

How Accurate Is the Assessment of Phosphorus Pools in Poultry Litter by Sequential Extraction?

Stefan Hunger,* J. Thomas Sims, and Donald L. Sparks

ABSTRACT

Amending poultry litter with Al sulfate (alum) has proven effective in reducing water-soluble P in the litter and in runoff from fields that have received litter applications. Although its effectiveness has been demonstrated on a macroscopic scale in the field or in the poultry houses, little is known about P speciation in either alum-amended or unamended litter. This knowledge is important for the evaluation of long-term stability and bioavailability of P, which is a necessary prerequisite for the assessment of the sustainability of intensive poultry operations. Here we report results from an investigation of alum-amended poultry litter (PL) that combined a chemical extraction sequence with solid-state ^{31}P nuclear magnetic resonance (NMR) spectroscopic analysis of the residues. Aluminum is predominantly found in the fine size separate ($<125\ \mu\text{m}$), indicating that the alum added to the poultry houses hydrolyzed without being completely dispersed in the litter. The NMR spectra confirmed the hypothesis that calcium phosphate phases are only dissolved during extraction with dilute acid and phosphate associated with Al is mainly dissolved during extraction with NaOH. Extraction of phosphate associated with Al was incomplete, however, as evidenced by ^{31}P NMR spectroscopy. It could also be demonstrated that the extraction sequence overestimates the calcium phosphate fraction by an order of magnitude in this particular sample. Results from sequential chemical extraction should therefore be used with caution when assessing the magnitude of different phosphate pools in poultry litter.

AGRICULTURE RELIES on the application of nutrients to soils to enhance food production. Animal manures have proven an especially inexpensive and effective way to improve soil quality and agricultural productivity. However, in areas with extensive animal operations, the quantities of animal manure that must be disposed of have led to the overapplication of nutrients, especially P. Phosphorus can be lost from soils to water bodies by erosion (particulate P) and by surface runoff and leaching (primarily soluble P).

Considering the pathways in which P enters the aquatic environment, part of the negative effects of the overapplication of animal manures can be remedied by lowering the water-soluble concentration of P and thus preventing loss of P by runoff. A promising approach is the addition of chemical amendments, such as lime, ferric chloride, or alum [$\text{Al}_2(\text{SO}_4)_3 \cdot 18\text{H}_2\text{O}$] (Moore and Miller, 1994; Moore et al., 1995a; Shreve et al., 1995). More recently, Dou et al. (2003) reported the effectiveness

of alum and coal combustion by-products in reducing soluble P in dairy, swine, and broiler manure.

Alum has received particular interest in the treatment of PL for its effectiveness in reducing the amount of water-soluble P, while at the same time reducing the litter pH. A lower litter pH has been shown to prevent the loss of N by ammonia volatilization, which otherwise diminishes air quality as well as the quality of the litter as fertilizer (Moore et al., 2000; Shreve et al., 1995). However, amending PL with alum does not reduce the total concentrations of P. Chemical amendments therefore do not prevent the accumulation of P in soils but merely reduce the possibility of loss by runoff and leaching. To our knowledge, no information is available on the long-term stability of P in chemically amended PL.

Although the effectiveness of alum as an amendment for PL has been proven on a macroscopic scale in poultry houses and in the field, the reactions involved have been speculative. Initially, it was assumed that phosphate in animal manures reacts with Al^{3+} to form solid Al phosphate (AlPO_4) (Moore and Miller, 1994), which is insoluble in the pH range typical for agricultural soils (Lindsay, 1979). Although this assumption has been recognized as oversimplified [e.g., Duffy and van Loon (1995)], little, if any, information about the mechanisms of P fixation in PL is available. This is mainly due to the lack of methods sufficiently sensitive to directly determine the species, or chemical form of P, in heterogeneous materials such as PL and soils.

Advances in spectroscopic techniques have recently permitted the spectroscopic investigation of P speciation in poultry litter and increased our understanding of P chemistry in PL. An X-ray absorption near edge structure (XANES) spectroscopic study of PL showed the presence of a calcium phosphate phase in the unamended PL samples, whereas features in the XANES spectra of the alum-amended PL samples indicated the formation of a P species similar to phosphate adsorbed to Al hydroxide (Peak et al., 2002). These results were confirmed and complemented by solid-state ^{31}P NMR spectroscopy (Hunger et al., 2004). In addition to the Ca- and Al-bound phosphate phases identified by XANES, a labile, physically sorbed phase was detected in both amended and unamended samples. The calcium phase detected by XANES, which was identified by ^{31}P NMR as either an amorphous tribasic calcium phosphate or a surface precipitate on calcium carbonate, was also found in the alum-amended samples. Using $^{31}\text{P}\{\text{Al}\}$ TRAPDOR (TRANSfer of Polarization during DOuble Resonance), a technique sensitive for Al and P nuclei in close proximity to each other, the presence of direct bonds between

S. Hunger, Department of Earth Sciences, University of Leeds, Leeds LS2 9JT, United Kingdom. D.L. Sparks and J.T. Sims, Department of Plant and Soil Sciences, University of Delaware, 152 Townsend Hall, Newark, DE 19711. Received 4 Mar. 2004. *Corresponding author (s.hunger@earth.leeds.ac.uk).

Published in J. Environ. Qual. 34:382–389 (2005).
© ASA, CSSA, SSSA
677 S. Segoe Rd., Madison, WI 53711 USA

Abbreviations: CP, cross-polarization; MAS, magic angle spinning; NMR, nuclear magnetic resonance; PL, poultry litter.

Al and phosphate could be confirmed (Hunger et al., 2004). The chemical shift range of the Al-bound phosphate species indicated a mixture of phosphate bound to Al hydroxide and an amorphous, uncondensed aluminum hydroxyl-phosphate phase, possibly a poorly ordered wavellite $[\text{Al}_3(\text{OH})_3(\text{PO}_4)_2 \cdot 5 \text{H}_2\text{O}]$. Both P-XANES and ^{31}P NMR confirmed that P in PL is present as orthophosphate (PO_4), rather than chains containing two or more phosphate groups or P species in a lower oxidation state.

These methods are not without limitations, however. Both P-XANES and ^{31}P NMR spectroscopy failed to detect or quantify organic P species, mainly due to the fact that positive identification requires a unique chemical environment around the phosphate group, which is not given in a heterogeneous matrix such as poultry litter. This limitation is especially severe because up to 60% of the total P extractable by 0.5 M NaOH and 50 mM EDTA has been identified as phytic acid (*myo*-inositol hexakisphosphate) by solution ^{31}P NMR spectroscopy (Turner, 2004). Furthermore, paramagnetic cations [e.g., Fe(II), Fe(III), or Mn(II)] in close proximity to phosphate groups distort the local magnetic field and cause a broadening of the NMR signal by facilitating the relaxation of the P nucleus (Blumberg, 1960; Smernik and Oades, 2002). This paramagnetic effect leads to signal loss, rendering phosphate groups in association with paramagnetic cations undetectable.

Nevertheless, spectroscopic techniques offer a much more detailed insight into P speciation than has been possible with established methods, such as sequential chemical extraction. Sequential chemical extraction schemes have been used to characterize P species in animal waste materials and soils by separating them into operationally defined groups according to their solubility in a series of extractants (Condrón et al., 1985; Dou et al., 2000, 2002; Hedley et al., 1982; Leinweber et al., 1997; Sharpley and Moyer, 2000). Dou et al. (2000) developed a very thorough extraction sequence from the one first proposed by Hedley et al. (1982). Manure samples are exhaustively extracted using first H_2O and NaHCO_3 to assess the labile species, followed by NaOH to extract Fe- and Al-bound species and occluded organic phosphate species, and finally HCl to dissolve calcium phosphate phases.

These assignments are, however, operationally defined and no information about chemical speciation is available from a sequential extraction. A very promising approach is to combine extraction techniques with spectroscopic techniques to verify assumptions about assignments made on a molecular level from macroscopic information. Frossard et al. (1994) characterized urban sewage sludge by sequential chemical fractionation with consecutive solid-state ^{31}P NMR analysis of the residual solids. They found that a complex mixture of phosphate solids was present in the sludge, including several calcium phosphates and Al phosphate. Corresponding to the proposed sequence of dissolution, the calcium phosphate phases were dissolved during extraction with HCl. The Al phosphate phase, which was tentatively assigned to a poorly ordered wavellite, was present in the residual

P fraction, although it was expected to dissolve during extraction with NaOH. It was reasoned that the poorly ordered wavellite reprecipitated during the extraction.

Sequential chemical extraction is a well-established method to characterize animal manures and estimate the risk of P loss after field application. Consequently, an extensive body of data exists in the literature. We report here results from an investigation combining sequential chemical extraction of alum-amended poultry litter with solid-state ^{31}P NMR spectroscopic analysis of the solid material before extraction and of the residues after each extraction step. Findings from both techniques are compared to verify assumptions made with regard to P species from chemical extraction.

MATERIALS AND METHODS

Sample Preparation and Extraction

Poultry litter that had received alum (approximately 10% by weight) was obtained from an on-farm evaluation of the effectiveness of alum as an amendment for PL conducted between January 1999 and May 2000. The details of this study are reported elsewhere (Sims and Luka-McCafferty, 2002). Although the litter sample analyzed for this study had been stored for 2 yr after sampling and had probably undergone significant alterations, the sample was chosen from this set because of the extensive complementary information available (Sims and Luka-McCafferty, 2002). One alum-amended sample (PL 181) was freeze-dried after 2 yr of storage at 4°C, ground using mortar and pestle, and separated into three size separates (841–420, 420–125, and <125 μm) using standard mesh sieves. The fine size separate (<125 μm) and the next coarser size separate (420–125 μm), were sequentially extracted and analyzed by both cross-polarization (CP)-magic angle spinning (MAS) and single-pulse MAS NMR spectroscopy. The third size separate (841–420 μm) was not used for extraction and solid-state NMR analysis because this fraction could not be packed in the MAS rotor to achieve even spinning.

Extractants were prepared by dissolving reagent-grade NaHCO_3 and NaOH in deionized water (>16 M Ω). Standardized 1.0 M HCl was used as received. The chemical extraction sequence was adapted from Hedley et al. (1982). The extractants were in their order of application: deionized water, 0.5 M NaHCO_3 , 0.1 M NaOH, and 1.0 M HCl. A standard extraction procedure was established as follows: 0.6 g of freeze-dried sample was shaken with 30 mL of extractant for 20 h (solid to solution ratio 1:50). The solids were separated from the supernatant by centrifugation (15 min, 15 000 rpm) and decanting. The supernatant was passed through 0.2- μm Supor filter paper (Gelman Laboratories, Ann Arbor, MI) and stored at 4°C until analysis. The solid to solution ratio employed in this work was higher than that published for manure analysis by Hedley et al. (1982) (1:60), Dou et al. (2000) (1:100), Sharpley and Moyer (2000), or Frossard et al. (1994) (both 1:200), but in accordance with the ratio recommended by Kuo (1996) for soils. This ratio was chosen to allow for the larger sample amount required for solid-state NMR analysis. Higher solid to solution ratios, however, can cause incomplete extraction due to saturation of the extractant with respect to the solid phase to be dissolved.

Concentrations of P, Al, Ca, and Fe in the extracts were determined by inductively coupled plasma atomic emission spectroscopy (ICP-AES). The total concentrations of these elements were measured after digestion of a dried, unextracted sample with concentrated HNO_3 and 30% H_2O_2 for 24 h at

Table 1. pH and elemental concentrations† before and after fractionation of poultry litter sample PL 181.

	Not fractionated	<125- μm size separate	125- μm < x < 420- μm size separate
pH‡	7.15	ND§	ND
P, g/kg	20.1	23.0	18.2
Al, g/kg	14.3	32.8	11.8
Ca, g/kg	ND	18.7	19.3
Fe, g/kg	ND	6.2	7.2

† Concentrations are given in g/kg of the freeze-dried litter, determined by inductively coupled plasma atomic emission spectroscopy (ICP-AES) after digestion.

‡ Determined as a suspension in deionized water (litter to water ratio 1:4). § ND, not determined.

50°C. A total number of four subsamples of each of the coarse and fine size separate of sample PL 181 were extracted. After each extraction step, one of the four samples was freeze-dried and subsequently used for ^{31}P NMR analysis. Elemental concentrations are therefore average values of four samples for the first (deionized water), three for the second (0.5 M NaHCO_3), and two for the third extraction step (1.0 M HCl). The residual amounts were calculated as the difference between the total concentrations determined by digestion and the sum of the extracted amounts. Total concentrations of P, Ca, Al, and Fe, and the pH of sample PL 181 are presented in Table 1. All concentrations are calculated as g/kg of dried litter.

Nuclear Magnetic Resonance Experiments

Solid-state ^{31}P NMR spectra were recorded on a Chemagnetics CMX Infinity spectrometer (Varian, Palo Alto, CA) with an Oxford 300 MHz wide-bore magnet (Oxford Instruments, Concord, MA) operating at a magnetic field of 7.04 Tesla, corresponding to resonance frequencies of 121.4 MHz for ^{31}P and 299.9 MHz for ^1H . To afford uniform distribution and homogeneous spinning of the rotor in the MAS probe, dried and ground samples were used. Spinning speeds were maintained constant at values from 7 to 10 kHz \pm 5 Hz.

The CP-MAS experiments used a proton $\pi/2$ -pulse of 3.5 μs , a contact time of 1.3 ms, and a pulse delay of 2 s. Depending on the P concentration, 2000 to 4000 scans were accumulated to give a signal to noise ratio of the most intense peak of at least 30:1. Single-pulse, proton-decoupled spectra were recorded using a ^{31}P $\pi/2$ -pulse of 3.7 μs and a relaxation delay of 60 s. For these experiments up to 512 scans were accumulated.

The spectra were processed and analyzed using the NUTS NMR utility transform software (Acorn NMR, 2000). A line broadening of 50 to 100 Hz was applied before Fourier transformation and phase correction. Isotropic chemical shifts are all reported in ppm relative to the signal of 85% H_3PO_4 as an external reference; positive values correspond to low-field or high-frequency shifts.

The spectra were deconvoluted using a minimal set of peaks as described by Hunger et al. (2004). The Lorentzian/Gaussian ratio was fixed at a value of 1 (Lorentzian peak) for the relatively sharp peak at $\delta = 3.0$ ppm and allowed to float for all other peaks. No further constraints were employed. An error margin of $\pm 5\%$ of the combined signal intensity of all signals is assumed for the peak areas of the deconvoluted peaks.

RESULTS AND DISCUSSION

Total Concentrations of Phosphorus, Calcium, Aluminum, and Iron in the Size Separates

Comparison of the elemental concentrations (Table 1) shows that both P and Al are enriched in the fine size

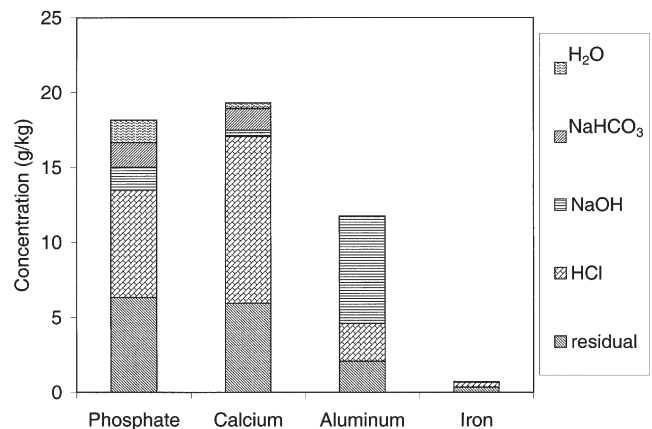


Fig. 1. Concentrations of P, Ca, Al, and Fe in the 125- to 420- μm size separate of the alum-amended poultry litter sample PL 181.

separate (<125 μm) but depleted in the coarse size separate (125–420 μm). No total concentrations of Fe and Ca were determined for the unfractionated litter, but the concentrations in the two size separates are not significantly different, suggesting that neither enrichment nor depletion took place during fractionation by size.

The strong enrichment of Al in the fine size separate is probably caused by the manner in which alum is applied. Alum was spread out in the poultry houses on top of existing layers of litter and bedding material (wood chips and saw dust) and incorporated into the upper few inches of the remaining litter by a spiked-tooth harrow (Sims and Luka-McCafferty, 2002). Further mixing was achieved by the birds' movements. No attempt was made to accomplish complete homogenization or dissolution; alum and its hydrolysis product [$\text{Al}(\text{OH})_3$] therefore remained in the litter as small particles in the fine size separate. As a consequence, sorption reactions of phosphate and metal ions occur at the interfaces of the Al hydroxide particles with the surrounding litter matrix and are therefore limited and rather slow. Phosphate is enriched in the fine size separate due to its high affinity for Al hydroxide.

Sequential Extraction

The amounts of P, Ca, Al, and Fe extracted from the two size separates of sample PL 181 with each extraction step are presented in Fig. 1 and 2, respectively. Most of the P is acid-extractable or recalcitrant to extraction, but P is released during each extraction step. Dou et al. (2003) reported lower amounts of acid-extractable and residual P in alum-amended broiler litter. This can be attributed to different reasons, particularly incubation time, litter origin and treatment, or diet. Dou et al. (2003) reacted fresh, moistened litter with approximately the same amount of alum as was used in our study for 3 d, whereas our sample was stored for 2 yr after sampling from a house that had received alum. Although Dou et al. (2003) and Dao et al. (2001) had found that a few days' difference in incubation time had little effect on the fraction of water-soluble P in alum-amended poultry manure, substantial changes can be

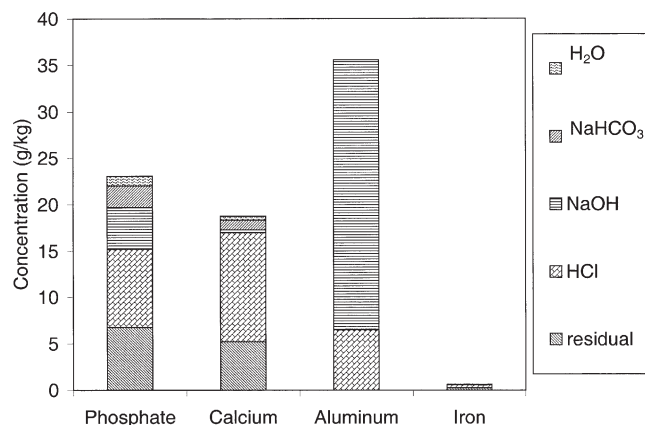


Fig. 2. Concentrations of P, Ca, Al, and Fe in the <125- μ m size separate of the alum-amended poultry litter sample PL 181.

expected over time scales of months and years. In a preliminary study of the effects of sample aging on the P speciation in alum-amended and unamended PL, Hunger et al. (unpublished data, 2005) were able to show that within 6 mo time the proportion of relatively insoluble calcium and Al phosphate phases increases in both amended and unamended litter.

From the coarse and fine size separates, 3.1 and 3.4 g/kg of P (17 and 15%) were extracted with deionized water and NaHCO₃, corresponding to the presumably plant-available P fraction (Dou et al., 2000). Small amounts of both Ca and Fe are concomitantly released. This indicates that Ca and Fe in these fractions were either not associated with P (e.g., as complexes with organic matter and as exchangeable cations), or present as colloidal calcium and iron phosphate, which did not settle during centrifugation and passed through the filter.

This sample had received alum and a significant amount of P was therefore expected to be associated with Al. Indeed, 1.5 and 4.5 g/kg of P (8.4 and 19%) are extracted from the coarse and fine size separates, respectively, by NaOH, concomitant with 7.2 and 29 g/kg of Al, respectively. More Al is present in the fine size separate than in the coarse size separate, which indicates that most of the Al hydroxide formed by hydrolysis of alum is present as particles smaller than 125 μ m. The fact that much more Al than P is released by NaOH indicates that the fraction of Al associated with P makes up only a small part of the total amount of Al in the samples. Furthermore, Sharpley and Moyer (2000) demonstrated that a significant amount of organic P was also extracted with 0.1 M NaOH; indeed, NaOH is the extractant of choice to quantitatively extract organic phosphate species from soils (Turner et al., 2003). The NaOH fraction therefore contains a heterogeneous mixture of P species, of which P formerly associated with Al is merely one part.

Significant amounts of Al are also extracted by HCl (2.5 and 6.5 g/kg in the coarse and fine size separates, respectively). This acid-extractable Al fraction is either a different Al phase or Al hydroxide that was not extracted by NaOH due to supersaturation of the extrac-

tant. No attempt was made to exhaustively extract each fraction by repeated treatment with the extractant, a very time-consuming procedure developed by Dou and coworkers (Dou et al., 2000). Also, the samples were extracted at a higher solid to solution ratio than reported in the literature. It cannot, therefore, be excluded that the extract was saturated with respect to amorphous Al(OH)₃. Using solid-state ³¹P NMR spectroscopy, Frossard et al. (1994) observed an Al phosphate phase in samples of biosolids after a similar extraction sequence. This phase, identified tentatively as a poorly ordered wavellite, was not observed in the residues after extraction with 0.1 M NaOH but rather after extraction with 1.0 M HCl. It was therefore concluded that it precipitated during extraction.

Most of the Ca in the samples is acid-extractable; however, some is present in the residual fraction. Similar results are seen for Fe, with an even higher proportion being recalcitrant to extraction. This has been attributed to insoluble or only slowly soluble crystalline calcium and iron phosphate minerals (Dou et al., 2000; Hedley et al., 1982). The major part of acid-extractable Ca can be attributed to calcium carbonate, which is contained in poultry feed. Calcium not absorbed during digestion precipitates as calcium carbonate under open atmosphere conditions and acts as a sorbent for phosphate (Griffin and Jurinak, 1973, 1974; Hinedi et al., 1992; Kuo and Lotse, 1972). Consequently, 37 and 39% of P are concomitantly extracted by 1.0 M HCl. The insoluble P not associated with metals has been mainly attributed to occluded phosphate species (Hedley et al., 1982).

Phosphorus-31 Nuclear Magnetic Resonance Spectroscopy

The ³¹P NMR spectra of the sequentially extracted coarse and fine size separates of sample PL 181 are presented in Fig. 3 and 4, respectively. The P concentrations of the samples after extraction with HCl were too low to give spectra of good quality in an acceptable experiment time. Of these spectra only the CP-MAS NMR spectrum of the fine size separate after acid extraction is therefore presented in Fig. 5.

The spectra of the unextracted samples are similar to the spectra observed by Hunger et al. (2004) in that they show complex unresolved resonances. The single-pulse MAS spectra contain a sharp peak at approximately 3.0 ppm that can be attributed to tribasic calcium phosphate, or a calcium phosphate surface precipitate on calcium carbonate. This peak is suppressed in the CP-MAS spectra. The CP-MAS spectrum of the coarse size separate of sample PL 181 contains a peak at 6.4 ppm, which was attributed to inorganic phosphate bound by hydrogen bonds to water molecules in the sample (Hunger et al., 2004). Contrary to the observations reported by Hunger et al. (2004), this peak does not disappear on extraction with deionized water, but is only removed by NaHCO₃.

There are several possible explanations for this behavior, the most obvious one being that the assignment is incorrect. Another explanation is that this P species

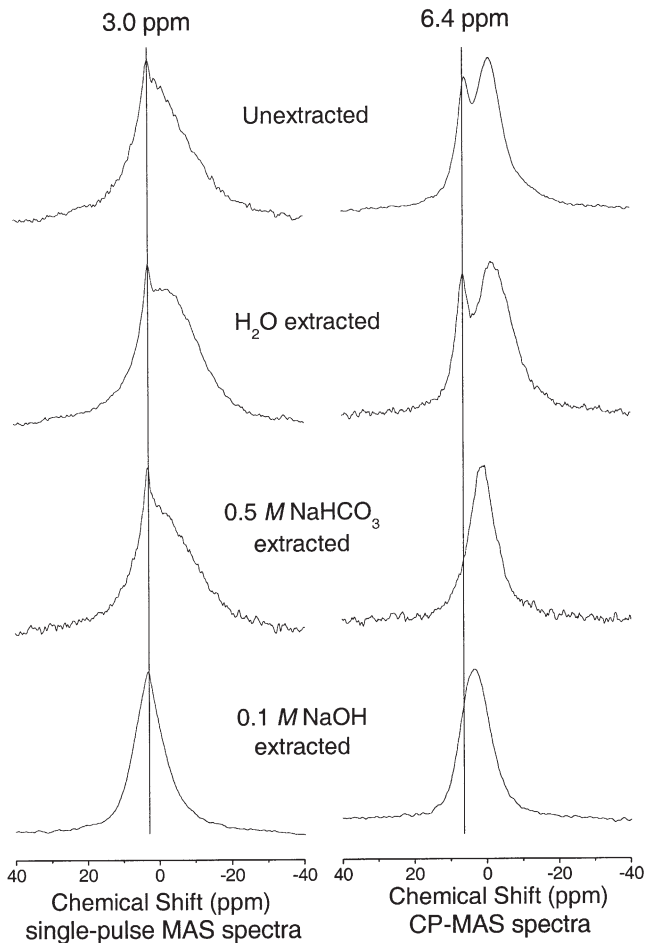


Fig. 3. Single-pulse, proton-decoupled magic angle spinning (MAS) nuclear magnetic resonance (NMR) spectra (left column) and cross-polarization magic angle spinning (CP-MAS) spectra (right column) of the coarse size separate of poultry litter sample PL 181. Samples are sequentially extracted from top to bottom up to the extraction step indicated.

is occluded, either in remnants of biological compartments, such as plant or microbial cells, or in humified organic matter or complexes of humified organic matter with inorganic polymers. Complexes between humified organic material and inorganic polymers, such as aluminosilicates and Al or iron hydroxide polymers, have been proposed for soils (Sparks, 2003; Stevenson, 1994). Myneni et al. (1999) demonstrated that changes in ionic strength and ionic composition of the background electrolyte cause changes in the shape and association of dissolved humic acid. Extracting PL with 0.5 M NaHCO₃ probably causes the organic-inorganic aggregations to break up and release any occluded phosphate. The different behavior of litter samples reported here and by Hunger et al. (2004) illustrates differences in litter compositions. The sample used in this study had received alum; more and stronger associations between humified material and the added Al are expected to form. The litter sample used in the study reported by Hunger et al. (2004) was unamended.

In general, the NMR spectra of the extracted samples confirm some of the molecular assignments of the mac-

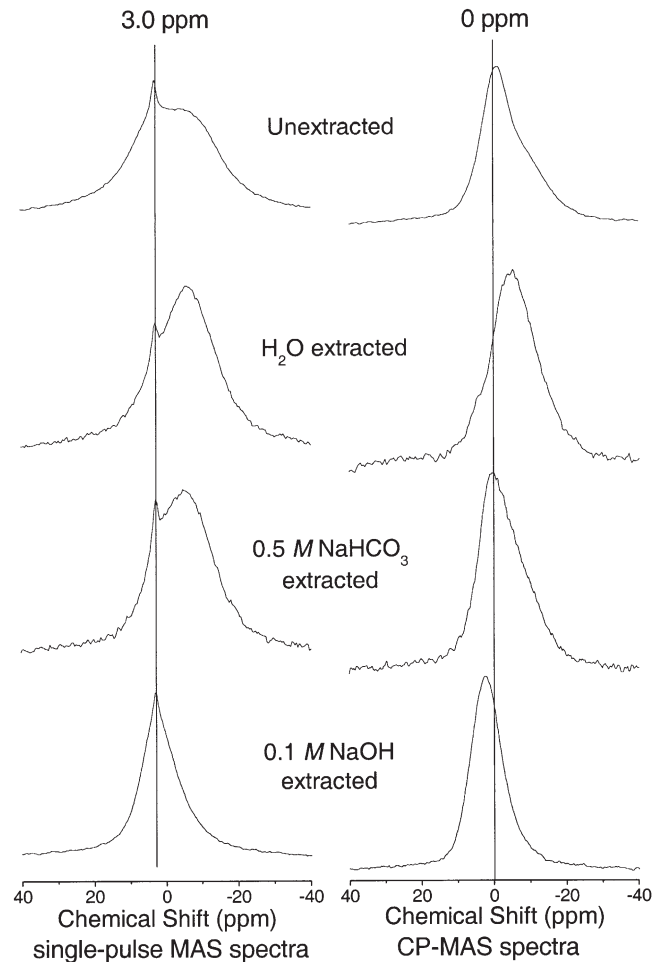


Fig. 4. Single-pulse, proton-decoupled magic angle spinning (MAS) nuclear magnetic resonance (NMR) spectra (left column) and cross-polarization magic angle spinning (CP-MAS) spectra (right column) of the fine size separate of poultry litter sample PL 181. Samples are sequentially extracted from top to bottom up to the extraction step indicated.

roscopic P fractionation. Phosphate associated with Al in the fine size separate, which can be detected as a shoulder on the upfield side of the broad peak in the right column of Fig. 4 (Hunger et al., 2004), is removed by NaOH. More phosphate is associated with Al in the fine size separate than in the coarse size separate, because the NMR spectra of the coarse size separate are lacking the peak in the corresponding chemical shift region (-4 to -10 ppm). This agrees with the observation made in the macroscopic fractionation that less Al and P are extracted by NaOH from the coarse size separate.

Furthermore, the calcium phosphate phase is present in all extracted samples (left column of Fig. 3 and 4) and is only removed by HCl (Fig. 5). The peak of the calcium phosphate phase is suppressed in the CP-MAS NMR spectra and not as sharp as in the single-pulse spectra, but the shift to more negative values is clearly visible in the bottom spectra of Fig. 5. The CP-MAS NMR spectrum of the sample after acid extraction further reveals that the residual P fraction contains a rather uniform chemical species, as evidenced by the narrow

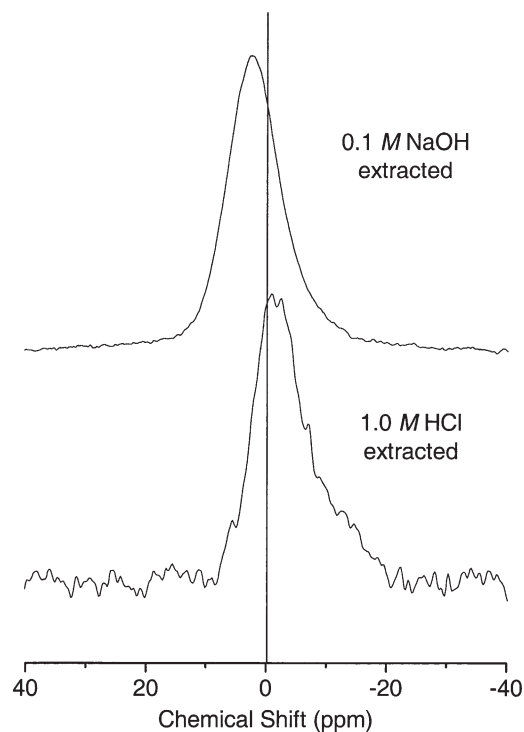


Fig. 5. Cross-polarization magic angle spinning (CP-MAS) ^{31}P nuclear magnetic resonance (NMR) spectra of the fine size separate of poultry litter sample PL 181 after extraction with 0.1 M NaOH (top) and 1.0 M HCl (bottom).

peak. The spectrum further contains a shoulder on the upfield side of the peak in the chemical shift range -5 to -20 ppm. This indicates the presence of phosphate species bound to Al (Hunger et al., 2004) that were not completely removed during the extraction sequence or precipitated due to supersaturation. The concentration of this phase is, however, very low and it is quite possible that the signal observed in the spectrum on the bottom of Fig. 5 is hidden in the complex signal in the spectrum at the top. All spectra were normalized to the most intensive peak and do therefore not allow direct quantitative comparison. This phosphate phase associated with Al is therefore most probably a residue that was not completely extracted. This is also a possible explanation for the observations by Frossard et al. (1994), who found an Al hydroxyl-phosphate phase in the residual fraction of urban biosolids after a similar extraction sequence. The need to repeat each extraction step until the phosphate concentration in the extract is below detection, as proposed by Dou et al. (2000), is evident.

Extraction of the samples with deionized water narrows the complex peaks in the NMR spectra (Fig. 3 and 4). Because only a small fraction of P was removed during this step, this indicates that removal of soluble metal cations has simplified the chemical environment of both inorganic and organic phosphate. The shift to more negative chemical shift values with extraction further indicates that inorganic phosphate also reacted with Al hydroxide to form surface complexes and Al hydroxyl-phosphate precipitates (Hunger et al., 2004). While the reaction mixture was quite heterogeneous during stor-

age in the chicken houses, it is more homogeneous during extraction, facilitating adsorption of soluble phosphate to Al hydroxide and/or calcium carbonate phases. Further extraction with NaHCO_3 and especially so with NaOH shifts the peaks to more positive values, centering them on the calcium phosphate peak at 3.0 ppm in the single-pulse MAS spectra and slightly lower values in the CP-MAS spectra. These extractants efficiently remove organic phosphate compounds and inorganic phosphate not bound in a solid phase (Cade-Menun and Preston, 1996; Turner, 2004), leaving mainly the calcium phosphate phase (3.0 ppm).

Comparison of the Quantification by Extraction and Phosphorus-31 Nuclear Magnetic Resonance

Deconvolution of the signals in the single-pulse spectra was not possible to the extent reported previously (Hunger et al., 2004). Only the peak at $\delta = 3.0$ ppm was reproducible over the course of the deconvolution. The main reason for this is possibly the limited number of peaks used for deconvolution, each of which consists of resonance lines of many different species that only differ in conformation and substitution (for organic phosphate) or the number and nature of cations complexed (for both organic and inorganic phosphate species). Extraction changes the proportions of these species, moving the apparent peak positions. The calcium phosphate phase, being the most homogeneous, did not undergo such profound changes. Therefore, only the fraction of calcium phosphate was determined from the single-pulse ^{31}P NMR spectra of the residues of the coarse and fine size separates after each extraction step (Table 2). The expected enrichment of the calcium phosphate phase during the course of the extraction is only observed in the coarse size separate. The changes in both size separates, however, are well inside the error margin ($\pm 5\%$) and therefore not significant. The acid-extractable phosphate fraction, which presumably contains predominantly calcium phosphate, was 39 and 37% in the coarse and fine size separates, respectively. The calcium phosphate content determined by ^{31}P NMR, however, is 4.3 and 3.6%, respectively. Clearly, sequential chemical extraction overestimates the calcium phosphate content by one order of magnitude by dissolving species other than calcium phosphate. As discussed previously, phosphate associated with Al is incompletely extracted with NaOH and makes up part of the phos-

Table 2. Determination of the calcium phosphate concentrations by ^{31}P nuclear magnetic resonance (NMR).

	Proportion of calcium phosphate [†]	
	125- to 420- μm size separate	<125- μm size separate
	%	
Before extraction	4.3	3.6
H_2O -extracted	4.9	2.7
NaHCO_3 -extracted	7.3	4.4
NaOH-extracted	8.8	3.2

[†] Determined as the fraction that the peak at $\delta = 3.0$ ppm occupies of the total signal area. Estimated error range: $\pm 5\%$.

phate fraction extracted by HCl. Furthermore, possible acid-extractable species that were not accounted for by ^{31}P NMR are complexes of phytic acid (inositol hexakisphosphate) with a variety of metal cations.

Because poultry is lacking the enzymes necessary to hydrolyze the phytic acid contained in the grain that makes up most of its diet, poultry manure and litter have therefore long been known to contain phytic acid (Taylor, 1965). A recent study by Turner (2004) using solution ^{31}P NMR demonstrated that phytic acid is a major component of poultry litter, comprising up to 60% of the total phosphate. Calcium and transition metal cations form complexes with phytate that are only soluble at low pH values (Bedot-Brigaud et al., 1999; Seaman et al., 2003) or in the presence of strong complexing agents such as EDTA (Turner, 2004). Due to the multitude of metals present in the litter (Jackson et al., 2003; Moore et al., 2000; Moore et al., 1995b), no single complex is formed with both phosphate and phytate; consequently only a broad, unresolved resonance was observed in the ^{31}P NMR spectra. The acid-extractable fraction therefore probably also contains phytic acid.

CONCLUSIONS

In conclusion, these results verified the assumption that Al and calcium phosphate phases are mostly extracted by NaOH and HCl, respectively. Aluminum phosphate phases are, however, present even after extraction with NaOH, as evidenced by the elevated concentrations of Al in the acid extract and the peaks in the chemical shift range of -5 to -20 ppm in the NMR spectra of the residues after extraction with 0.1 M NaOH and 1.0 M HCl . A more thorough, repeated extraction is therefore necessary for a complete assessment of Al-associated phosphate species. Calcium phosphate species, on the other hand, are overestimated by the extraction sequence due to extraction of phytate at low pH values. Although chemical sequential extraction is a valuable tool to assess P fractions of differing availability, this research indicates that it is inadmissible to draw conclusions about P species in the material extracted.

The results of this study are, however, limited by the fact that only one sample was extracted and subsequently analyzed by solid-state ^{31}P NMR. This sample had furthermore been stored for 2 yr and had probably undergone major changes toward more stable phosphate species (Hunger et al., unpublished data, 2005). It is therefore questionable whether these results are applicable to fresh poultry litter, particularly considering the variability of possible litter compositions. The general conclusion, however, remains that sequential chemical extraction schemes employing nonspecific reagents have to be interpreted with caution. Further research comparing speciation using different techniques is clearly needed to validate chemical extraction sequences.

Comparing these results with solution ^{31}P NMR data of PL extracts (Turner, 2004) demonstrates that a combination of solid-state and solution ^{31}P NMR spectroscopy

will offer a much deeper insight into P speciation than each technique on its own. While solid-state ^{31}P NMR spectroscopy is an invaluable tool to quantitatively assess the proportions of Ca and Al phases, solution ^{31}P NMR spectroscopy of alkaline extracts of poultry litter allows the quantification of organic P species that are not detected by solid-state NMR spectroscopy due to the heterogeneity of the material. Both techniques, however, require sophisticated analytical equipment that is not available for routine measurements of animal manures in general. A combination of both techniques with established methods such as sequential chemical extraction, on the other hand, will allow the validation of results gained from those routinely employed methods. This will also allow the in-depth study of the effects of storage and chemical amendments on the phosphate speciation in poultry litter in particular and animal manures in general.

ACKNOWLEDGMENTS

The NMR experiments were performed at the Environmental Molecular Sciences Laboratory (a national scientific user facility sponsored by the U.S. DOE Office of Biological and Environmental Research) located at Pacific Northwest National Laboratory, operated by Battelle for the DOE. We especially appreciate the assistance of Dr. Sarah Burton and Dr. Joseph Ford in conducting the NMR experiments at EMSL, and of Dr. Herman Cho with helpful discussion and guidance. This manuscript benefited from valuable input from the Environmental Soil Chemistry research group of the University of Delaware. Stefan Hunger appreciates the support of a Delaware Water Resources Center graduate research fellowship.

REFERENCES

- Acorn NMR. 2000. NUTS NMR utility transform software. Acorn NMR, Livermore, CA.
- Bedot-Brigaud, A., C. Dange, N. Fauconnier, and C. Gérard. 1999. ^{31}P NMR, potentiometric and spectrophotometric studies of phytic acid ionization and complexation properties toward Co^{2+} , Ni^{2+} , Cu^{2+} , Zn^{2+} and Cd^{2+} . *J. Inorg. Biochem.* 75:71–78.
- Blumberg, W.E. 1960. Nuclear spin-lattice relaxation caused by paramagnetic impurities. *Phys. Rev.* 119:79–84.
- Cade-Menun, B.J., and C.M. Preston. 1996. A comparison of soil extraction procedures for ^{31}P NMR spectroscopy. *Soil Sci.* 161:770–785.
- Condron, L.M., K.M. Goh, and R.H. Newman. 1985. Nature and distribution of soil phosphorus as revealed by a sequential extraction method followed by ^{31}P nuclear magnetic resonance analysis. *J. Soil Sci.* 36:199–207.
- Dao, T.H., L.J. Sikora, A. Hamasaki, and R.L. Chaney. 2001. Manure phosphorus extractability as affected by Al- and iron by-products and aerobic composting. *J. Environ. Qual.* 30:1693–1698.
- Dou, Z., K.F. Knowlton, R.A. Kohn, Z. Wu, L.D. Satter, G. Zhang, J.D. Toth, and J.D. Ferguson. 2002. Phosphorus characteristics of dairy feces affected by diets. *J. Environ. Qual.* 31:2058–2065.
- Dou, Z., J.D. Toth, D.T. Galligan, C.F. Ramberg, and J.D. Ferguson. 2000. Laboratory procedures for characterizing manure phosphorus. *J. Environ. Qual.* 29:508–514.
- Dou, Z., G.Y. Zhang, W.L. Stout, J.D. Toth, and J.D. Ferguson. 2003. Efficacy of alum and coal combustion by-products in stabilizing manure phosphorus. *J. Environ. Qual.* 32:1490–1497.
- Duffy, S.J., and G.W. van Loon. 1995. Investigations of Al hydroxyphosphates and activated sludge by ^{27}Al and ^{31}P MAS NMR. *Can. J. Chem.* 73:1645–1659.
- Frossard, E., P. Tekely, and J.Y. Grimal. 1994. Characterization of

- phosphate species in urban sewage sludges by high-resolution solid-state ^{31}P -NMR. *Eur. J. Soil Sci.* 45:403–408.
- Griffin, R.A., and J.J. Jurinak. 1973. The interaction of phosphate with calcite. *Soil Sci. Soc. Am. J.* 37:847–850.
- Griffin, R.A., and J.J. Jurinak. 1974. Kinetics of phosphate interaction with calcite. *Soil Sci. Soc. Am. J.* 38:75–79.
- Hedley, M.J., J.W.B. Stewart, and B.S. Chauhan. 1982. Changes in inorganic and organic soil phosphorus fractions induced by cultivation practices and by laboratory incubations. *Soil Sci. Soc. Am. J.* 46:970–976.
- Hinedi, Z.R., S. Goldberg, A.C. Chang, and J.P. Yesinowski. 1992. A ^{31}P and ^1H NMR study of phosphate sorption onto calcium carbonate. *J. Colloid Interface Sci.* 152:141–160.
- Hunger, S., H. Cho, J.T. Sims, and D.L. Sparks. 2004. Direct speciation of phosphorus in alum-amended poultry litter: A solid-state ^{31}P -NMR investigation. *Environ. Sci. Technol.* 38:674–681.
- Jackson, B.P., P.M. Bertsch, M.L. Cabrera, J.J. Camberato, J.C. Seaman, and C.W. Wood. 2003. Trace element speciation in poultry litter. *J. Environ. Qual.* 32:535–540.
- Kuo, S. 1996. Phosphorus. p. 869–919. *In* D.L. Sparks (ed.) *Methods of soil analysis. Part 3.* SSSA Book Ser. 5. SSSA, Madison, WI.
- Kuo, S., and E.G. Lotse. 1972. Kinetics of phosphate adsorption by calcium carbonate and Ca-kaolinite. *Soil Sci. Soc. Am. J.* 36:725–729.
- Leinweber, P., L. Haumaier, and W. Zech. 1997. Sequential extractions and ^{31}P -NMR spectroscopy of phosphorus forms in animal manures, whole soils and particle-size separates from a densely populated livestock area in northwest Germany. *Biol. Fertil. Soils* 25:89–94.
- Lindsay, W.L. 1979. *Chemical equilibria in soils.* John Wiley & Sons, New York.
- Moore, P.A., Jr., T.C. Daniel, and D.R. Edwards. 2000. Reducing phosphorus runoff and inhibiting ammonia loss from poultry manure with Al sulfate. *J. Environ. Qual.* 29:37–49.
- Moore, P.A., Jr., T.C. Daniel, D.R. Edwards, and D.M. Miller. 1995a. Effect of chemical amendments on ammonia volatilization from poultry litter. *J. Environ. Qual.* 24:293–300.
- Moore, P.A., Jr., T.C. Daniel, A.N. Sharpley, and C.W. Wood. 1995b. Poultry manure management: Environmentally sound options. *J. Soil Water Conserv.* 50:321–327.
- Moore, P.A., Jr., and D.M. Miller. 1994. Decreasing phosphorus solubility in poultry litter with Al, calcium and iron amendments. *J. Environ. Qual.* 23:325–330.
- Myneni, S.C.B., J.T. Brown, G.A. Martinez, and W. Meyer-Ilse. 1999. Imaging of humic substance macromolecular structures in water and soils. *Science (Washington, DC)* 286:1335–1337.
- Peak, D., J.T. Sims, and D.L. Sparks. 2002. Solid-state speciation of natural and alum-amended poultry litter using XANES spectroscopy. *Environ. Sci. Technol.* 36:4253–4261.
- Seaman, J.C., J.M. Hutchison, B.P. Jackson, and V.M. Vulava. 2003. In situ treatment of metals in contaminated soils with phytate. *J. Environ. Qual.* 32:153–161.
- Sharpley, A., and B. Moyer. 2000. Phosphorus forms in manure and compost and their release during simulated rainfall. *J. Environ. Qual.* 29:1462–1469.
- Shreve, B.R., J.P.A. Moore, T.C. Daniel, D.R. Edwards, and D.M. Miller. 1995. Reduction of phosphorus in runoff from field-applied poultry litter using chemical amendments. *J. Environ. Qual.* 24:106–111.
- Sims, J.T., and N.J. Luka-McCafferty. 2002. On-farm evaluation of Al sulfate (alum) as a poultry litter amendment: Effects on litter properties. *J. Environ. Qual.* 31:2066–2073.
- Smernik, R.J., and J.M. Oades. 2002. Paramagnetic effects on solid state carbon-13 nuclear magnetic resonance spectra of soil organic matter. *J. Environ. Qual.* 31:414–420.
- Sparks, D.L. 2003. *Environmental soil chemistry.* 2nd ed. Academic Press, San Diego.
- Stevenson, F.J. 1994. *Humus chemistry: Genesis, composition, reactions.* 2nd ed. John Wiley & Sons, New York.
- Taylor, T.G. 1965. The availability of the calcium and phosphorus of plant materials for animals. *Proc. Nutr. Soc.* 24:105–112.
- Turner, B.L. 2004. Optimizing phosphorus characterization in animal manures by solution phosphorus-31 nuclear magnetic resonance spectroscopy. *J. Environ. Qual.* 33:757–766.
- Turner, B.L., N. Mahieu, and L.M. Condron. 2003. Phosphorus-31 nuclear magnetic resonance spectral assignment of phosphorus compounds in soil NaOH-EDTA extracts. *Soil Sci. Soc. Am. J.* 67:497–510.