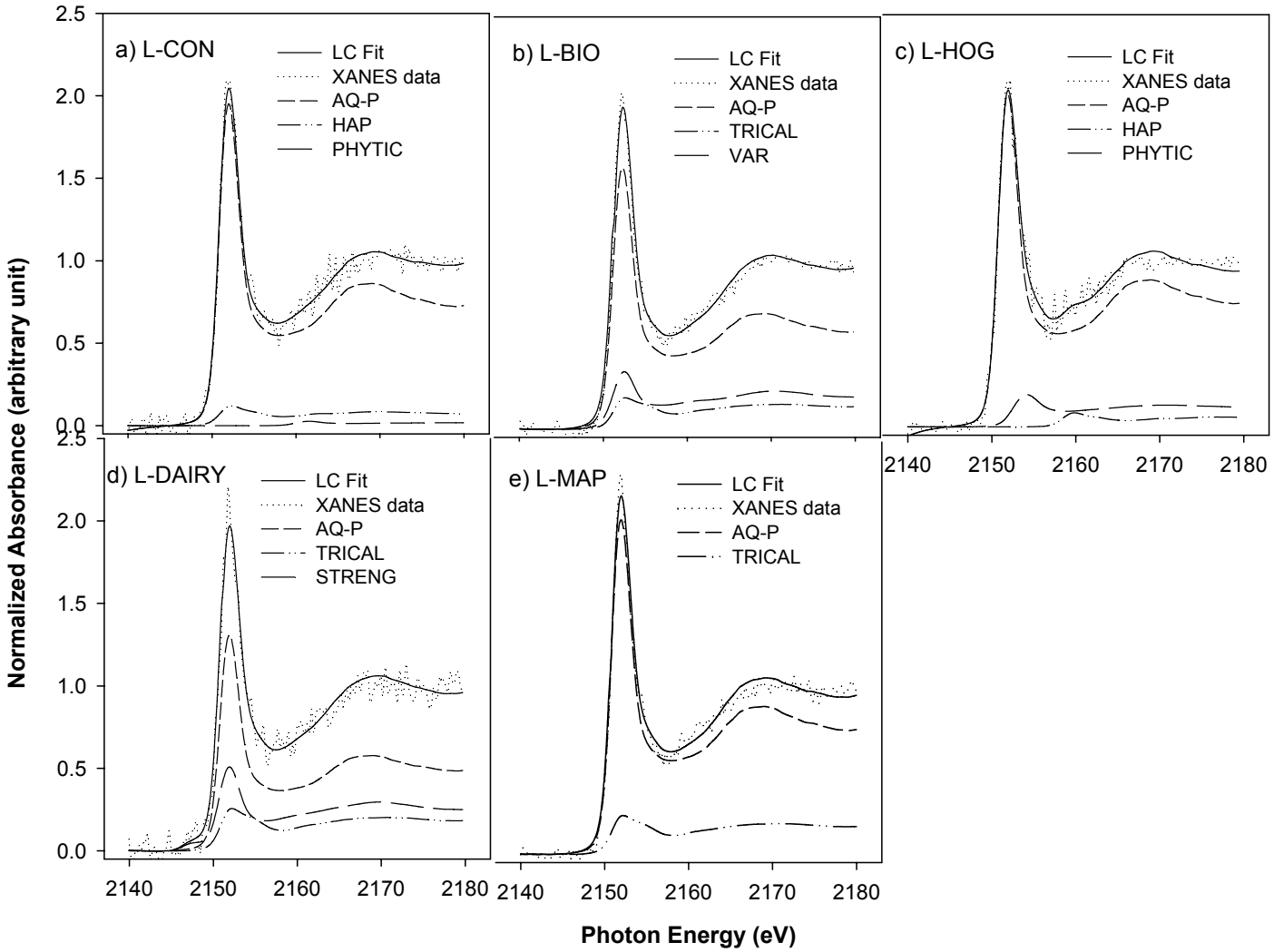

Figure 4.3. Linear combination fitting of amended Lakeland soil.

From an environmental standpoint, the 'soluble and adsorbed P' in these amended soils will constitute a source of P while the CaP identified as HAP and TRICAL will have limited solubility considering the alkaline pH of these soils. Although the proportions of HAP and TRICAL were smaller in comparison with AQ-P, they could remain in the soil in a non-exchangeable form. Lombi et al. (2006) also reported that HAP and TRICAL formed within the vicinity of a granular MAP fertilizer was less labile and contributed less to the isotopically exchangeable P pool in comparison with readily soluble CaP like monocalcium phosphates.

4.5 Conclusion

This study showed the result from solid-phase speciation of P in organically amended and fertilized soil using the P 1s XANES. The quantitative analysis of the XANES spectra of both amended Osborne and Lakeland soils using LC fitting showed that aqueous phosphate, representing 'soluble and adsorbed P' was the dominant P species, but its proportion relative to other species should be interpreted with caution. For the Osborne soil, the unamended and soil amended with biosolids and MAP contained appreciable amount of HAP, the most thermodynamically favoured CaP. In addition, soil amended with biosolids, hog and dairy manures contain TRICAL, which is a more soluble CaP than HAP. The amended Lakeland soil contained variable species in addition to the dominant 'soluble and adsorbed P'. While TRICAL was found in all amended Lakeland soil except in that amended with hog manure, HAP was present in appreciable amount only in the unamended soil. The P 1s XANES analysis provided chemical information that supports previous findings on the different level of extractable

P in the amended Osborne soil, but not in those of Lakeland soils. In addition, the result from XANES analysis did not support previous hypothesis on the differences between Osborne and Lakeland soils amended with MAP; XANES showed that adsorption may be the dominant retention mechanism in both soils contrary to previous speculation about precipitation being dominant in Osborne soil and a combination of adsorption and precipitation in Lakeland soil. The 'soluble and adsorbed P' in these amended will constitute a source of P into the environment and those soils with appreciable amount of HAP and TRICAL will have limited solubility considering the alkaline pH of these amended soils. Overall, this result shows that the feasibility of P 1s XANES spectroscopy in speciating P in amended soils may not be entirely independent of chemical extraction data during interpretations. For example, the similarity in the spectra of some organic P compounds, aqueous P (soluble and adsorbed P), and adsorbed P on Al oxides means that the choice of reference compounds selected for LC fitting will be highly dependent on the chemical extraction data of the sample. The lack of distinguishing features in many reference P compounds used in quantitative P 1s XANES analysis is a major limitation of this technique. It may be worthwhile to explore the near edge regions of other 'edges' such as L-edge for distinguishing features that may be used to optimize quantitative analysis of P species in environmental samples.

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5. EXPERIMENTAL VALIDATION OF QUANTITATIVE X-RAY ABSORPTION SPECTROSCOPIC ANALYSIS FOR PHOSPHORUS SPECIATION *

5.1 Abstract

The quantitative approach used in X-ray absorption spectroscopy (XAS) experiments is often times based on statistical goodness-of-fit criteria, which do not explain the accuracy of the components obtained from the fittings. This study was carried out to validate the linear combination (LC) approach used in quantitative XAS analysis by estimating the accuracy of this procedure. Near-edge $K\alpha_1$ fluorescence XAS spectra were acquired for known binary mixtures of calcium, aluminum, and iron phosphates in varying proportions and for the individual compounds. All combinations of the spectra of model compounds were fitted to the spectra of the known mixtures to obtain their relative abundance. The binary combinations produced the best fit with χ^2 values ranging from 0.02 - 0.25. The relative error associated with the fitting ranged from as low as 0.8 % to 41.6 % for thoroughly mixed samples. The relative error was small when the proportion of calcium phosphate in the mixture was high but the error was large at low abundance of this component in the mixture. Because the interpretation of the XANES results depends

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largely on the relative proportion of species in the sample obtained by LC, we therefore recommend acquiring a spectrum for a mixture of certified reference compounds that mimics the composition of the sample being investigated at the beamline to estimate the accuracy of the proportions obtained from quantitative XANES analysis.

5.2 Introduction

X-ray absorption spectroscopy (XAS) is fast becoming a versatile tool for the speciation of phosphorus in complex environmental samples. The near-edge region of XAS spectra, also known as XANES (X-ray absorption near-edge structure), is especially sensitive to the local structural and electronic environment of the P atom and can distinguish between precipitated and adsorbed phases of P (Hesterberg et al. 1999; Beauchemin et al. 2003; Sato et al. 2005). Studies have shown that each P species has its own fingerprints in the XANES region (Hesterberg et al. 1999; Khare et al. 2004), and the spectra of two or more P species can be used quantitatively to reveal the abundance of these species in a sample. Quantitative speciation using XANES data is highly dependent on the quality of the data and how well the chosen standards match the real species in the samples of unknown composition (Pickering et al. 1995; Beauchemin et al. 2003). In addition, the spectral features of identified components must be clearly distinguishable from one another to perform a reliable quantitative XANES analysis.

The quantitative approach used in the speciation of P and other elements in XAS usually involves principal component analysis (PCA) combined with target transformation (TT), and least-squares linear combination (LC) fitting (Beauchemin et al. 2002; Beauchemin et al. 2003; Ressler et al. 2005). The PCA uses a multivariate

statistical procedure to identify the number of independent orthogonal components that constitute the sample spectra while TT identifies the actual chemical species. The LC can be used as a stand-alone procedure for quantitative XANES analysis or in conjunction with PCA and TT to reduce the number of reference compounds needed in the fitting, which could be a big time-saver. The LC is usually done by fitting the reference spectra directly to unknown spectra while adjusting the fraction of each component assumed in the fit model and energy offset (O'Day et al. 2004). Beauchemin et al. (2003) reported that PCA lacked sensitivity to P 1s XANES data in that the maximum number of chemical species that can be accepted based on TT depends on the number of orthogonal components considered as sufficient to reconstruct the XANES data in a reduced space. In fact, these authors achieved the best characterization of their soils by using ternary combinations of P standards in the LC fitting contrary to the binary combination suggested by the result from PCA analysis. In using S XANES to characterize chemical species of humic acid in soil, Beauchemin et al. (2002), also reported that the number of potential targets (identified by TT) was more than the number of dominant components indicated by PCA. In a recent study on P speciation in fertilized soil with granular and liquid monoammonium phosphate (MAP), only two of the three species accepted by TT (SPOIL value <3) was used in the LC fitting because PCA indicated that only two orthogonal components are sufficient to reconstruct the P XANES data (Lombi et al. 2006). Therefore, using either a binary or ternary combination of P compounds in the LC fitting may affect the LC result and conclusion made from such a procedure. For example, in the study by Lombi et al. (2006), the relative contribution of the third component that was discarded could be significant and may have a different implication from an environmental perspective.

In using LC for quantifying the relative proportion of reference standards in the XANES spectra of the sample, very few studies have examined the accuracy of the estimated proportion of standard compounds in known mixtures of these compounds (O'Day et al. 2004). As at the time of writing this thesis, no study has been reported in the literature where the accuracy of the quantitative methods of XAS analysis of P using known mixtures was investigated. The goodness-of-fit criteria reported for the LC fitting are usually statistical measures of error, which do not explain whether the components assumed in the fits are correct or not. This systematic error has not been well characterized and is difficult to estimate by statistical measure alone (IXS 2000; O'Day et al. 2004). Therefore, an empirical measure of accuracy of the XANES fit of known components may be necessary as a first step in using LC for quantitative XANES analysis in order to obtain a reliable fit. This will serve as a more robust estimation of the error in using LC fitting than the goodness-of-fit criteria obtained for the LC fitting.

The objective of the study was to use known mixtures of P compounds to validate the LC fitting procedure used in quantitative XANES analysis as a means of estimating its accuracy.

5.3 Materials and Methods

Three classes of P compounds commonly found in the environment were selected for this study. The compounds were CaP: hydroxyapatite, HAP ($Ca_{10}(PO_4)_6(OH)_2$); AlP: variscite, VAR ($AlPO_4.2H_2O$); and FeP: phosphosiderite, PSIDER (monoclinic $FePO_4 \cdot 2H_2O$). Reagent-grade samples of these compounds were ground with mortar and pestle and mixed in a g-weight proportion of the compounds equivalent to $[HAP]_x$ +

$[VAR]_{1-x}$, and $[HAP]_x$ + $[PSIDER]_{1-x}$ with x = 0.25, 0.5, 0.75. The relative contribution of P, based on atomic mass fraction, by the individual reference compounds constituting the mixtures varied. However, the total P in the mixtures was similar with ~ 19 wt-% in the HAP+VAR mixtures and ~ 17-18 wt-% in the HAP + PSIDER mixtures (Table 5.1). The mixtures and pure standard compounds were diluted with boron nitride (BN) to bring the P content to ~ 5 wt-% and then spread as a thin film on a double-sided C tape to minimize self-absorption which may distort fluorescence measurements. The XANES spectra of the mixtures and individual P compounds were acquired using the DCM beamline of the Canadian Synchrotron Radiation Facility (CSRF) at the Synchrotron Radiation Center (SRC), University of Wisconsin-Madison operating at 800 MeV ring energy and maximum beam current of ~230 mA. Standard operating parameters for this beamline have been explained in detail earlier (Lombi et al., 2006). Duplicate spectra of $K\alpha_1$ fluorescence emission, involving the ejection of an 1s electron into the continuum and simultaneous filling of the core-hole with a $2p_{3/2}$ electron, were recorded as a fluorescence yield (FY) from 2140 - 2200 eV for each mixture.

The energy range of the spectra was re-calibrated to correct for any edge shift in the spectra of the standards used in calibrating the beamline. This was done by using the edge energy offset between the energy of the while line of $Na_4P_2O_7$ analyzed with the sample and that recorded during the calibration of the beamline. This offset value was used as pre-processing parameter for all other spectra. The FY data were averaged, background corrected by a linear regression fit through the pre-edge region and a cubic spline through post edge region. The spectra were then normalized to a unit edge jump. All the data reduction and XANES analysis were performed using ATHENA ver 0.8.049 (Ravel and Newville 2005). Linear combination fitting was performed without *a priori*

Table 5.1 Binary mixtures of P compounds showing the total P in the fractions

Mixtures	Mass fraction of P compounds (P in the fraction)		Total P g-wt (g in mixture)	% P [z]
	HAP	VAR		
25 % HAP + 75 % VAR	0.515 (0.049)	0.485 (0.158)	0.207 (1.0696)	19.33
50 % HAP + 50 % VAR	0.761 (0.097)	0.239 (0.105)	0.203 (1.064)	19.05
75 % HAP + 25 % VAR	0.905 (0.146)	0.095 (0.053)	0.199 (1.0583)	18.78
	HAP	PSIDER		
25 % HAP + 75 % PSIDER	0.473 (0.049)	0.527 (0.124)	0.173 (1.0364)	16.69
50 % HAP + 50 % PSIDER	0.729 (0.097)	0.271 (0.083)	0.180 (1.0418)	17.30
75 % HAP + 25 % PSIDER	0.890 (0.146)	0.1101 (0.041)	0.187 (1.0472)	19.90

[z] based on total mass of the mixture

assumptions about the number of components in the mixtures by using all combinations of the three model spectra (HAP, VAR, and PSIDER). The fitting was performed over the relative energy range of -11 to 49 eV. During the LC fitting, the threshold energy, E_0, was allowed to vary, but the maximum energy shift observed for any of the components in the mixture was much less than the step size (0.25 eV) used during data acquisition. The goodness-of-fit was judged by the residual factor (R-factor) and chi square values (χ^2); the fit with the least R-factor and χ^2 and the least standard deviation of the estimated proportion was chosen as the most likely fit.

5.4 Results and Discussion

The LC fit of known binary mixtures of P standard compounds are shown in Fig. 5.1. The various proportions of standard mixtures and the overall LC fit are plotted together with normalized XANES data acquired for the mixture. Visual inspection confirmed that the LC is a good representation of the XANES data (Fig. 5.1). The statistical goodness-of-fit value ranged from 0.15 to 0.25 for different combinations of HAP and VAR mixtures and from 0.02 to 0.09 for HAP and PSIDER mixtures (Table 5.2). The mixing efficiency of these P standards, though not verified, was assumed to be within the range for dry mixing of two powder systems, which was reported to be between 0.62 and 0.82 (Chen and Yu, 2004). The relative error between the theoretical proportions of the standards in the mixtures and proportions obtained by LC fitting of the XANES spectra ranged from as low as 0.8 % to 17 % for the HAP/VAR mixtures and from 2.9 to 41.6 % for the HAP/PSIDER mixtures (Table 5.2). The relative error was small when the proportion of HAP in the mixture was high but the error was large at low abundance of

this component in the mixture (Table 5.2). It should be noted that the XAS spectrum of HAP has rich features especially in the near-edge region and this result suggests that such features are easily optimized for in the LC fitting when they are more abundant than other component features in the mixture. Although the 25 % HAP+75 % PSIDER mixture had the greatest relative error, the statistical uncertainty in the LC fit was least in comparison with other mixtures. This fit also had the best statistical goodness-of-fit (Fig. 5.1d, Table 5.2). There are some explanations for this observation. First, it is possible that the mixing efficiency was not perfect, which in turn resulted in hotspots of PSIDER in the mixture analyzed by XAS. Another reason may be the abundance of PSIDER in the mixture coupled with similarity in the energy position of the post-edge resonance occurring at approximately 2162 eV in both PSIDER and HAP, and the lack of a shoulder feature in the XANES data of the mixture. This makes the pre-edge feature the only structure that was optimized during the LC fitting, hence the overestimation of PSIDER. In addition, the low proportion of HAP in the mixture and the absence of a feature that distinguishes it from PSIDER in the post-edge region, may be limiting the quantitative XANES analysis. Studies have shown that quantitative XANES cannot detect a trace of one chemical species of an element in presence of a large excess of another species of the same element (Pickering et al. 1995; Pickering et al. 2000) or for that matter, other element with similar XANES features.

Because the interpretation of the XANES result depends on the relative proportion of species in the sample obtained by LC, we propose that the accuracy of the fitting should be reported alongside the fit result. This can be accomplished by acquiring the XANES spectrum for a mixture of certified reference compounds that mimics the

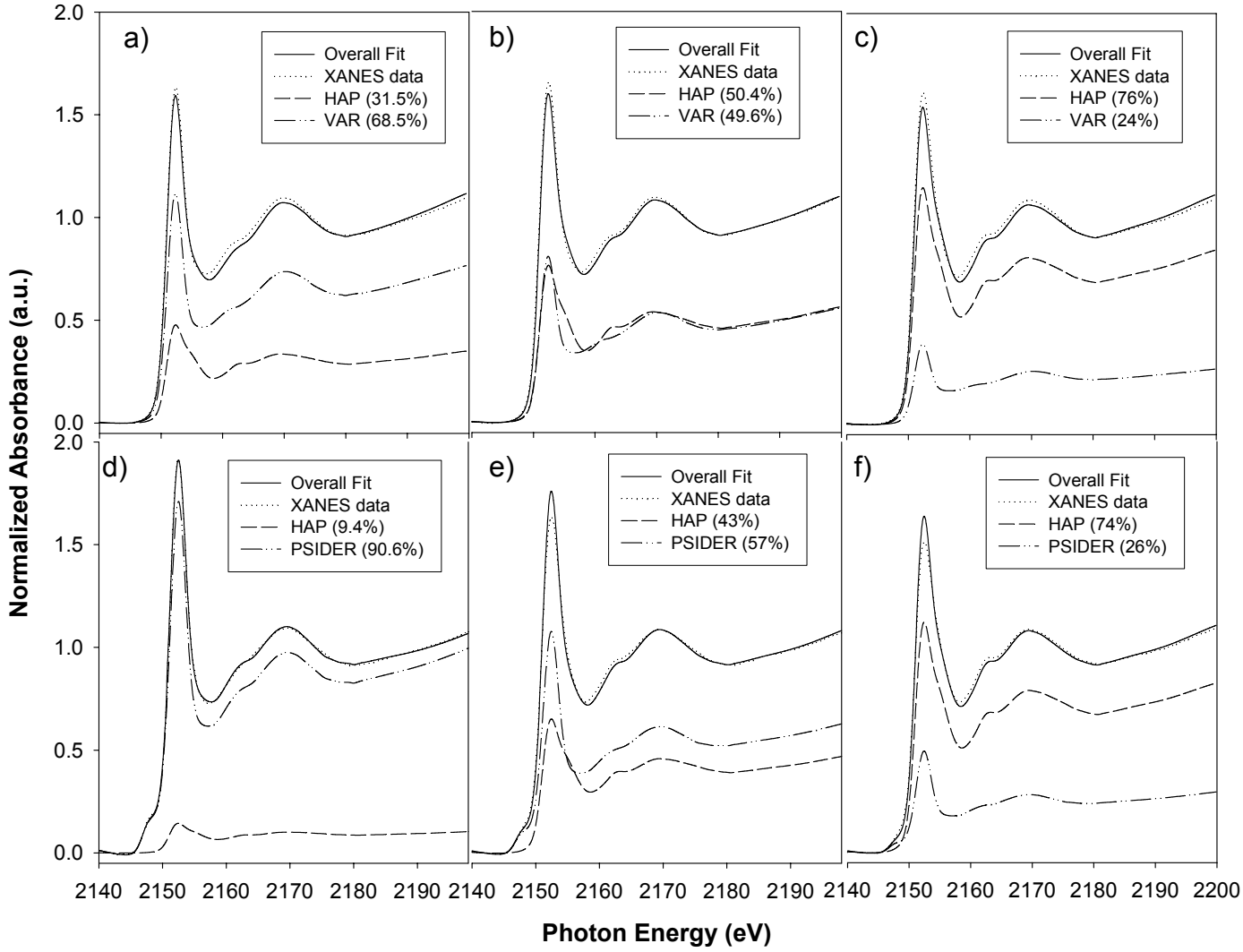

Fig. 5.1. LC fit of binary mixture of phosphate standards containing (a) 25 % HAP +75 % VAR, (b) 50 % HAP + 50 % VAR, (c) 75 % HAP + 25 % VAR, (d) 25 % HAP + 75 % PSIDER, (e) 50 % HAP + 50 % PSIDER, and (f) 75 % HAP + 25 % PSIDER.

Table 5.2. Summary of linear combination (LC) fittings of XANES spectra of binary mixtures of hydroxyapatite (HAP), variscite (VAR), and phosphosiderite (PSIDER).

	Standard Mixtures [z]	Goodness-of-fit [y]		LC Fittings		Relative Error [w]
		R-factor	χ^2			
		($\times 10^{-4}$)		---------- wt % ---------		%
				HAP	VAR	
	25 % HAP +75 % VAR	6.2	0.15	31.5 ± 2 [x]	68.5 ± 2	17.3
CaP + AlP	50 % HAP + 50 % VAR	10.1	0.25	55.2 ± 2.6	44.8 ± 2.6	0.8
	75 % HAP + 25 % VAR	8.7	0.22	76 ± 2.4	24 ± 2.4	2.7
				HAP	PSIDER	
	25 % HAP + 75 % PSIDER	0.7	0.02	9.4 ± 0.5	90.6 ± 0.5	41.6
CaP + FeP	50 % HAP + 50 % PSIDER	3.0	0.07	43 ± 2.6	57 ± 1.8	14.0
	75 % HAP + 25 % PSIDER	3.6	0.09	73.9 ± 2.4	26.1 ± 1.9	2.9

[z] based on g-wt

[y] $R = \frac{\sum_{i=1}^{N}\left[y_{measured}(i) - y_{fitted}(i)\right]^2}{\sum_{i=1}^{N}\left[y_{measured}(i)\right]^2}$; $\chi^2 = \frac{1}{\sigma^2}\sum_{i=1}^{N}\left[y_{measured}(i) - y_{fitted}(i)\right]^2$

where $y_{measured}(i)$ = *ith* normalized absorbance data point measured for the sample, $y_{fitted}(i)$ = *ith* normalized absorbance data point fitted for the sample, σ = uncertainty (or experimental error) estimate, and N = number of data points.

[x] ± standard deviation

[w] average of the mixture constituents

composition of sample under investigation at the beamline.

5.5 Conclusion

This study examined the accuracy of LC fitting of reference standards to known composition of samples. Overall, this result confirms that the relative proportion of different species in a mixture can be obtained from an LC fit of XANES data with some confidence. The relative error associated with the fitting ranged from as low as 0.8 % to 41.6 % for thoroughly mixed sample. An unusually large relative error was associated with a particular mixture involving calcium and iron phosphates, which suggested that capability of quantitative XANES in detecting a species of small proportion with no distinct spectral feature in comparison with other species in the post edge region may be limited. We therefore recommend that a mixture of certified reference materials similar to the sample being investigated should be analyzed during the XAS experiment to estimate the accuracy of the proportions obtained from quantitative XANES analysis.

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6. OVERALL SYNTHESIS

Given the overall objective of this dissertation, which was to improve the understanding of P species in organic amendments and manure-amended soil at a molecular level using advanced spectroscopy techniques such as synchrotron-based X-ray absorption spectroscopy (XAS) and nuclear magnetic resonances (NMR), the following improvements have been made to existing knowledge. Prior to this study, it was unclear whether or not centrifugation of reconstituted extract was a necessary step in solution ^{31}P NMR analysis of organic amendments. This study clearly showed that centrifugation of ample reconstituted extract is necessary to obtain well-resolved NMR spectra that can be confidently used for spectra assignments, especially when the total P concentration in the sample is low. Obtaining well-resolved spectra is very important because it is expensive to operate an NMR spectrometer. Centrifuging the reconstituted extracts prior to NMR analysis proved useful in subsequent analysis of sequential extracts of organic amendments.

In speciating the P in organic amendments sequential fractions using NMR, the predominance of inorganic P in the labile fractions of organic amendments was suggested to be due to the proneness of labile organic P to alkaline hydrolysis in comparison with the non-labile fractions. The XANES analysis further showed these labile P species were mainly readily soluble calcium and aluminum phosphates. This clearly indicates that an advanced *in situ* techniques such as XANES needs to be combined with the conventional

sequential chemical extraction procedure to unravel the intricacies of P molecules of environmental relevance in these organic amendments.

The insight on the P species in organic amendments, coupled with previous solubility and fractionation studies of amended soils provided a standard for comparing the *in situ* analysis of these amended using XANES. The P 1s XANES analysis provided chemical information that supports previous findings on the different levels of extractable P in the amended Osborne soil, but not in those of Lakeland soil. In addition, the result from XANES analysis did not support previous hypotheses on the differences between Osborne and Lakeland soils amended with MAP (described in Chapter 4) because XANES showed that adsorption may be the dominant retention mechanism in both soils, contrary to previous speculation about adsorption being dominant in Osborne soil and a combination of adsorption and precipitation in Lakeland soil. This result further confirms that P 1s XANES can provide insight on P species in amended soils if combined with chemical extraction data.

Another contribution to knowledge emanating from this dissertation was the suggested improvement in reliability of the linear combination fitting employed in quantitative XANES analysis. One of the major problems encountered in applying P 1s XANES to P speciation in organic amendments was the difficulty in estimating the relative errors associated with the curve fitting procedure. Statistical uncertainty obtained along with the proportions of species from LC fitting is not a measure of accuracy. The validation of the quantitative XANES analysis may be considered as a first step towards estimating the relative error associated with this technique in that it was limited to binary mixtures. Future investigations should include mixtures of P compounds commonly

found across a wide range of soil pH. Such investigations may include an analysis of mixtures of loosely adsorbed and aqueous phosphates, additional binary mixtures involving AlP and FeP, ternary and more complex mixtures like freeze dried insoluble minerals and a wet "sludge" mixture of insoluble minerals and phosphate solution in a liquid cell. However, based on the result from the experiment to validate the accuracy of the fitting procedure, I recommend that a mixture of certified reference material similar to the sample being investigated be analyzed with XAS technique. The mixtures can be prepared after species in the experimental samples have been determined by PCA or LC fitting, to estimate the accuracy of the proportions obtained from quantitative XANES analysis.

Despite all the advances reported on the application of solution ^{31}P NMR in speciating P in organic amendments, certain gaps in knowledge need to be filled before solution ^{31}P NMR results can be regarded as conclusive. One of such gaps is the improvement in the extraction efficiency of P in the organic amendments. While there is little agreement in the literature on the most suitable extractant for NMR analysis, a mixture a NaOH and some chelating agents, such as EDTA, has been proven to be reliable. This alkaline extractant is necessary to bring the solution to a pH range (>13) suitable for consistent chemical shift for ^{31}P. In soil and organic amendments analyses, NaOH has been interpreted to extract P associated with organo-complexed Fe and Al, hence the reason why its extraction efficiency (relative to the total P) varies depending on the sample, and is not exactly 100 %. A convergence in the extraction of P in different samples will therefore be an important advancement to the interpretation and comparison of P species in different environmental samples.

Another gap in knowledge that is worth exploring is the likely underestimation of organic P due to its hydrolysis in the extract. More information on the kinetics of hydrolysis of specific organic P in alkaline solutions will be useful in setting the time limit for an NMR experiment that completely quantifies all organic P species. Specific organic P, such as phospholipids, may be extracted with chloroform and methanol and its hydrolysis kinetics monitored using NMR spectroscopy.

Another gap that needs to be explored is the optimization of the colorimetric analysis of P in organic amendments. Given the apparent overestimation of inorganic P in some organic amendments by the colorimetric approach as reported in this thesis, further investigation is recommended to determine the cause and magnitude of this error. The possibility of matrix effect needs to be thoroughly investigated. At present, the quantitation of P in the extracts is often done by direct calibration with orthophosphate standards that involves graphing of the absorbance of light by the molybdate blue complex against the concentration of standard solutions. In an ideal situation, the standard used in the calibration should match the composition of the samples to be analyzed, not only with respect to the analyte concentration but also with the concentrations of other species in the sample matrix. This is to ensure that the effect of various components of the sample on the measured absorbance is minimized. Unfortunately, reproducing the exact sample matrix of complex materials, such as organic amendments and amended, in the calibration standard is often impossible or extremely difficult to achieve. The best available practice has been the preparation of the standards and blank with the same solution used in extracting the samples, and neglecting possible interferents in the sample. Possible interferents in the colorimetric analysis of P

include As^{3+}, As^{5+}, Fe^{3+}, Cu^{2+}, Si^{4+}, V^{5+}, Ge^{4+} but their concentrations are not usually measured alongside P. A method of standard addition should be used in extrapolating the orthophosphate concentration in the extract whenever matrix interference is present. However, the method of standard addition may be impractical where a high throughput is required, which may explain why it is not used in routine analysis. An automation of the method of standard addition will therefore be a significant contribution to the analysis of P for agro-environmental purposes.

Essential to a successful application of XAS techniques are good sample preparation, high quality data made possible by good signal detection, and thorough data analysis. The improvement in the XAS signal detection in environmental samples with low concentration of P species therefore warrants further investigation. Many P transformation processes in the environment will be understood using the XAS technique if the detection methods can be improved and a precision limit set.

The application of XANES in all the studies reported in this thesis was limited to P 1s involving K-shell electron. A major limitation in the identification and quantitation of P species shown in this thesis is the lack of distinct features in the P 1s XANES spectra of certain P compounds that may be present in the samples. The near edge spectra of transitions and decay processes involved with other core level electrons apart from 1s needs to be investigated for P compounds. For example, P L-edge XANES spectra of these P compounds should be investigated for their uniqueness. Clearly distinct spectra of all reference P compounds used in XANES speciation of P in environmental samples will be a significant improvement of this technique.

In conclusion, the results presented in this dissertation provided an improved understanding of P species in organic amendments and amended soils at a molecular level that can aid decision-making on the environmentally sustainable fertilization of agricultural soils. The implication of the finding from speciation of organic amendments, from an agro-environmental standpoint, is that amendments such as biosolids that contain HAP may pose less risk to the environment compared with other amendments, and its labile P, which was dominated by VAR, may be readily bioavailable at neutral to alkaline soil pH like those used in this study. In addition to what has been reported in the literature, the result from XANES study of amended soil further implies that the 'soluble and adsorbed P' in these amended soils will constitute a source of P into the environment while those amended soils with appreciable amount of HAP and TRICAL will have limited solubility considering the alkaline pH of these soils.