

## Liming Poultry Manures to Decrease Soluble Phosphorus and Suppress the Bacteria Population

R. O. Maguire,\* D. Hesterberg, A. Gernat, K. Anderson, M. Wineland, and J. Grimes

### ABSTRACT

Stabilizing phosphorus (P) in poultry waste to reduce P losses from manured soils is important to protect surface waters, while pathogens in manures are an emerging issue. This study was conducted to evaluate CaO and Ca(OH)<sub>2</sub> for killing manure bacterial populations (pathogens) and stabilizing P in poultry wastes and to investigate the influence on soils following amendment with the treated wastes. Layer manure and broiler litter varying in moisture content were treated with CaO and Ca(OH)<sub>2</sub> at rates of 2.5, 5, 10, and 15% by weight. All treated wastes were analyzed for microbial plate counts, pH, and water-soluble phosphorus (WSP), while a few selected layer manures were analyzed by phosphorus X-ray absorption near edge structure (XANES). A loamy sand and a silt loam were amended with broiler litter and layer manure treated with CaO at rates of 0, 2.5, 5, 10, and 15% and soil WSP and pH were measured at times 1, 8, and 29 d. Liming reduced bacterial populations, with greater rates of lime leading to greater reductions; for example 10% CaO applied to 20% solids broiler litter reduced the plate counts from 793 000 to 6500 mL<sup>-1</sup>. Liming also reduced the WSP in the manures by over 90% in all cases where at least 10% CaO was added. Liming the manures also reduced WSP in soils immediately following application and raised soil pH. The liming process used successfully reduced plate counts and concerns about P losses in runoff following land application of these limed products due to decreased WSP.

LOSSES OF P and pathogens in runoff are two of the greatest concerns for protecting surface water quality. Phosphorus losses from soils following manure applications have been linked to the solubility of P in the manure, therefore any process which decreases manure P solubility should decrease soluble P losses in runoff (Kleinman et al., 2002). Recent changes in state and federal laws have restricted the amounts and times when poultry manure can be land applied based on nitrogen and P standards. The USEPA may also develop regulations to cover pathogens in manures, especially when they are transported off-farm.

The use of lime [CaO and Ca(OH)<sub>2</sub>] to kill pathogens in biosolids (sewage sludge) is a well established process. In the United States, the EPA Part 503 rule regulates the land application of biosolids and requires treatment to reduce pathogens before land application is permitted (USEPA, 1999). When lime is the option used for pathogen reduction, it is required that sufficient lime

is added to raise the pH to 12 for 2 h to kill pathogens (USEPA, 1999). Liming of biosolids has been shown to reduce the solubility of P in the treated product, probably due to the formation of calcium phosphates (Penn and Sims, 2002). When the limed biosolids are applied to soils the reduced solubility of P can persist relative to soils amended with unlimed biosolids (Maguire et al., 2001). However, biosolids treated with lime for pathogens have normally gone through preliminary treatments before lime is added and would therefore be expected to differ in characteristics from animal wastes. For example, biosolids treatment often involves digestion and reduction in nitrogen concentration, so the liming of animal wastes would necessitate the use of an ammonia scrubber to capture the volatilized ammonia.

Manure production has become more intensive and localized; for example, the number of livestock operations decreased by 24% in the United States between 1982 and 1997, even though total animal production numbers were fairly stable (Sims and Maguire, 2004). A national strategy has been developed in the U.S. to deal with confined animal feeding operations and the manure they produce, with the aim of limiting P losses from such agricultural operations (USDA and USEPA, 1999). As a result of this legislation, many states have developed P Indices that assess environmental risk for P losses to surface waters (Sharpley et al., 2003). One factor frequently used in P Indices is the solubility of P in manures that are land applied, with a greater solubility leading to a higher risk of P loss. Therefore, a treatment process that reduces P solubility in manures, such as lime stabilization, would be expected to reduce P losses in runoff (Penn and Sims, 2002). Moore and Miller (1994) have demonstrated that WSP in broiler litter can be decreased from >2000 mg P kg<sup>-1</sup> litter to <1 mg P kg<sup>-1</sup> litter with the addition of quicklime or slaked lime. Moore et al. (1995) showed that alum additions to poultry litter greatly decreased ammonia volatilization. However, when alum additions were combined with lime additions, reductions in ammonia volatilization were not as great as for alum additions alone, indicating that the lime increased ammonia volatilization (Moore et al., 1995).

If regulations to control pathogens in poultry wastes are developed, and regulations covering land application of wastes continue to evolve as expected, then there is a great need for a viable process that can control pathogens and stabilize P in animal wastes. In areas with acidic soils the liming of manure may have the further benefit of reducing soil acidity, as was demonstrated for limed biosolids by Maguire et al. (2001). Therefore, the

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Published in *J. Environ. Qual.* 35:849–857 (2006).

Technical Reports: Waste Management

doi:10.2134/jeq2005.0339

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**Abbreviations:** WSP, water-soluble phosphorus; XANES, X-ray absorption near edge structure.

objectives of this study were to (i) evaluate different combinations of lime and manure moisture on pathogen reductions and P solubility, (ii) add treated manures from the most effective treatments to soils to ascertain the effect of adding limed manures to soils on soil pH and P solubility, and (iii) identify the specific forms of P that are formed by the liming process.

## MATERIALS AND METHODS

### Lime Treatment of Layer Manure and Broiler Litter

Layer manure and broiler litter were sampled from commercial poultry houses and mixed thoroughly. Moisture content was measured at 105°C and adjusted to 90, 85, and 80% (10, 15, and 20% solids) with deionized water before lime treatment. Lime in the form of quicklime (CaO) or hydrated lime [Ca(OH)<sub>2</sub>] was added at rates of 0, 2.5, 5.0, 10, and 15% to approximately 500 g of layer manures and broiler litters by weight for each of the three moisture contents [except the 20% solids broiler litter for Ca(OH)<sub>2</sub> as this was considered to be unfeasible due to preliminary studies showing insufficient heat generation]. Samples were incubated at room temperature for 1 d and then refrigerated at 4°C until analysis and inclusion in the incubation study.

### Analysis of Manures and Litters

Microbial plate counts were conducted on samples collected in an aseptic manner and transported to the laboratory (Dey et al., 1998, p. 4–1 to 4–14). Two samples were collected per treatment group, one before and the other after the addition of CaO or Ca(OH)<sub>2</sub>. Each of the samples were thoroughly mixed. A 1-g sample was placed in 10 mL of buffered peptone broth, then serially diluted to 10<sup>-9</sup> to capture the microbial load in the fresh manure samples. The treated samples were handled in the same manner, with analyses done in duplicate. The dilutions were made in the laboratory to allow for accurate determination of the microbial loads in the samples. Standard Methods Agar (STD) was used for obtaining microbial plate counts. The units used in the figures are log<sub>10</sub> colony forming unit (CFU) g<sup>-1</sup> of samples. Colony forming unit less than a log of 0.20 was below our testing sensitivity.

Water-soluble P was extracted in moist manures at a 1:10 manure to deionized water ratio. After shaking horizontally at 300 rpm for 1 h, extracts were centrifuged at 1000 × *g* for 1 h and the supernatant filtered through Whatman (Maidstone, UK) #40 filter papers. The filtered supernatant was then acidified with concentrated HCl and analyzed for P by inductively coupled plasma–atomic emission spectrometry (ICP–AES). The WSP was then converted to a dry matter basis using the moisture content determined at 105°C. Total P was extracted by adding 8 mL of concentrated nitric acid to 2.5 g manure, drying on a steam plate, and combusting at 500°C in a muffle furnace over night. Once the samples had cooled, 4 mL of 6 M HCl was added, dried on a steam plate, then rehydrated with 4 mL of 6 M HCl and warmed before being transferred into a volumetric flask and diluted with deionized water. The resulting solution was filtered through Whatman #40 filter paper, and then analyzed for P, Al, and Fe by ICP–AES. The pH of the samples were measured 30 min after mixing twice at a manure to deionized water ratio of 1:1.

### Phosphorus XANES Analysis

The XANES analyses were performed at the phosphorus K-edge (approximately 2150 eV) to evaluate transformations of

solid-phase P species as affected by lime treatments applied to layer-manure samples with 20% solids. Moist manure samples for each treatment (and the nontreated control) were mounted using the techniques described by Khare et al. (2004) for moist samples. XANES data were also collected on the following standards to use in quantitative fitting of sample spectra: hydroxyapatite, monetite, brushite, octacalcium phosphate, variscite, and noncrystalline Fe-phosphate (same standards as used by Beauchemin et al., 2003); phosphate adsorbed at pH 6 on boehmite (500 mmol kg<sup>-1</sup>) or ferrihydrite (600 mmol kg<sup>-1</sup>) (prepared as Khare et al., 2004); and purchased samples of Ca- and Na-phytate. Adsorbed standards of PO<sub>4</sub> on boehmite and ferrihydrite were used as surrogates for PO<sub>4</sub> adsorbed on Al- and Fe-oxide minerals in general. Mineral standards were ground and diluted to 400 mmol kg<sup>-1</sup> in boron nitride to decrease self-absorption effects for fluorescence-mode XANES analysis.

The XANES data were collected at Beamline X-19A at the National Synchrotron Light Source (NSLS), Brookhaven National Laboratory, Upton, NY. An electron beam energy of 2.5 GeV and a maximum beam current of 300 mA were utilized. A dual-crystal Si(111) monochromator was detuned by 50% to reject higher-order harmonics, and calibrated to an energy of 2149 eV at the phosphorus K edge for a standard of variscite (Khare et al., 2004). The calibration was checked several times during the course of analyzing samples and standards to ensure that the energy scale did not shift over time. Data were collected in fluorescence mode between 2119 and 2249 eV (–30 to 100 eV relative to the 2149 eV calibration energy) using a passivated implanted planar silicon (PIPS) detector mounted into a He<sub>(g)</sub> purged sample chamber (Khare et al., 2004). Two to five scans with consistent baselines were ensemble averaged. Normalization of XANES spectra was done using the Athena data analysis software (Ravel and Newville, 2005). Average spectra were linear-baseline corrected over a linear portion of the spectra between –30 and –10 eV (relative to the 2149 eV reference energy). Background normalization was done using a linear background calculated at a featureless energy interval between 29 and 55 eV (relative energy). Normalization removes effects of differences in P concentrations on the intensity of spectral features so that differences in normalized spectra can be related to solid-phase P speciation only. To eliminate any slope in the post-edge region of normalized spectra, the “flatten” feature in Athena was invoked. This algorithm corrects the post-edge slope to near zero while not affecting the near-edge (white line) region of the spectra. The least-squares, nonlinear fitting (linear combination fitting) routine in Athena was used to obtain the proportions of up to three standard spectra summed to yield the best fit (least chi-squared value) to sample spectra (Beauchemin et al., 2003). Spectra were fit over a relative energy range of –7 to 30 eV, with no energy shifts allowed in the fitting. All possible combinations of the 10 standards were considered, and the best-fit combination for each sample is reported here. The sum of proportions of standards calculated for the best fits ranged from 1.04 to 1.12, so the proportion of each standard was normalized to yield a sum of 1.00 (100%) (Beauchemin et al., 2003).

### Incubation of Selected Manures and Litters with Soils

Two agriculturally important soils in North Carolina were selected that varied in physical and chemical properties. The two soils were (i) Herndon silt loam (Fine, kaolinitic, thermic Typic Kanhapludults) and (ii) Norfolk loamy sand (fine-loamy, kaolinitic, thermic Typic Kandudults). The soils were air dried and passed through a 2-mm sieve before analysis and inclusion in the incubation experiment.

**Table 1. Plate counts for the layer manure and broiler litter ( $\pm$ standard error) with three moisture contents that were treated with four rates of CaO or Ca(OH)<sub>2</sub>. These values relate to unamended layer manure and broiler litter that had plate counts of 3 770 000 and 793 000, respectively.**

Rate of CaO or Ca(OH) <sub>2</sub>	Plate counts				
	CaO			Ca(OH) <sub>2</sub>	
	Solids (%)				
	10	15	20	10	15
%	count mL <sup>-1</sup>				
	<b>Layer manure</b>				
2.5	60 000 $\pm$ 0	62 500 $\pm$ 7 500	31 000 $\pm$ 2 000	2 690 000 $\pm$ 570 000	761 500 $\pm$ 210 500
5.0	15 000 $\pm$ 1 500	37 000 $\pm$ 3 000	26 500 $\pm$ 500	37 000 $\pm$ 10 000	14 000 $\pm$ 5 000
10	4 500 $\pm$ 500	7 000 $\pm$ 0	12 500 $\pm$ 4 500	45 500 $\pm$ 12 500	26 000 $\pm$ 1 000
15	4 000 $\pm$ 3 000	3 000 $\pm$ 2 000	3 000 $\pm$ 1 000	20 000 $\pm$ 6 000	17 000 $\pm$ 3 000
	<b>Broiler litter</b>				
2.5	180 000 $\pm$ 32 000	223 500 $\pm$ 4 500	148 500 $\pm$ 33 500	175 500 $\pm$ 13 500	309 000 $\pm$ 109 000
5.0	14 500 $\pm$ 5 500	23 500 $\pm$ 4 500	34 500 $\pm$ 9 500	114 000 $\pm$ 4 000	102 000 $\pm$ 5 000
10	1 500 $\pm$ 500	500 $\pm$ 500	6 500 $\pm$ 2 500	3 500 $\pm$ 500	3 500 $\pm$ 1 500
15	1 000 $\pm$ 1 000	2 500 $\pm$ 1 500	15 000 $\pm$ 4 000	5 500 $\pm$ 500	5 000 $\pm$ 2 000

The layer manure and broiler litter with 20% solids and treated with CaO were selected for inclusion in the incubation, on the basis of good pathogen control and WSP reductions as described later (Tables 1 and 2). The greater solid content would also make the process more practical, as less moisture makes handling easier. Each of the manures or litters was incorporated into 100 g soil, in triplicate. A rate of 150 kg total P ha<sup>-1</sup> (assuming 2242 Mg topsoil ha<sup>-1</sup>) was chosen, as this is similar to the P rate when manures are added according to N-based nutrient management. Approximate field capacity of the soils was determined by weighing 50 g of soil into a plastic cup with pinholes in the base, placing in a shallow tray of water until the soils became saturated, and leaving covered for 2 d to drain. The amended and unamended control soils (no litter or manure added) were incubated at 70% of field capacity in polyethylene containers for 29 d at 25°C. Two holes were poked in the top of the incubation cups to maintain aerobic conditions while preventing rapid drying. Soil moisture content was maintained on a weight basis by adding deionized water at weekly intervals.

After 1 and 29 d, 2-g subsamples were removed and analyzed for WSP in the soil at a soil to deionized water ratio of 1:10 (dry weight basis) on moist soils, 1 h shaking time, 15 min centrifugation at 1000  $\times$  g, followed by filtration through a 0.45- $\mu$ m Millipore (Billerica, MA) membrane. Molybdate reactive P was determined in the extract by the method of

Murphy and Riley (1962). The soil pH was measured at a soil to deionized water ratio of 1:1, 30 min after mixing twice. Before and after the incubation, subsamples of the soils were air-dried, and analyzed for Mehlich-3 P (Mehlich, 1984). The extracts were analyzed for P by ICP-AES.

### Statistical Analyses

Standard errors were determined using Microsoft Excel (Microsoft Corporation, 2000). Separation of means was performed using least significant differences calculated with the PROC GLM procedure of the Statistical Analysis System, Version 8 (SAS Institute, 1998) with alpha set to 0.05.

## RESULTS AND DISCUSSION

### Impact of Liming on Plate Counts in Wastes

The total plate counts measure bacterial populations but are also an indicator of pathogenic organism populations (Table 1). Based on the pH and temperature that was achieved following lime addition, the thermal D values of the organisms (where they are killed) likely present in the samples would follow the same pattern in that the death curves would be similar and fit those described by Lambert (2003). This means bacterial

**Table 2. The pH of the layer manure and broiler litter ( $\pm$ standard error) with three moisture contents that were treated with four rates of CaO or Ca(OH)<sub>2</sub>. These values relate to unamended layer manure and broiler litter that had pH of 8.89 and 7.97, respectively.**

Rate of CaO or Ca(OH) <sub>2</sub>	pH				
	CaO			Ca(OH) <sub>2</sub>	
	Solids (%)				
	10	15	20	10	15
%	<b>Layer manure</b>				
2.5	8.27 $\pm$ 0.02	8.19 $\pm$ 0.02	8.40 $\pm$ 0.03	8.21 $\pm$ 0.00	7.89 $\pm$ 0.03
5.0	11.15 $\pm$ 0.07	11.30 $\pm$ 0.07	11.31 $\pm$ 0.06	10.23 $\pm$ 0.00	10.82 $\pm$ 0.04
10	11.58 $\pm$ 0.05	11.84 $\pm$ 0.02	11.89 $\pm$ 0.02	11.70 $\pm$ 0.00	12.12 $\pm$ 0.02
15	11.67 $\pm$ 0.01	11.82 $\pm$ 0.00	11.92 $\pm$ 0.01	11.40 $\pm$ 0.02	12.18 $\pm$ 0.00
	<b>Broiler litter</b>				
2.5	8.08 $\pm$ 0.01	7.98 $\pm$ 0.02	7.69 $\pm$ 0.01	8.34 $\pm$ 0.02	8.21 $\pm$ 0.03
5.0	9.65 $\pm$ 0.01	9.53 $\pm$ 0.01	9.82 $\pm$ 0.01	9.17 $\pm$ 0.01	9.18 $\pm$ 0.01
10	11.78 $\pm$ 0.02	11.58 $\pm$ 0.01	11.92 $\pm$ 0.09	10.68 $\pm$ 0.02	10.86 $\pm$ 0.03
15	11.97 $\pm$ 0.04	12.06 $\pm$ 0.03	12.11 $\pm$ 0.01	11.90 $\pm$ 0.01	11.94 $\pm$ 0.01

population counts would reflect the fate of many organisms, including those such as *Salmonella*.

The plate counts in the unlimed wastes were very high, being 3 770 000 counts mL<sup>-1</sup> in the layer manure and 793 000 counts mL<sup>-1</sup> in the broiler litter. Liming of the wastes proved very effective at reducing plate counts in many cases, but was affected by lime type, rate, and initial moisture content of wastes (Table 1). For all moisture contents and both lime types and waste types, plate counts decreased greatly from no lime to 2.5% lime to 5% lime. There were then further small decreases from 5, to 10 to 15% lime rates. For example, across all moisture contents and lime types, for layer manure the mean plate counts were 3 770 000, 721 000, 25 900, 19 100, and 9400 counts mL<sup>-1</sup> for the 0, 2.5, 5, 10, and 15% lime rates, respectively.

Comparing similar rates and moisture contents for CaO vs. Ca(OH)<sub>2</sub>, the CaO was more effective at reducing plate counts. For example, for broiler litters the mean plate counts for 10 and 15% solids were 19 000 counts mL<sup>-1</sup> for 5% CaO and 108 000 counts mL<sup>-1</sup> for 5% Ca(OH)<sub>2</sub>. This is in line with what would be expected, as Ca(OH)<sub>2</sub> had released its heat of hydration before addition to the waste, while CaO released this energy on contact with moisture after addition to the waste. Trends in plate counts across moisture contents of wastes were not consistent. The Ca(OH)<sub>2</sub> generally reduced plate counts to a greater extent in the wastes with 15% solids, compared to those with 10% solids. However, the plate counts were generally lower in 20% solids wastes at 2.5% CaO compared to 10% solids, but higher in the 20% solids wastes at 10 and 15% CaO rates relative to 10% solids.

### Impact of Liming on pH in Wastes

The pH of the limed wastes increased with increasing lime rate as would be expected (Table 2). However, the pH of the unlimed layer manure was slightly greater than in layer manures receiving the lowest rate of lime. The pH of the unlimed layer manure (8.89) was greater than that of the unlimed broiler litter (7.97), but when the same rate of lime was added to both materials this greater pH in the layer manures did not always persist. For example, the layer manure with 20% solids amended with 15% CaO had a pH of 11.92, while the equivalent broiler litter had a pH of 12.11. Addition of CaO led to higher pHs in the broiler litters compared to Ca(OH)<sub>2</sub>, but both sources of lime led to similar increases in pH in the layer manures. The CaO would be expected to raise pH more due to it being a more concentrated form of lime. The pH of the limed wastes generally increased with increasing solids content, for both CaO and Ca(OH)<sub>2</sub>, and for layer manure and broiler litter. Changes in pH were not measured in the hours following lime amendment, so from this study it is not possible to tell which treatments met the EPA Part 503 rule for biosolids that requires sufficient lime to be added to raise the pH to 12 for 2 h to kill pathogens before land application is permitted (USEPA, 1999). However, even after storage for a few weeks, the pH values in the wastes limed with the greatest rate of lime

(15%) were frequently above 12. It is also likely that pH 12 was reached for several of the other treatments, and the impact of raised pH can be seen through the reduced plate counts as already discussed (Table 1).

### Impact of Liming on Water-Soluble Phosphorus in Wastes

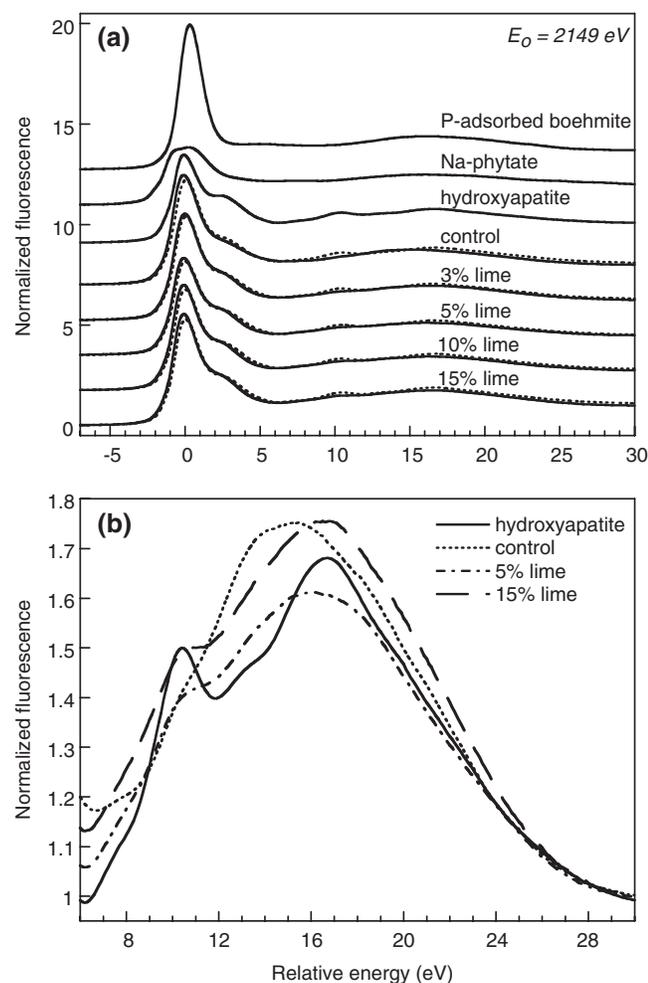
As for plate counts, liming was effective at reducing WSP in wastes with higher rates leading to greater reductions (Table 3). Both CaO and Ca(OH)<sub>2</sub> were capable of decreasing WSP in layer manure and broiler litter. Liming has previously been shown to reduce the solubility of P in biosolids, probably due to the formation of calcium phosphates (Maguire et al., 2001; Penn and Sims, 2002). The greatest reductions in WSP per unit increase in CaO rate were for the 2.5 and 5% rates, with generally little change in WSP from the 10% to the 15% CaO rate. The untreated layer manure had less WSP (1854 mg kg<sup>-1</sup>) than the unamended broiler litter (2750 mg kg<sup>-1</sup>), and this trend continued after liming with the limed layer manures almost always having lower WSP than the equivalent limed broiler litters. The 2.5% rate of Ca(OH)<sub>2</sub> was not as efficient at reducing WSP as the equivalent rate of CaO. However, the 5, 10, and 15% rates of CaO and Ca(OH)<sub>2</sub> reduced WSP by generally the same amount in layer manures and broiler litter. The WSP rapidly decreased in both layer manures and broiler litters with greater rates of Ca(OH)<sub>2</sub>, being reduced from an average of 1445 mg kg<sup>-1</sup> with 2.5% Ca(OH)<sub>2</sub> to an average of 125 mg kg<sup>-1</sup> with an application of 5% Ca(OH)<sub>2</sub>. There were no consistent trends for changes in WSP across solids contents for the CaO treatments, with 10 and 15% CaO being effective at reducing WSP across all solids contents in both layer manure and broiler litter. The WSP was consistently less for the 15% solids than the 10% solids for the Ca(OH)<sub>2</sub> treatments, with the 15% rate proving most effective at reducing WSP for layer manure and broiler litter.

**Table 3. Water-soluble P for the layer manure and broiler litter (± standard error) with three moisture contents that were treated with four rates of CaO or Ca(OH)<sub>2</sub>. These values relate to unamended layer manure and broiler litter that had water-soluble P of 1854 ± 59 and 2750 ± 23 mg kg<sup>-1</sup>, respectively.**

Rate of CaO or Ca(OH) <sub>2</sub>	Water-soluble P				
	CaO			Ca(OH) <sub>2</sub>	
	Solids (%)				
	10	15	20	10	15
%	mg kg <sup>-1</sup>				
	Layer manure				
2.5	973 ± 59	1148 ± 64	310 ± 21	1302 ± 43	947 ± 189
5.0	153 ± 20	231 ± 42	337 ± 5	433 ± 52	212 ± 48
10	62 ± 3	133 ± 4	95 ± 47	172 ± 5	134 ± 18
15	86 ± 1	84 ± 5	96 ± 6	84 ± 6	101 ± 6
	Broiler litter				
2.5	1171 ± 127	578 ± 69	850 ± 40	2122 ± 62	1408 ± 221
5.0	505 ± 47	249 ± 85	401 ± 5	166 ± 19	144 ± 23
10	169 ± 23	100 ± 43	175 ± 15	453 ± 11	335 ± 32
15	137 ± 23	124 ± 12	105 ± 15	201 ± 19	112 ± 45

## Phosphorus Speciation by XANES in Limed and Unlimed Wastes

Figure 1a shows phosphorus XANES spectra for samples of lime-treated layer manure samples (20% solids) along with standards that yielded the best fits in linear-combination fitting analysis as reported in Table 4. Based on the standard-set used for fitting, results indicated that all samples contained between 76 and 85 mol % of P as hydroxyapatite [ $\text{Ca}_5(\text{PO}_4)_3\text{OH}$ ] (Table 4). Fitting results for  $\text{PO}_4$  adsorbed on boehmite indicated that minor concentrations of total P were sorbed with Al, for example, on an Al-oxide mineral or perhaps with Al(III) bound to organic materials. Including up to 6 mol % of the Na-phytate standard improved the goodness-of-fit for some samples, whereas Ca-phytate did not improve fits. A dominance of a Ca-phosphate in the samples is shown by the shoulder between 3 and 5 eV relative energy ( $E_0 = 2149$  eV) on the high-energy side of the strong white-line peak near 1 eV. This post-white-line shoulder peak is unique for



**Fig. 1.** Stacked, normalized phosphorus X-ray absorption near edge structure (XANES) spectra for lime-treated layer manure samples (and a nontreated control) and standards yielding the best fit to the data (a), and magnification of the post-edge region for selected spectra (b). Overlays of linear combination fitting results reported in Table 4 are shown as dashed lines in Fig. 1a.

**Table 4.** Best-fit results from linear combination fitting of phosphorus X-ray absorption near edge structure (XANES) spectra for lime-treated poultry waste samples and a control treatment. Fitting range was  $-7$  to  $30$  eV (relative energy) using 10 possible standards.

Lime treatment	Hydroxyapatite	$\text{PO}_4$ on boehmite <sup>†</sup>	Phytate	$1000 \times R$ factor <sup>‡</sup>
%	— mol % of standard ( $\pm$ uncertainty <sup>§</sup> ) —			
0 (control)	$76 \pm 3$	$18 \pm 1$	$6 \pm 1$	8.0
2.5	$82 \pm 1$	$19 \pm 10$		2.7
5	$84 \pm 2$	$11 \pm 1$	$6 \pm 1$	3.3
10	$86 \pm 2$	$13 \pm 1$	$1.2 \pm 0.9$	5.2
15	$85 \pm 1$	$15 \pm 8$		6.0

<sup>†</sup> Phosphate adsorbed on boehmite at  $500 \text{ mmol kg}^{-1}$  and pH 6.

<sup>‡</sup>  $R$  factor =  $\Sigma(\text{data} - \text{fit})^2 / \Sigma(\text{data}^2)$ , where the sums are over the data points included in the fitting range ( $-7$  to  $30$  eV).

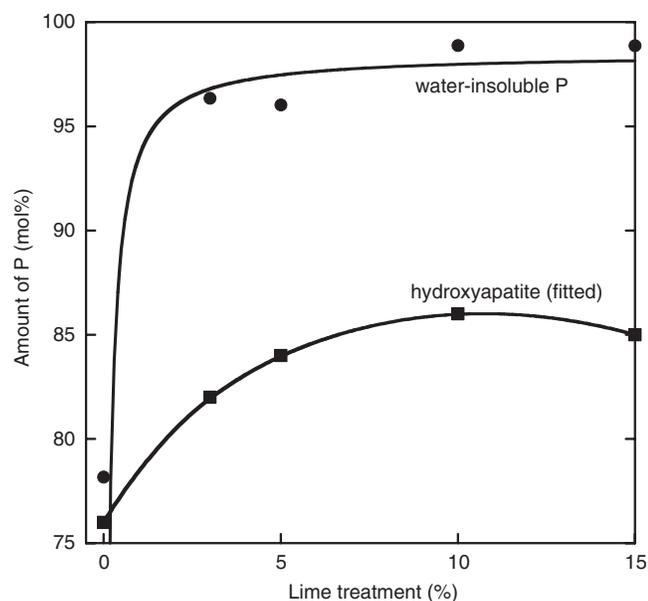
<sup>§</sup> Uncertainties on fitting parameters were calculated by the Athena program and normalized by the proportionality constant used to yield  $\Sigma \text{ mol \%} = 1$ .

Ca-phosphates (Hesterberg et al., 1999), and is more distinct than that found by Peak et al. (2002) for natural poultry litter samples. Although the XANES spectra show definitively that the manure samples all contain Ca-P, the white-line peak for these samples is more intense than those for Ca-phosphate mineral standards (Hesterberg et al., 1999; Beauchemin et al., 2003). As noted by Beauchemin et al. (2003) and Khare et al. (2005), a strong white line peak is characteristic of P adsorbed on oxide minerals (see, for example, the standard of  $\text{PO}_4$  on boehmite in Fig. 1a).

Total elemental analyses of the layer manure samples yielded Ca to P molar ratios ranging from 7 to 20, indicating that sufficient Ca was always present to bind P as hydroxyapatite (detailed results not shown). Molar ratios of Al to P and Fe to P were  $0.11 \pm 0.04$  and  $0.05 \pm 0.01$ , suggesting that a maximum of 15 mol % of P could be associated with Al if in a 1:1 molar ratio (as occurs in Al-phosphate minerals), and  $\leq 5$  mol % of P could be associated with Fe. Based on the Al to P ratio, the fitting analysis probably overestimated amounts of  $\text{PO}_4$  associated with Al (as an oxide or organic-bound Al) (Table 4). The accuracy of fitting XANES spectra for organic P forms depends on the amounts and types of organic P present (Beauchemin et al., 2003). Regardless of the minor species present, the dominance of a hydroxyapatite-like phase is clear (Fig. 1a, Table 4).

The XANES results also showed that the crystallinity of the hydroxyapatite in the layer manures tended to increase with increasing rate of liming (Fig. 1b). Phosphorus in the control sample yielded a broad fluorescence peak between 14 and 16 eV (relative energy) with a very weak shoulder discernible at 10 eV. With increasing lime treatment, this shoulder became more pronounced and the strongest peak shifted toward that of the hydroxyapatite standard (selected data shown in Fig. 1b). The more distinct spectral features indicate that a greater proportion of phosphorus atoms are contained in a well-ordered, local-molecular bonding environment, consistent with larger hydroxyapatite crystallites (i.e., greater mineral crystallinity).

Phosphorus XANES results provide one explanation for the decrease in water-soluble P induced by liming



**Fig. 2.** Amounts of phosphorus determined by X-ray absorption near edge structure (XANES) analysis to be bound as hydroxyapatite in layer manure samples (20% solids) treated with different lime rates, and the proportion of total phosphorus that was not water soluble. A smooth curve for hydroxyapatite and a Langmuir-type fit [ $y = abx/(1 + bx)$ ] to the water-insoluble P data are included to illustrate trends.

of layer manure (Table 3). As shown in Fig. 2, both hydroxyapatite and water insoluble P increased with increasing amounts of reacted lime. The mole fractions of water insoluble P were calculated as the proportion of mean total P in the layer manure control sample ( $270 \pm 40 \text{ mmol kg}^{-1}$ ) that was not water extractable (Table 3). However, the molar increase in hydroxyapatite concentration derived from XANES fitting could not entirely account for the increase in water-insoluble P (Fig. 2). Increased crystallinity (Fig. 1b) would also decrease the water solubility of hydroxyapatite. Furthermore, a greater pH (Table 2) and greater aqueous activity of  $\text{Ca}^{2+}$  in equilibrium with  $\text{Ca}(\text{OH})_2$  formed by hydration of added CaO in limed samples would also decrease the water solubility of hydroxyapatite (Lindsay, 1979). However, when limed manure is added to an acid soil, the decrease in pH would tend to increase the solubility of hydroxyapatite contained in the manure, while this would not occur in alkaline soils.

### Impact of Liming Wastes on pH in Amended Soils

Only the layer manures and broiler litters with 20% solids and limed with CaO were selected for the incubation study, due to the good performance of this treatment in terms of reduced plate counts and WSP, as well as being more practical as dryer manures are easier and less bulky to handle. The application of limed wastes to the silt loam and loamy sand increased soil pH in all cases (Table 5), which would be expected as lime is used in agriculture to raise soil pH. Application of unlimed layer manure or broiler litter also increased soil pH at 1 d, due to the alkaline pH of these wastes. Increases in

**Table 5.** Effect of quicklime application to manures on the pH of amended soils.

Lime rate	Layer manure		Broiler litter	
	Day 1	Day 29	Day 1	Day 29
<b>Herndon silt loam</b>				
0	7.37c†	7.29c	6.93c	7.14c
2.5	7.41c	7.35c	6.99c	7.17c
5	7.50bc	7.47bc	7.04c	7.20bc
10	7.68ab	7.65ab	7.39b	7.41ab
15	7.84ab	7.78ab	7.70a	7.58ab
Unamended soil	6.58d	6.86d	6.58d	6.86d
<b>Norfolk loamy sand</b>				
0	8.08b	6.63e	6.74b	4.97f
2.5	7.74c	6.94d	6.87b	5.60d
5	7.83c	7.22c	6.84b	6.29c
10	8.23b	7.88b	7.51a	6.82b
15	8.70a	8.16a	7.69a	7.57a
Unamended soil	5.72d	5.19f	5.72c	5.19e

† Means within one column followed by different letters are significantly different at the 0.05 probability level.

soil pH following amendment with limed layer manure or broiler litter were greater in the loamy sand than the silt loam. This is almost certainly due to the greater pH buffer capacities of more finely textured soils. As expected, the higher rates of CaO led to greater increases in soil pH, due to the higher pH of the wastes amended with higher rates of CaO (Table 2). The raised pH relative to the unamended soil persisted throughout the 29 d of the incubation. Application of layer manures led to greater increases in soil pH than application of the equivalent limed broiler litter. This again suggests that the layer manures have a greater buffer capacity for pH than the broiler litters, as was shown for changes in pH following liming of the layer manures and broiler litters (Table 2). For the silt loam the pH was fairly constant between 1 and 29 d, but the pH in the loamy sand soil consistently decreased in waste-amended and unamended soils between 1 and 29 d. The reason for this is not clear. Agronomic pH recommendations for most crops are generally in the range 5.8 to 6.2 for mineral soils in North Carolina, so the relatively large increases in pH caused especially by some of the higher rates of CaO would be problematic (Crozier and Hardy, 2003). However, the two soils used were already close to the agronomic optimum pH range (Table 5) and the liming quality of these limed manures would be of value to acidic soils where a rise in pH would be beneficial. If these limed products are going to be used in an agronomic setting, care should be taken to avoid overapplication of lime especially if higher rates of lime are used.

### Impact of Liming Wastes on Water-Soluble Phosphorus in Amended Soils

Application of limed or unlimed layer manure or broiler litter increased WSP in the silt loam and loamy sand in all cases at 1 d, relative to the unamended soil (Fig. 3a and 3b). The unamended silt loam and loamy sand both had approximately  $4.5 \text{ mg WSP kg}^{-1}$ , despite the silt loam having greater Mehlich-3 P than the loamy sand (Table 6). The greatest increase in WSP was always for the unlimed layer manure or broiler litter, indicating

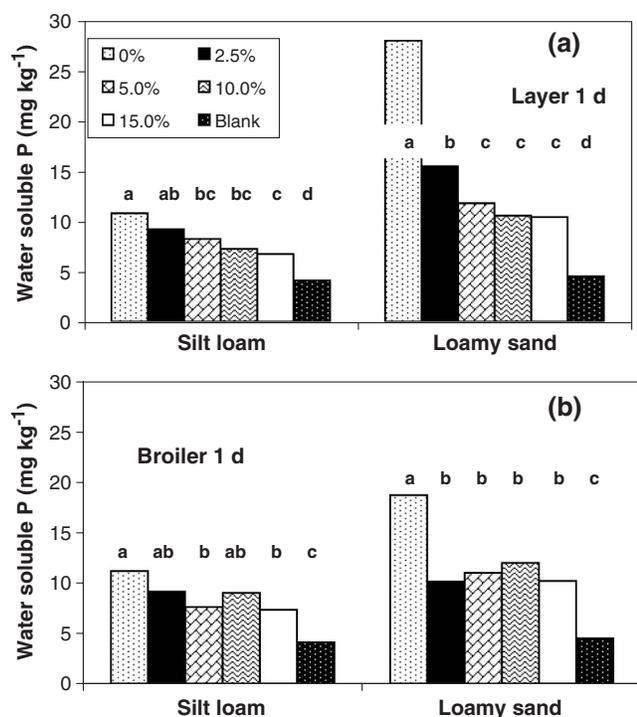


Fig. 3. Water-soluble P in the silt loam and loamy sand 1 d after amendment with (a) layer manure and (b) broiler litter treated with different rates of quicklime (CaO). Different letters indicate significant differences at the 0.05 probability level.

that all rates of CaO reduced the solubility of P not only in layer manure and broiler litter, but also in manure-amended soils. There was a clear trend for decreasing WSP in soils with increasing rate of CaO for the layer manure, but this trend was not as clear for the broiler litter. As for WSP in the wastes, the greatest reduction in WSP in soils per unit application of CaO was for the initial application of 2.5% CaO. There was a decreasing return in terms of reducing soil WSP with further applications of CaO to the waste. For example, for the loamy sand an addition of 2.5% CaO reduced the WSP in the soil from 28.0 to 15.5 mg kg<sup>-1</sup>, a reduction of 12.5 mg kg<sup>-1</sup>. However, addition of 15% CaO only led to a further reduction of 5.1 mg kg<sup>-1</sup> in soil WSP compared

Table 6. Effect of quicklime application to manures on Mehlich-3 P in amended soils at Day 29 of the treatment.

Lime rate	Layer manure	Broiler litter
	mg kg <sup>-1</sup>	
	<b>Herndon silt loam</b>	
0	153a†	135b
2.5	146a	131b
5	154a	140b
10	153a	141b
15	156a	138b
Unamended soil	128b	128b
	<b>Norfolk loamy sand</b>	
0	166a	171a
2.5	151ab	148b
5	137b	134b
10	143b	148b
15	139b	150b
Unamended soil	109c	109c

† For each soil, means within one column followed by different letters are significantly different at the 0.05 probability level.

to the 2.5% rate (Fig. 1a). The increase in WSP in the silt loam following addition of the limed manure or litter was not as great as that in the loamy sand. For example the unamended layer manure increased WSP by 23.5 mg kg<sup>-1</sup> in the loamy sand, but only by 6.7 mg kg<sup>-1</sup> in the silt loam. This would be expected, as finer textured soils generally have greater clay and Al and Fe contents that retain P in soils and therefore buffer changes in soil WSP (Maguire et al., 2002; Moughli et al., 1993).

The WSP in all soils amended with limed or unlimed layer manure or broiler litter decreased between 1 and 29 d (Fig. 3 and 4). Other researchers have also observed decreases in soil WSP following amendment with animal manures or inorganic fertilizer. For example, van Riemsdijk et al. (1984) attributed this decrease in P availability with time to the diffusion of P into the interior of metal (Al and Fe) oxide particles, where they became harder to extract.

The WSP at 29 d in the unamended silt loam was similar to WSP in soils to which limed and unlimed layer manure and broiler litter had been added. However, the WSP in the loamy sand consistently and significantly ( $P < 0.05$ ) followed the trend unamended manure or litter > all limed manures and litters > unamended soil (Fig. 2). This was probably due to the ability of finer textured soils to buffer changes in soluble P (Maguire et al., 2002; Moughli et al., 1993). Unlike 1 d where greater CaO rates tended to lead to larger decreases in soil WSP, all lime rates led to similar reductions in soil WSP by 29 d. The ability of liming manures to reduce the WSP at 1 d should have a large impact on P losses in

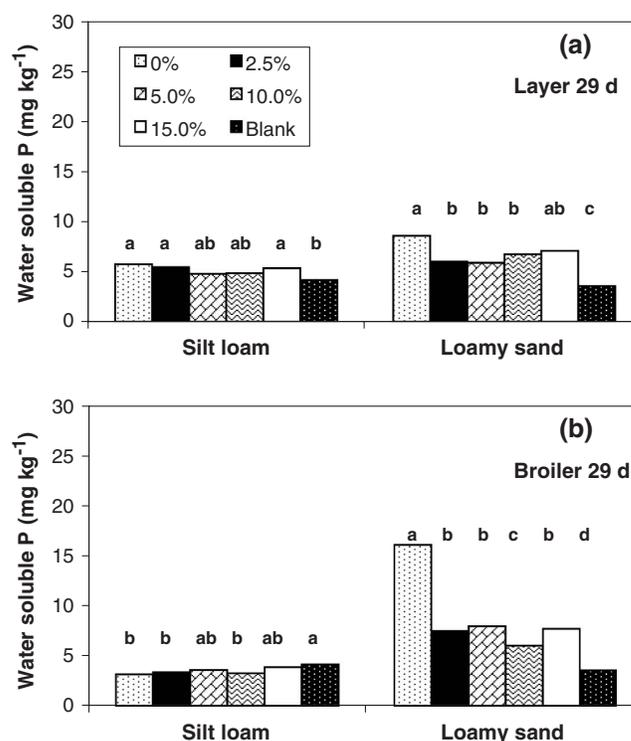


Fig. 4. Water-soluble P in the silt loam and loamy sand 29 d after amendment with (a) layer manure and (b) broiler litter treated with different rates of quicklime (CaO). Different letters indicate significant differences at the 0.05 probability level.

runoff, even though the impact was not as great by 29 d, because soluble P losses in runoff are generally greatest immediately following application of a manure to a soil (Maguire et al., 2005).

### Impact of Liming Wastes on Mehlich 3-Extractable Phosphorus in Amended Soils

Application of limed or unlimed layer manure or broiler litter increased the Mehlich-3 P measured at 29 d relative to the unamended soils and this was significant ( $P < 0.05$ ) in all cases except for broiler litter addition to the silt loam (Table 6). Limed manures did not significantly impact Mehlich-3 P in the silt loam, but liming did significantly decrease Mehlich-3 P in the loamy sand. As for WSP, increases in Mehlich-3 P following addition of limed or unlimed manure or litter were greater for the loamy sand than the silt loam. The rate of CaO addition to the layer manure and broiler litter had no significant impact on Mehlich-3 P in either the silt loam or the loamy sand soil at 29 d. The Mehlich extract is acidic and would therefore be expected to extract some of the calcium phosphates formed by the liming of the layer manure and broiler litter (Mehlich, 1984). However, some of the P added to these soils would almost certainly be sorbed by Al and Fe hydroxides present in the soils, especially if the P were in a soluble form such as WSP. Mehlich 3 extracts P associated with Al and Fe as well as the calcium phosphates in the limed wastes (Mehlich, 1984; Sims et al., 2002), thus masking the effect of reduced solubility due to liming the layer manure and broiler litter.

### CONCLUSIONS

As regulations are developed to control pathogens in manures and P losses from manure-amended soils, the development of processes to kill pathogens and reduce the solubility of P will become crucial for the economic survival of intensive animal production. Our results suggest that lime treatment can successfully reduce bacterial populations and greatly reduce P solubility in layer manure and broiler litter. When comparing initial solids content and different rates and types of lime, the efficiency of the process varies depending on the method of evaluation. After measuring bacterial population reductions, pH and WSP in the limed manures and pH and WSP in soils following application of the limed manures, a 10% CaO rate with manures at 20% solids seemed most efficient. Beyond this rate of CaO, there was little benefit in terms of further reductions in bacterial populations and WSP in the limed manures. Soil pH and WSP would also not benefit from further additions of CaO making greater rates of CaO economically wasteful. From phosphorus XANES spectra, we concluded that adding Ca and raising the pH through lime treatment of layer manure samples increased the proportion and crystallinity of hydroxyapatite, which contributed to the decreased water solubility of P in the limed samples. Hydroxyapatite would likely dissolve over time after amending acid soils with a limed layer

manure. The 20% solids was the highest solids content tested, and it was easier and less bulky to handle. CaO was preferred over  $\text{Ca}(\text{OH})_2$ , because it too was less bulky and lower rates were necessary to achieve the same goals.

If regulations to cover pathogen transport come into force as expected, this liming procedure for manures could foreseeably meet these regulations and the liming value of the product could make off-farm transport economically viable. Overall the liming process was able to reduce WSP in the manures by more than 90%, which should greatly reduce concerns about P losses in runoff following land application of these materials. However, the calcium phosphates formed in the process would be expected to become soluble over time following application to acidic soils, and therefore care must still be taken to try to achieve a long-term balance between P inputs and removal from agricultural systems.

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