# XANES Spectroscopic Analysis of Phosphorus Speciation in Alum-Amended Poultry Litter

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Aluminum sulfate (alum; $Al_2(SO_4)_3 \cdot 14H_2O$) is used as a chemical treatment of poultry litter to reduce the solubility and release of phosphate, thereby minimizing the impacts on adjacent aquatic ecosystems when poultry litter is land applied as a crop fertilizer. The objective of this study was to determine, through the use of X-ray absorption near edge structure (XANES) spectroscopy and sequential extraction, how alum amendments alter P distribution and solid-state speciation within the poultry litter system. Our results indicate that traditional sequential fractionation procedures may not account for variability in P speciation in heterogeneous animal manures. Analysis shows that NaOH-extracted P in alum amended litters is predominantly organic (~80%), whereas in the control samples, >60% of NaOH-extracted P was inorganic P. Linear least squares fitting (LLSF) analysis of spectra collected of sequentially extracted litters showed that the P is present in inorganic (P sorbed on Al oxides, calcium phosphates) and organic forms (phytic acid, polyphosphates, and monoesters) in alum- and non-alum–amended poultry litter. When determining land application rates of poultry litter, all of these compounds must be considered, especially organic P. Results of the sequential extractions in conjunction with LLSF suggest that no P species is completely removed by a single extractant. Rather, there is a continuum of removal as extractant strength increases. Overall, alum-amended litters exhibited higher proportions of Al-bound P species and phytic acid, whereas untreated samples contained Ca-P minerals and organic P compounds. This study provides in situ information about P speciation in the poultry litter solid and about P availability in alum- and non-alum–treated poultry litter that will dictate P losses to ground and surface water systems.

The link between phosphorus (P) influx to surface waters and their subsequent decline in overall health has created a focus on P-based management in recent environmental legislation. The excessive, long-term use of high-P animal manures, such as poultry litter, as fertilizers for crops is of the utmost concern. The Delmarva Peninsula, the area encompassing parts of Delaware, Maryland, and Virginia, is a major farming community where broiler production dominates the agricultural economy. It is imperative to find environmentally responsible means of dealing with the 50,000 Mg of manure that is generated annually (Mozaffari and Sims, 1996). Recent federal nutrient management laws impose strict regulations on manure management practices, requiring nutrient budgets and implementation of best management practices (BMPs) for confined animal feeding operations (e.g., poultry production facilities). In addition to dietary supplements (Maguire et al., 2003), composting, and pelletizing, the use of chemical amendments, primarily aluminum sulfate (alum, $Al_2(SO_4)_3 \cdot 14H_2O$), alter litter chemistry, thereby decreasing soluble P release (Moore et al., 1999; Moore et al., 2000). In short-term studies, alum amendment reduces soluble P transport from farms; however, little is known about how this chemical treatment changes P speciation and distribution to limit transport.

Forty to fifty percent of total phosphorus in poultry wastes is in the organic form, with four main types of organic P compounds: inositol phosphates, sugar phosphates, nucleic acids, and phospholipids (Barnett, 1994a; Toor et al., 2006). The lability of organic P compounds depends on the ability of phosphatase enzymes to hydrolyze organic P forms to inorganic P (Toor et al., 2006). Other minor organic P compounds commonly found in poultry waste include labile monoesters (sugar phosphates, mononucleotides) and diesters (DNA, RNA, phospholipids), which are soluble in water. Phosphate sorbed on the surfaces of clay minerals and Fe or Al (hydr)oxides are important inorganic P species that are generally less bioavailable in water (Toor et al., 2006). Additionally, P incorporated into mineral-phase litters (potassium phosphates, pyrophosphate, aluminum phosphate) is commonly found in poultry litters. Polyphosphates (adenosine diphosphate [ADP]) and phosphonates (aminoethyl phosphate [AEP]) represent another class of inorganic P-compounds found in organic wastes. Therefore, it is essential to take organic and inorganic compounds into consideration when considering P speciation in poultry litter and predicting environmental P mobility.

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**Abbreviations:** BMP, best management practices; ICP-AES, inductively coupled plasma atomic emission spectroscopy; LLSF, linear least squares fitting; MRP, molybdate-reactive phosphorus; NMR, nuclear magnetic resonance; PC, principal component; PCA, principal component analysis; XANES, X-ray absorption near edge structure.

To understand why BMP, such as alum treatments, are effective, there must be an in situ means of characterizing manures and litters after they have been subjected to various treatments. Sequential extractions are used to describe the relative distribution of an element between different species, or pools, in soil and heterogeneous systems. In agronomic-based studies, it is useful to know in which soil (litter) fraction an element, such as P, is bound and with what other substances or surfaces it is interacting, so that predictions can be made as to its reactivity, mobility, or likelihood to cause risk. Fractionation procedures are based on the assumption that these forms are differentially extractable, with reactivity of one form being clearly distinguishable from another (Dou et al., 2000; Eneji et al., 2001; Eneji et al., 2003; He and Honeycutt, 2001; Hunger et al., 2004; Sharpley and Moyer, 2000; Chang and Jackson, 1957). One sequential extraction method, The Hedley procedure (Hedley et al., 1982), uses sequential extractions of water, sodium bicarbonate, sodium hydroxide, and hydrochloric acid. These extractants presumably remove bioavailable P, labile inorganic and organic P, "microbial P," aluminum (Al)- and iron (Fe)-chemisorbed P, and mineral-occluded P. The sequential extraction method of McAuliffe and Peech (Hedley et al., 1982; Barnett, 1994a, 1994b) was modified to draw more conclusive conclusions about relative abundance and forms of soil organic and inorganic P (He et al., 2003; Levy and Schlesinger, 1999).

These sequential extraction procedures have been complemented by spectroscopic techniques to determine the forms of phosphorus that were removed from the solid phase of alum-amended poultry litter through the Hedley fractionation procedure (Hunger et al., 2004). After each extraction, solid-state $^{31}$P-nuclear magnetic resonance (NMR) was used to analyze the remnant P phases in sample residue. Although the combination of these two techniques confirmed the extraction of Al- and Ca-bound P in the NaOH and HCl fractions, the authors note that they did not provide enough information to determine how these extractants react with the different species of P in the poultry litter. Turner and Leytem (2004) used solution NMR to investigate the P speciation within the supernatant after each extraction step, using a modified Hedley procedure. In this study, the authors noted the importance of phospholipids, nucleic acids, phosphate monoesters, and phytic acids in swine, beef-cattle, and poultry manure. Before this study, there had been little focus on organic P because none of the soil-based interpretations can accurately account for the variability in organic P compounds in poultry litter. Understanding solid-phase P speciation remaining after extraction product removal is important, so that these products can be correlated with the extracted solid phase P species, providing a systematic P mass balance; however, solid-state P-NMR is not sensitive enough to resolve P-speciation in heterogeneous materials.

X-ray absorption near edge structure (XANES) spectroscopy offers a means of analyzing solids at in situ moisture contents, ambient pressures, and temperatures without extensive alteration to the samples (Brown, 1990; Peak et al., 2002). Shober et al. (2006) used XANES analysis to speciate P in a series of manures, including poultry litter. Organic and inorganic P species were detected in treated and nontreated poultry litters. The properties of sequential extraction residue with respect to P chemical specia-

tion are investigated here. Using principal component analysis (PCA) in combination with linear least squares fitting (LLSF) procedures, allows for the spectra from poultry litter samples to be described in terms of their P-containing components. Therefore, the overall objective of the study was to investigate P speciation in alum-amended and non-alum–amended poultry litter samples using a combination of sequential extraction and XANES spectroscopy to determine if alum is an effective BMP.

## Materials and Methods

### Sample Characterization

All poultry litter samples were supplied by Dr. J.T. Sims (University of Delaware) from a previously conducted study (Sims and Luka-McCafferty, 2002). The samples were chosen based on physicochemical characteristics and elemental composition. The samples were passed through an 841-μm sieve (number #20 mesh screen) without drying or grinding. Although this size fraction is useful for XANES studies, it is also relevant to size fractionation of poultry litter, which contains a nutrient-dense fine fraction and a low-nutrient coarse material that could be recycled as bedding or fuel (Ndegwa et al., 1991). Water content was estimated by drying several grams of the sieved material at 65°C (limiting organic compound volatilization) until constant weight was achieved, usually for 48 h. Total Al, Fe, Ca, and P were determined by digesting 0.5 g of litter with 10 mL of 1:1 concentrated $HNO_3$:$H_2O$ overnight followed by the addition of 3 mL of 30% $H_2O_2$ for 1 h. The mixture was heated to105°C for 2 h with a DigiPREP MS digestion block (SCS Science, Quebec, Canada). Digested samples were diluted to a final volume of 50 mL and vacuum filtered through 0.45-μm Millipore filter paper before analysis by inductively coupled plasma atomic emission spectroscopy (ICP-AES). For determination of water-soluble constituents, a 1:10 solid:$H_2O$ mixture was shaken for 1 h on an end-over-end shaker, centrifuged at 2500 rpm for 15 min, and vacuum filtered through 0.45-μm Millipore filter paper. Supernatants were analyzed for P, Al, and Ca using ICP-AES. All results are presented on a dry weight basis, with samples dried as described previously.

### Phosphorus Sequential Extraction

Molybdate-reactive phosphorus (MRP) (inorganic P), as a percentage of the amount of P removed in each extraction, was determined using the ascorbic acid-phosphomolybdenum blue method (He et al., 1998; Murphy and Riley, 1962; Turner et al., 2002). Upon comparison to the total P data determined by ICP-AES, a relative distribution of P between inorganic and organic phases within an extract was determined.

The sequential extraction method of Dou et al. (2000) was used for P fractionation. This method is briefly described as follows: A 0.3-g sample of poultry litter, in its in situ moisture content, was placed in a 50-mL polyethylene centrifuge tube, suspended in 30 mL of deionized $H_2O$ (1:100 solid:solution ratio), shaken for 1 h on an end-over-end shaker, and centrifuged for 15 min at 2500 rpm. Supernatants were removed using a glass Pasteur pipette; care was taken not to remove solid from the bottom of the centrifuge tube. This solution was vacuum filtered and analyzed for P, Al,

Fe, and Ca by ICP-AES and the MRP method. These steps were repeated, using the residues from the previous extraction, with 30 mL each of 0.5 $M$ NaHCO$_3$ (to extract labile P), 0.1 $M$ NaOH (to extract Al and Fe associated P, plus chemically and physically protected organic forms), and 1 $M$ HCl (to extract Ca associated P).

## Spectroscopic Analysis

After each step in the sequential extraction procedure, residue from one of the replicates was used for XANES analysis. Sufficient replicates were used so that at the end of the extraction profile triplicates remained for statistical analysis. The samples were shock-frozen with liquid nitrogen and freeze dried for 24 h before analysis. Freeze-dried litter samples were ground with a mortar and pestle to achieve uniform thickness before being mounted on adhesive tape attached to the sample holder. The amount of P in adhesive tape is negligible compared with the concentration of P in the samples. Aqueous standards were mounted on acrylic sample holders (1 mm thick) with a disc-shaped cut-out and covered with polypropylene (5 µm thick) X-ray transparent film, which is deficient in P. Standards were collected on polypropylene film with a small amount of petroleum jelly used as an adhesive. All standards were crushed into fine particles to limit self-adsorption effects, and transmission data were collected when possible (not shown) to ensure such artifacts did not affect the data quality or interpretation.

XANES spectroscopy was conducted at beamlines X-19A and X-15B at the National Synchrotron Light Source on the premises of Brookhaven National Laboratory, Upton, NY (Caliebe et al., 2004; Yang et al., 1990). Beamlines X-19A and X-15B are tender X-ray lines designed for light element X-ray analysis. Experimental and standard data were collected on beamline X-19A using a monochromator with Si(111) crystals and a flux at the P edge of $5 \times 10^{10}$ photons s$^{-1}$. A helium-purged sample chamber with a Canberra-PIPS fluorescence detector was used in data collection at this line. Standard spectra were collected at beamline X-15B also using a monochromator with Si(111) crystals and a flux of $1 \times 10^{12}$ photons s$^{-1}$. A He-purged sample box and solid-state Ge detector were used to maximize sample signal. The monochromator was calibrated with a NaH$_2$PO$_4$ powder standard, and the maximum of the white line of the standard spectra was assigned an energy value of 2158.5 eV (Peak et al., 2002). In this paper, this value is referred to as the white line energy, and shifts are expressed as a difference from this value. The same scan parameters were used when collecting both sets of data with increased detail paid to the white line region of the spectra. At least three reproducible scans were collected for each sample or standard, and many samples were run a number of times to collect representative spectra.

## Data Analysis

Using WINXAS 3.1 (Ressler, 1998), scans for each sample were normalized using a macro that included energy conversions and a two-polynomial normalized fit. The macro ensured a standard procedure for normalization of all samples. Reproducible scans were then averaged using Athena 0.8.050 (Ravel and Newville, 2005). SixPack (Webb, 2005) software powered by IFEFFIT 1.2.8 (Newville, 2001) was used for PCA, target transformation, and LLSF. Principal component analysis was used to determine the smallest number of mathematical functions, or principal components (PC), required to sufficiently describe a composite data set of the experimental spectra. During this procedure, the number of PCs is increased until a local minimum in the change in sum square total occurs, indicating that further addition of a PCs is not statistically improving the quality of the fit. The number of PCs was determined when the addition of another component did not improve the fit by 20%. These separate mathematical functions are represented graphically and used, in combination, for the target transformation. Principal component analysis was performed on the data as two sets: the spectra from alum-amended litters and then those from control litters.

In target transformation, the spectra from reference compounds are individually compared with the collection of mathematical PCs. The software "transforms" the experimental reference spectra to fit the number of PCs selected during the previous step. The degree to which the reference must be changed to fit the PCs is measured in a SPOIL value. A high SPOIL value indicates a poor fit, giving a first suggestion that this standard is not well represented in the collection of sample spectra (represented by the PCs). The reference spectra were then split into groupings based on their SPOIL values and given a rating of excellent (0–1.5), good (1.5–3), fair (3–4.5), or poor (>4.5).

The last step in data analysis is LLSF. In this step, individual sample spectra (from extracted litters) are fit to the reference spectra to determine what reference functions best compose the unknown sample. During the fit, the residuals were minimized, and the best fit is achieved when a combination of references produces the lowest possible reduced chi squared value (at least a $10^{-5}$ red chi squared value). All samples were analyzed from 2150 to 2190 eV and were processed similarly to maintain uniformity. This technique's detection limit is 5 to 10%, so components totaling less than 10% should be considered lightly (Roberts et al., 2002).

# Results and Discussion

## Characterization and Sequential Extractions

The two control litters are similar in composition, with the greatest variability being P and Al concentrations (Table 1). Alum-amended litters #181 and 182 are almost identical in composition, whereas #129 is significantly different in all components (Table 1). These differing compositions may have an effect on the fate of P in the samples.

There is a distinct difference in P solubility between alum-amended and control litters (Fig. 1A). First, the H$_2$O-extractable fraction is significantly lower for all of the alum-amended litters when compared with the values for the controls, supporting the use of alum to reduce P losses from poultry litter (Moore and Miller, 1994; Moore et al., 1999). According to traditional interpretations of these extractions, NaOH removes P bound to Al (Hedley et al., 1982), and because alum addition is thought to create additional Al-surfaces for P to bind (Peak et al., 2002), it is not surprising that the NaOH-extractable P increases in the amended poultry litters. The percentages of P removed by NaHCO$_3$ and HCl did not follow a trend that would suggest that alum treatment affected these fractions.

**Table 1. Elemental analysis for the 841-μm fraction of all poultry litter samples.**

| Litter sample | Treatment | Total† | | | | Al:P | Water soluble‡ | |
|---|---|---|---|---|---|---|---|---|
| | | P | Al | Fe | Ca | | P | Al |
| | | —mg kg⁻¹— | | | | | —mg kg⁻¹— | |
| 525 | control | 17,802 | 976 | 406 | 29,000 | 0.055 | 866 | 10.3 |
| 519 | control | 20,734 | 680 | 398 | 29,225 | 0.033 | 1192 | 6.53 |
| 182 | alum | 22,943 | 27,577 | 841 | 28,196 | 1.20 | 521 | 22.3 |
| 181 | alum | 24,601 | 27,629 | 871 | 31,231 | 1.12 | 485 | 23.7 |
| 129 | alum | 18,235 | 12,789 | 437 | 27,154 | 0.70 | 331 | 22.9 |

† Concentrations are calculated on a dry weight basis and are the mean of duplicate experiments.

‡ Concentrations are calculated on a dry weight basis and are the mean of triplicate determinations.

Although some acid hydrolysis of organic and condensed P compounds can occur during the colorimetric technique used, Mo-reactive P is thought to be mostly inorganic P (Turner et al., 2002). The MRP procedure can overestimate organic P percentage in highly organic media (Turner et al., 2006). Taking these into consideration, on comparison to the total P data (as measured by ICP) in Table 1, a relative distribution of P between inorganic and organic phases within an extract can be estimated. In all samples, the majority of $H_2O$- and $NaHCO_3$–extractable P was Mo reactive (inorganic), and <10% of the HCl extractable P was inorganic (Fig. 2). Because the HCl fraction is the largest P fraction for all of the litters (Fig. 1), this suggests that most of the P in these litter samples is organic. The alum-amended and control litters differ greatly in the amount of NaOH P that is Mo reactive. In the control samples (#519 and #525), the NaOH-extractable P is almost entirely inorganic, whereas the opposite is true of the alum-amended samples. Changes in the extractability of Al between alum-amended and control poultry litter are easily distinguishable (Fig. 1B). For the control litters, a majority of the Al (93% for litter 519 and 66% for litter 525) is recalcitrant to removal by all but the strongest extractant (1M HCl), whereas in the alum amended litter, the majority of Al was removed by the alkaline extraction step (0.1M NaOH). This may be because the Al in the controls is partitioned in the structure of clay minerals from soil mixed into the litter while in the poultry house. Additionally, amorphous AlOOH is thought to be the dominant Al phase in alum-amended poultry litter and would be easily dissolved in the high pH of the NaOH extraction.

For Ca, the only fraction that was clearly different due to alum treatment was that extracted by $H_2O$ (Fig. 1C). The pH of the alum-amended litter (7.2) was slightly lower than that of the control (7.75), which may have provided for more dissolved Ca in the material. In all of the litters, the Ca-HCl fraction was the largest, indicating that most of the Ca in these systems is stable and nonreactive, except in highly acidic conditions. The enzyme phytase is added, often as a calcium salt, to help monogastrics digest phytate in corn grain, allowing assimilation of the organic P in the feed. Turner et al. (2003) showed that this stable orthophosphate monoester does not degrade in the presence of NaOH; therefore, it is likely that the large portion of Ca-HCl originates from calcium phytate. Other inorganic
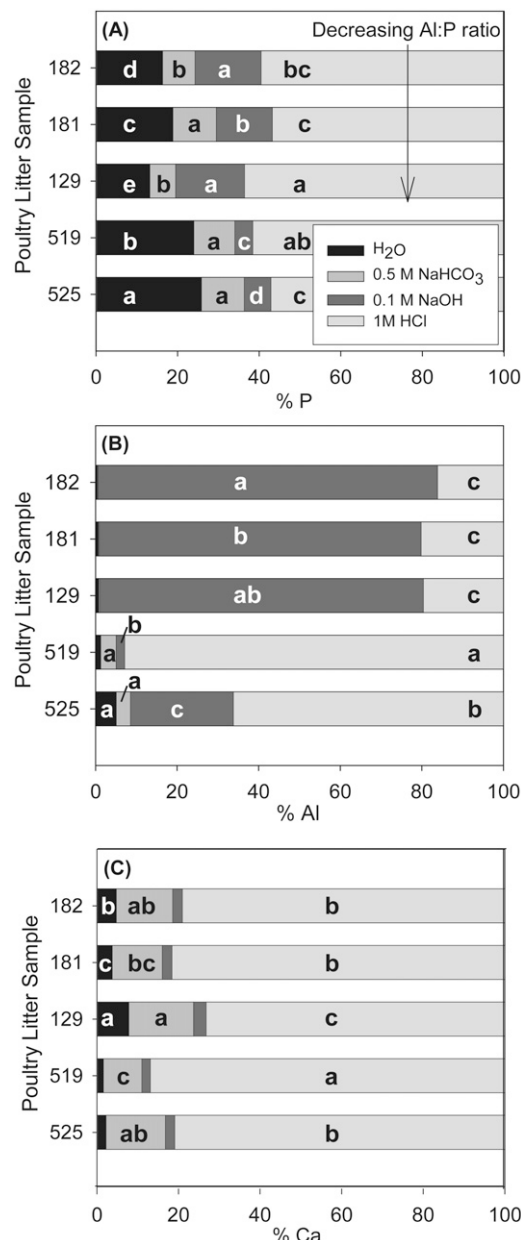


Fig. 1. Percent (calculated from sum of the four fractions) of P (A), Al (B), and Ca (C), extracted in 1:100 suspension of poultry litter and extractant (1 h shake time). Statistically different means (significant at *P* < 0.001) between litters within the same extraction are noted with different letters. Samples 519 and 525 are controls, and samples 129, 181, 182 are alum amended.

calcium salts and minerals that were taken into account include calcium triphosphate, calcium pyrophosphate, and apatite.

## Principal Component Analysis and Linear Least Squares Fitting

Applying a finger-printing data analysis technique to the XANES spectra from this study would be extremely difficult and possibly inaccurate because of how similar the spectra appear on visual inspection. Figure 3 shows the spectra from alum-amended poultry litter #129. A more quantitative alternative to visual inspection and comparison is desirable for differentiating the P

species represented in these XANES data. Principal component analysis and LLSF provide a more quantitative alternative to visual inspection and comparison for differentiating the P species represented in the XANES data shown in Fig. 3.

Principal component analysis determined that each set of experimental spectra in the datasets (control and alum) could be described using four components. This value also reflects the number of PCs that yielded the most "excellent," "good," "fair," and "poor" SPOIL values during the target transformation, although no reference compounds were fit with an "excellent" value (Table 2). These data show that the reference materials used in the fitting procedures fit the alum-amended and control samples differently. The alum-amended litters had more organic P species with "good" spoil values. In comparison, the control litter provided less "good" spoil values for organic P species, indicating a dominance of inorganic P in untreated litters. The N-phosphates provided a "fair" value in the alum litter, whereas they had a "poor" fit in the control litter. These shifts in P speciation between these two types of poultry litter are thoroughly described in the LLSF discussion.

The spectra of reference materials that were dominant in the LLSF are presented in Fig. 4. Figure 5 is a complete representation of the LLSF results for each of our sample spectra. For instance, in Fig. 5A, the first bar shows the relative amounts of four references (inositols, monoesters, Al phosphates, and polyphosphates) needed to achieve a linear combination fit for the "129 As Is" spectra (collected from the alum-amended litter #129 before any extractions were conducted). By examining which references make up each spectra and how the quantities of those references change across the extraction series, the effects of the sequential extraction procedure on the litter samples can be determined. These fits represent a theoretical fit to abstract components (Manceau et al., 2002) and are representative of relative P concentrations in the samples.

Although LLSF is a powerful analytical technique, it has some limitations. Low P concentration and sample alteration due to beam-induced changes and can cause XANES data to become very noisy and therefore difficult to fit using this method of data analysis. The "182 As Is" and "525 $H_2O$" samples were omitted due to poor data quality and an inability to fit references consistent with other samples from the extraction series. The best possible conclusions are considered here. Second, not all components that were dominant in the LLSF fits achieved a "Good" spoil value in the target transformation. It is possible that the references used may not represent the exact forms of P that are present in the poultry litter.

Al-P species are present in most of the alum-amended samples (Fig. 5A–C). According to the target transformations and the LLSF the P speciation is dominated by Al-P species represented as $PO_4^{3-}$ sorbed onto aluminum oxide. The polyphosphates are present in all samples except for the "As Is" samples. Phytic acid species are found in most of the samples. The phytic acid concentration decreases, and in sample #129 disappears, as the strength of the extractant increases (Fig. 5A). On the whole, there is a stronger presence of phytic acid in the alum-amended litters than the control litters. This result correlates with the work of Shober et al. (2006), who found increased levels of phytic acid and Al-P in the alum litters. This phytic acid accumulation is likely due to the presence
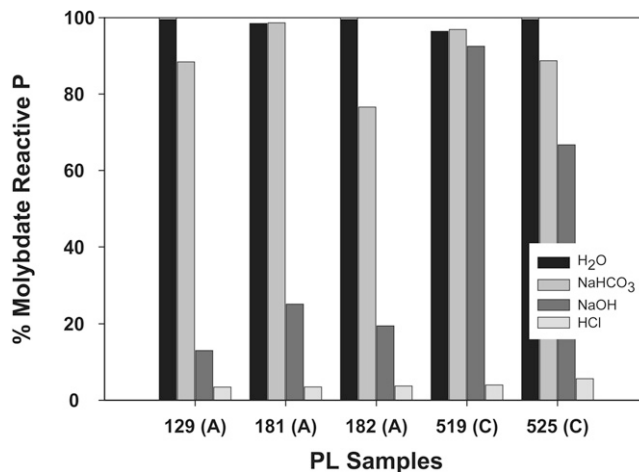


Fig. 2. Fraction (calculated from the sum of the four fractions) of P in the supernatant (from the sequential extractions, the results of which are shown in Fig. 1), which was molybdate reactive. Samples 519 and 525 are controls (C), and samples 129, 181, 182 are alum amended (A). Molybdate-reactive P is considered to be predominantly an inorganic P species.

of alum because when phytic acid binds it becomes less soluble and therefore less bioavailable (Shang et al., 1996; Shang et al., 1990). Phytic acid is a siderophore and binds strongly to $Fe^{3+}$ (Witter et al., 2000); therefore, based on stoichiometry and charge, phytic acid should react similarly with $Al^{3+}$ in the alum.

The "As Is" samples contain aqueous $PO_4^{3-}$, inositols, monoesters, Al-P, and Ca-P. Although there is noticeable variability
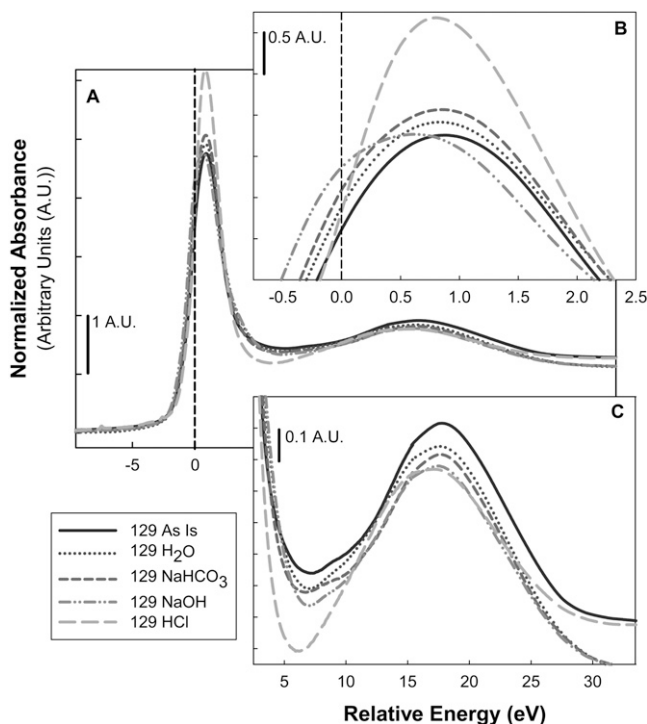


Fig. 3. XANES data collected from poultry litter #129. (A) Entire sequential extraction sequence as a function of relative energy (eV). (B) Magnification of the white line region (0 eV) of the XANES spectra presented in (A). (C) Magnification of the oxygen oscillation (17 eV) from the XANES spectra shown in (A).

**Table 2. Target transformation results for alum-amended and control poultry litter samples.**

| | Reference compounds | |
|---|---|---|
| **Spoil** | **Alum-amended litter** | **Control litter** |
| Good (1.5–3.0) | dGMP (monoester) | ADP (polyphosphates) |
| | DNA (diester) | $K_2HPO_4$ (K phosphates) |
| | phytic acid (inositol) | phytic acid (inositol) |
| | phytate (inositol) | phytate (inositol) |
| | P sorbed onto $Al(OH)_3$ (Al phosphate) | P sorbed onto $Al(OH)_3$ (Al phosphate) |
| | RNA (diester) | brushite ($CaHPO_4 \cdot 2H_2O$) (Ca phosphate) |
| | aluminum phosphate ($AlPO_4$) (Al phosphate) | $CaH_2PO_4$ (Ca phosphate) |
| | brushite ($CaHPO_4 \cdot 2H_2O$) (Ca phosphate) | |
| | $CaH_2PO_4$ (Ca phosphate) | |
| Fair (3.0–4.5) | Ca pyro phosphate ($Ca_2P_2O_7$) (Ca phosphate) | Ca pyro phosphate ($Ca_2P_2O_7$)(Ca phosphate) |
| | aqueous $PO_4$ (aqueous phosphate) | DNA (diester) |
| | fluoroapatite ($Ca(PO_4)_3F$) (Ca phosphate) | phosphatidyl choline (phospholipid) |
| | ammonium phosphate (($NH_4)_3PO_4$) (N phosphate) | RNA (diester) |
| | | fluoroapatite ($Ca(PO_4)_3F$) (Ca phosphate) |
| Poor (4.5–10.0) | ADP (polyphosphates) | AEP (phosphonate) |
| | AEP (phosphonate) | dGMP (monoester) |
| | $K_2HPO_4$ (K phosphates) | aqueous $PO_4$ (aqueous phosphate) |
| | phosphatidyl choline (phospholipid) | glucose (monoester) |
| | glucose (monoester) | glycerol (phospholipid) |
| | phosphocreatine (phospholipid) | ammonium phosphate (($NH_4)_3PO_4$) (N phosphate) |
| | tri-calcium phosphate (Ca phosphate) | phosphocreatine (phospholipid) |
| | | tri-calcium phosphate (Ca phosphate) |

between the two alum "As Is" amended samples, there is a large organic P component found in both. The $H_2O$ extraction samples are similar in all three alum-amended samples with inositols, diesters, polyphosphates, Ca-P, and Al-P present. The diesters are removed after the $NaHCO_3$ extraction and replaced with monoesters. These monoesters are then removed in litters #181 and #182 after the NaOH extraction. The composition of alum-amended litter #129 is different from litters #181 and 182, which may affect the P speciation in the sample. Litters #181 and #182 have inositols, polyphosphates, and Al-P present in the NaOH samples. The samples after the HCl extraction are all similar in composition (aqueous $PO_4^{3-}$, inositol, Al-P, Ca-P, and polyphosphates). The presence of Al-P indicates that not all of the Al is removed in the NaOH step.

The "As Is" spectra for the control litter (Fig. 5D and 5E) are fit with a combination of aqueous $PO_4^{3-}$, N, and Al Phosphates and K or Ca phosphates (519, 525 respectively), similar to the findings found by Toor et al. (2005). The dominant P species in the control litter samples are inorganic, which differs from the alum-treated litters. Ammonium phosphate $(NH_4)_3PO_4$ is only present in the "As Is" samples for the control litters. This fraction is removed after the $H_2O$ extraction. Alum amendments reduce the amount of ammonia created and released in the litter by lowering the pH; hence, inhibiting enzyme and microbial activity (Moore et al., 2000). The alum-amended litters do not contain a significant portion of ammonia-phosphate, which indicates that the amount of $NH_4^+$ created is limited or it is no longer bound to P.

The organic constituents become dominant in the spectra after the next two extractions, $H_2O$ and $NaHCO_3$, which are often grouped to describe the weakly bound P pool (Fig. 5D and 5E). These organic P species are not initially seen because the signal is masked by other P species or because the extractants may be alter-

ing organic P content (dissolving compounds and sorption surfaces). Contributions from inositols, monoesters, polyphosphates, and Ca phosphates are present in the samples. The ratio of inorganic to organic species differs greatly from the "As Is" samples. The inositol species are evident after these extractants, although the amount decreases after the $H_2O$ extraction in sample 519. The inositol species then disappear in both the NaOH and HCl samples. It is possible that the phytic species have not found sufficient surfaces to bind and are easily removed with the stronger extractants. After the NaOH extraction, the litters contain similar compositions (monoesters, polyphosphates, Ca-phosphates), except for the phospholipids component in sample #525 and the disappearance of the insitols. The P distribution in both HCl samples is identical, with only aqueous phosphates, polyphosphates, and Al-phosphates remaining in the sample. Unlike previous interpretations of sequential extractions (Dou et al., 2000; Hedley et al., 1982), the presence of Al-phosphates at the end of the extraction series indicates that the Al-P component is not removed entirely in the NaOH step.

For both control litter samples, polyphosphates persisted after all four extraction steps. Turner et al. (2003) initially noted that these organic phosphates are not fully degraded by the NaOH extractant, which may explain their persistence in these samples. Turner and Leytem (2004) have shown that organic polyphosphates could be removed by the NaOH extraction. Therefore, the presence of polyphosphates in these samples could be explained by the duration (1 h) over which the extraction was completed and/or the challenges involved when comparing solution-based techniques to a solid, in situ technique. Turner and Leytem (2004) used solution $^{31}P$ NMR spectroscopy, whereas our study used solid residues for XANES data analysis.

The control litter samples have a strong Ca-P contribution throughout all of the fractions, which is not surprising consider-

ing Ca in the form of limestone and other minerals is commonly added to poultry litter or contained in the poultry diets (Leytem et al., 2007). Calcium most likely formed Ca-P compounds due to the relatively low concentration of other P-complexing elements (i.e., Al and Fe). The strong presence of Ca-P compounds in the control litters is not solely due to Ca levels because the Ca levels in these litters are not higher than the alum litters (Table 1).

Combination fitting gives a composition based on dominant species contributing to the XANES spectra, which may explain the lack of consistency in the trends within the sample series (e.g., 129 NaOH and the alum "As Is" samples). Therefore, components that are present are not always calculated in a fit because there are dominant signals from other references that mask the weaker, less significant components. This makes it difficult to make direct correlations between the "As Is" spectra and each step in the fractionation series. Furthermore, PCA is sensitive only to variations within sample spectra. Thus, in a series of samples that contain the same fraction of species, PCA may not be able to detect common species as a separate component every time (Manceau et al., 2002).

## Conclusions

Direct identification of phosphorus species in solid materials is a challenging process. When using macroscopic techniques, such as sequential extractions, care must be taken when making assumptions about chemical speciation. This is especially true for heterogeneous materials containing a mix of inorganic nutrients and biologically important organic compounds. Knowledge of P speciation in wastes, gained by physiochemical fractionation procedures coupled with spectroscopic methods, can provide invaluable information about the potential bioavailability of P forms.

This study provides information on inorganic and organic P speciation in poultry litters that will be useful when determining nutrient budgets and implementation of BMPs for confined animal feeding operations, like poultry production facilities. The solubility of P species varies greatly and therefore needs to be considered independently when determining BMPs. Not all organic P is soluble. Polyphosphates and monoesters, the backbone of metabolic processes, are able to withstand the strongest extractants, meaning that these compounds are less likely to pose a threat to the environment. However, other organic P species, such as phytic acid, aqueous phosphate, and diesters, are easily removed ($H_2O$ and $NaHCO_3$) and may need to be considered when determining nutrient management plans.

According to LLSF results, aqueous phosphate, diesters, inositols, and loosely bound components are easily extracted with $H_2O$ and $NaHCO_3$ in alum- and non-alum–amended litters. When using the MRP method on alum-treated litter, it showed that organic P was removed during the NaOH extraction, indicating that these organic P species, including inositols, are bound to the Al oxides. The spectroscopic data demonstrate that no extraction method removes all of a species of P in one step; rather, P-compounds are removed across a continuum of increasingly astringent extractants.

The macroscopic and molecular-scale data support previous studies that suggest that adding alum to litter reduces the amount of soluble P available to leach into soil and water environments
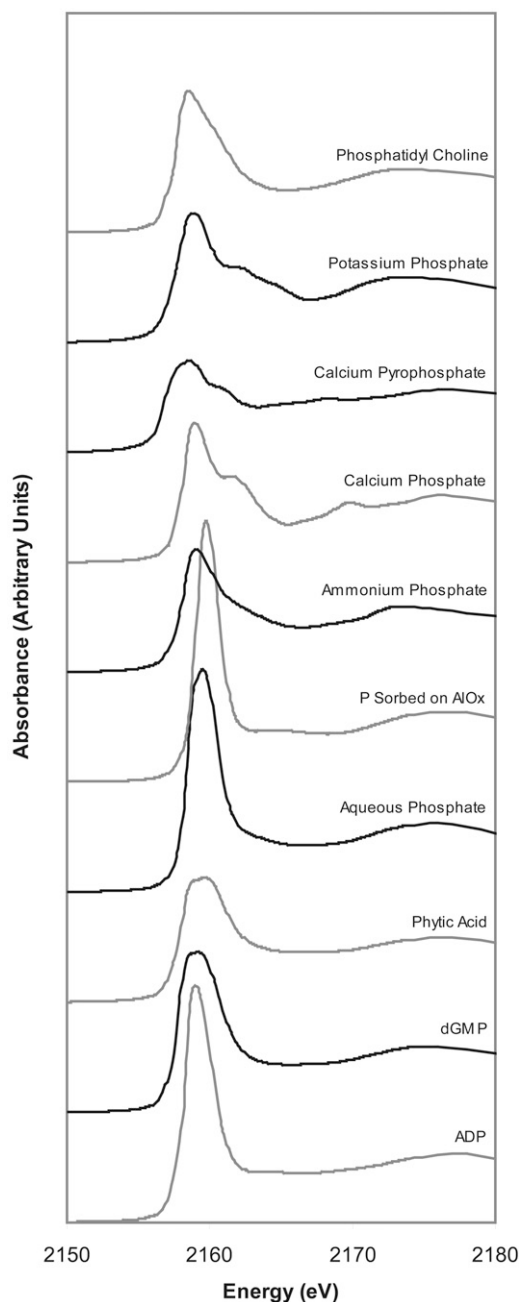


Fig. 4. Spectra collected from reference materials, both organic and inorganic, that were dominant species in litter samples.

(Moore and Miller, 1994; Moore et al., 1999). The data also support the use of alum to alter $NH_4^+$ content in litter samples (Moore et al., 1999). The results indicate a noticeable change in $NH_4^+$ content between alum- and non-alum–amended litters. The acidification of the litter due to the alum amendments may be limiting the creation of ammonia, thus improving air quality in the poultry house and reducing potential transport of soluble N. The LLSF demonstrates that phytic acid species remain throughout the entire sequential extraction process and are more likely to persist in the presence of alum. The data suggest that land-applying alum-amended litter to Delmarva soils will decrease the amount of soluble and bioavailable P (and potentially N) being introduced
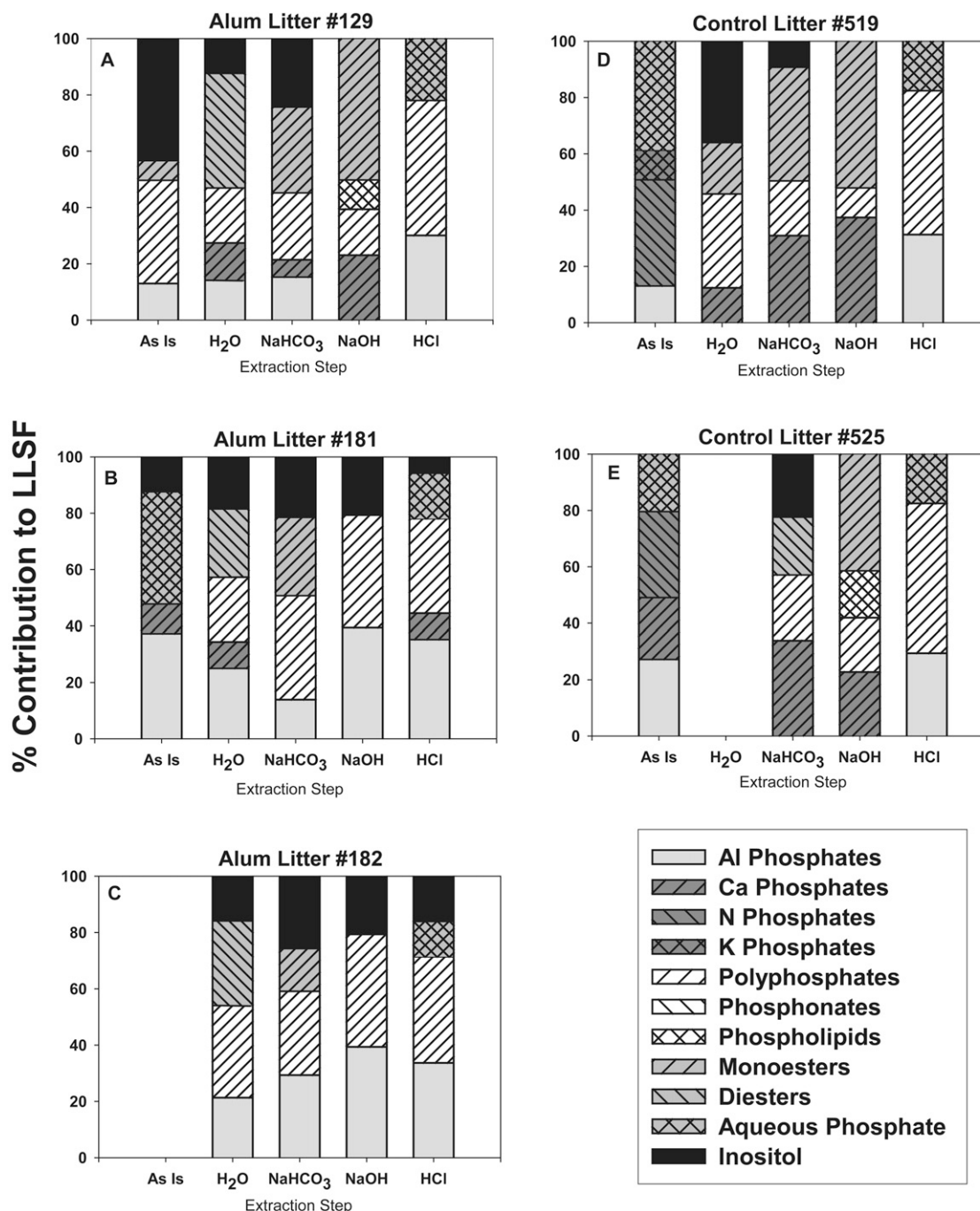
**Fig. 5. Linear combination fitting results for all poultry litter samples by extraction step. The *y* axis represents the percent contribution of a specific component to the overall linear combination fit (summing to 100). Each graph is divided into four or five bars, and each bar is divided into the components required to make up the linear least squares fitting for the averaged spectra from a sample.**

into the land and water system, therefore limiting P input to the fragile water systems of the area. Alum and aluminum oxides are not stable at acidic conditions under pH 3.5. Because litter application increases soil pH and the natural pH of these soils is above this value (pH 4.0–6.5), dissolution of alum and hence the release of Al-bound P is not likely under natural conditions.

This work aimed to determine the effect of alum amendment on P distribution and speciation within poultry litter. Speciation provides valuable information about the fate P may take in the soil and water environments. This information is especially important in the Delmarva Peninsula, which has soils that are acidic and sandy and has seasonally high water-tables; all these conditions are conducive to solute transport.

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