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## Neutral Ammonium Citrate Extraction of Biosolids Phosphorus

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**Abstract:** Matching biosolids application rates to crop phosphorus (P) needs requires quantifying the P fertilizer replacement value of biosolids. Neutral ammonium citrate (NAC) extraction of P, used for assessing available P in mineral fertilizers, was evaluated for 35 different biosolids. Biosolids NAC-P was not statistically different ( $p = 0.05$ ) from total P using strong acid digestion (EPA 3051-P). High P recovery by NAC was attributed to dissolution of P-containing iron (Fe)/aluminum (Al) oxides under the aggressive extracting conditions (0.88 M citrate at 65°C). Citrate effectively dissolves P-binding Fe/Al hydrous oxides, the very components that reduce phytoavailability when biosolids are land applied. Greenhouse studies with pasture grass (*Paspalum notatum* Flugge) grown in P-deficient soils amended with biosolids revealed P phytoavailability was not correlated ( $r^2 = 0.10$ ) with biosolids NAC-P. Phytoavailability was inversely correlated ( $r^2 = 0.66$ ) with biosolids total Al + Fe content. The NAC extraction, designed for commercial fertilizers, is inappropriate for quantifying biosolids phytoavailable P.

**Keywords:** Biosolids, phosphorus, phytoavailability, neutral ammonium citrate

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## INTRODUCTION

Nearly 7 million dry tons of biosolids were produced in the United States in 1998 (USEPA 1999). Biosolids represent a substantial resource of useable organic matter and nutrients, and land application is the most common beneficial use method. Application rates are normally determined by the nitrogen (N) requirements of the vegetation at the site. However, the nutrient (N:P) ratio in biosolids is normally much narrower than commercial crop requirements. If applied to meet crop N needs, biosolids can supply 5–10 times crop P needs. This surplus P is not normally an agronomic problem, but can it promote accelerated eutrophication in P-limited waterbodies if transported off the application site.

Accurate nutrient management planning, implemented to reduce P losses from fields, requires accounting for the differences in phytoavailability of P in various biosolids. The USEPA process design manual (USEPA 1995) suggests a 50% relative effectiveness of biosolids P compared with fertilizer P, and Ontario regulations (OME 1996) recommend a value of 40%. Research suggests, however, that P phytoavailability ranges from nearly 0% up to 100% of the total P (de Haan 1981; Häni et al. 1981; McCoy et al. 1986; Frossard et al. 1996; Wen et al. 1997; Smith et al. 2002; O'Connor et al. 2004). Many of these studies found biosolids-P phytoavailability depended on wastewater treatment processes, which influence the forms and solubility of P in the biosolids. Use of a single value for all biosolids could result in sub-optimal soil P levels for plant growth *or* excess labile P that may adversely impact water quality.

The use of relative effectiveness factors for biosolids P (USEPA 1995; OME 1996) tacitly acknowledges that not all P in biosolids is phytoavailable. There are, however, few published investigations aimed at identifying a suitable biosolids extractant for predicting a priori the plant-available P of land-applied biosolids. This is, in part, because of uncertainties about P release behavior and the P retention characteristics of the amended soil. With the legislative trend toward P-based nutrient management, some reliable measure of P phytoavailability will be needed.

States have existing protocols for verifying the P content of mineral P fertilizers. In Pennsylvania, for example, the neutral ammonium citrate (NAC) extraction (AOAC 1995) is the official method for determining guaranteed “available” P (i.e., P available for plant uptake) in commercial fertilizers. Some states, such as Maine (MEDEP 1999), require that plant-available P be determined for land-applied biosolids. However, the suitability of tests designed for mineral fertilizers has not been systematically evaluated for biosolids. Thus, the purpose of this study was to determine the P extraction efficiency of NAC for biosolids generated from a variety of treatment processes. Greenhouse phytoavailability data (O'Connor et al. 2004) were collected for a subpopulation of the biosolids tested, allowing correlation of biosolids NAC-P to plant P uptake. The appropriateness of the NAC-P for calculating biosolids application rates under P-based nutrient management is addressed.

## MATERIALS AND METHODS

### Biosolids Materials

Thirty-five biosolids, originating from facilities with flow rates of less than  $380 \text{ m}^3 \text{ d}^{-1}$  to greater than  $7.6 \times 10^5 \text{ m}^3 \text{ d}^{-1}$  ( $<0.1$  to  $>200$  million gallons per day), were collected from Pennsylvania, New York, Maryland, and Florida (Table 1). Samples were chosen to include materials from a wide variety of biosolids treatment processes: aerobic digestion, anaerobic digestion, lime stabilization, lime post treatment, composting, autothermal thermophilic aerobic digestion (ATAD), heat drying and pelletization. Both chemical precipitation (Al and Fe salt addition) and biological P removal (BPR) processes were represented in the facilities surveyed because these processes have been shown to impact the environmental availability of biosolids P (Kyle and McClintock 1995; Penn and Sims 2002; Elliott et al. 2002). Biosolids were analyzed for total elemental content by inductively coupled plasma atomic emission spectroscopy (ICP) following USEPA Method 3051, a microwave-assisted, strong acid digestion technique (USEPA 1994). Percent solids and pH were determined by using standard methods (Page 1982).

### NAC Extraction Procedure

The NAC extraction used was based on fertilizer methods 960.03 and 960.63 of the Association of Official Analytical Chemists (AOAC 1995). The extracting solution was prepared by dissolving 370 g of crystalline citric acid into 1500 mL of distilled, deionized (DI) water. This solution was mixed while adding 345 mL of concentrated (28–29%) ammonium hydroxide. The solution was cooled to room temperature. Then the pH was adjusted to 7.0 by using either the citric acid or  $\text{NH}_4\text{OH}$ . The specific gravity was adjusted to 1.09 at  $20^\circ\text{C}$  by adding DI water. The resulting NAC concentration was 0.88 M.

Because of the heterogeneity of some biosolids materials, four replicates were extracted for each biosolids sample. One gram (dry weight equivalent) of the sample was weighed onto a dry Whatman No. 4 filter paper. This was placed into a 250-mL Erlenmeyer flask containing 100 mL of NAC solution preheated to  $65^\circ\text{C}$  in a shaker bath. The flask was sealed and handshaken vigorously for 15 s to disintegrate the filter paper and disperse the sample. The flask was then placed in the shaker bath for 1 h at  $65^\circ\text{C}$ . After removal from the bath, the sample pH was recorded, and the sample was transferred to a 500-mL plastic bottle and diluted to 500 mL with DI water (preheated to  $65^\circ\text{C}$ ). The official procedure of filtration through Whatman No. 5 paper proved unacceptable because of clogging by the biosolids samples. Therefore, samples were centrifuged for 15 min at approximately 3250 rpm, and the centrate was then filtered through a  $0.45\text{-}\mu\text{m}$  filter. A 49.5-mL

**Table 1.** Properties of the biosolids

Biosolids	pH	Al <sub>T</sub> (% dw)	Fe <sub>T</sub> (% dw)	Ca <sub>T</sub> (% dw)	Al <sub>T</sub> + Fe <sub>T</sub> (% dw)	Al <sub>NAC</sub> + Fe <sub>NAC</sub> (% dw)	P <sub>T</sub> (g kg <sup>-1</sup> )	P <sub>NAC</sub> (g kg <sup>-1</sup> )	P <sub>NAC</sub> /P <sub>T</sub> (%)
UAJA compost	7.10	1.32	0.21	1.28	1.53	1.02	16.3	15.3	93.6
Ephrata	7.56	0.72	6.92	4.36	7.64	5.56	38.9	35.6	91.5
Reading	7.89	2.09	1.05	3.79	3.14	1.74	20.1	18.3	91.1
Bellefonte	6.74	2.19	0.74	2.28	2.93	2.17	25.4	23.4	92.1
Hanover	7.73	3.71	2.99	2.9	6.70	5.6	30.6	31.3	102.3
Valley Forge	12.18	0.81	1.23	8.13	2.04	1.14	9.9	8.54	86.3
Alcosan	12.16	1.38	2.63	10.58	4.01	2.57	10.1	10.9	107.9
Alcosan raw sludge	6.40	1.87	3.94	1.87	5.81	3.99	14.1	15.0	106.6
Altoona West	7.24	1.87	2.11	2.04	3.98	2.19	23.16	23.4	100.8
UAJA raw sludge	6.41	2.53	0.39	1.72	2.92	2.46	27.1	29.2	107.7
Altoona East	7.28	2.50	2.11	3.32	4.61	1.13	17.2	16.9	98.2
Allentown	8.12	1.21	0.94	3.47	2.15	0.96	22.2	19.8	89.2
Dover	6.22	1.60	2.03	1.93	3.63	2.28	24.9	23.7	95.5
DRWPCC	12.0	0.40	1.62	9.29	2.02	1.42	13.2	10.8	81.9
Middletown	12.47	0.55	1.00	30.00	1.55	0.99	7.96	6.14	77.0

York raw	8.01	0.90	0.98	3.21	1.88	1.19	31.8	29.7	93.5
Butler compost	7.90	0.76	0.38	12.38	1.14	0.85	3.82	4.45	116.4
University Plant	7.40	0.76	1.22	3.49	1.98	1.19	18.4	16.5	89.8
Philadelphia compost	5.90	1.31	7.54	2.33	8.85	6.9	24.3	30.0	123.3
Philadelphia cake	8.32	1.18	5.62	2.40	6.80	5.75	27.7	33.7	121.7
Baltimore pellets	6.84	1.88	4.99	2.10	6.87	5.21	28.8	30.2	104.9
Baltimore cake	8.51	1.68	5.07	4.53	6.75	5.79	30.5	35.7	117.0
Tarpon Springs pellets	8.71	3.39	0.96	3.60	4.35	3.44	37.7	37.1	98.4
Largo cake	7.87	1.01	0.95	2.95	1.96	1.57	37.0	39.5	106.6
Quakertown	7.10	5.80	1.31	2.45	7.11	5.63	31.0	34.1	110.0
Lancaster raw sludge	5.85	1.11	0.61	1.36	1.72	1.2	20.9	19.9	94.9
Penn Township	6.83	0.90	4.46	1.42	5.36	4.35	22.1	22.6	102.5
Columbia	10.0	3.33	0.74	18.87	4.07	2.74	40.2	34.8	86.7
Terre Hill	7.56	6.68	1.25	2.41	7.93	5.85	26.9	30.6	113.6
Syracuse pellets	7.51	1.01	5.95	4.20	6.96	3.87	24.6	19.9	81.0
Syracuse N-Viro <sup>®</sup>	11.4	0.55	1.09	72.81	1.64	1.2	4.4	5.27	119.7
Syracuse compost	7.37	1.01	5.35	3.87	6.36	4.9	23.1	24.7	107.0
Syracuse raw	7.13	1.08	7.35	4.35	8.43	6.89	28.7	29.7	103.3
Tarpon Springs N-Viro <sup>®</sup>	12.10	1.41	0.66	16.0	2.07	0.68	3.7	4.59	124.1
Largo pellets	6.42	0.69	0.61	3.06	1.30	0.72	28.7	21.6	75.2

aliquot of filtrate was combined with 0.5 mL of  $\text{HNO}_3$  (1 + 1) to give a final extract volume of 50 mL. Extracts were refrigerated until analyzed via ICP. Because the NAC procedure predates ICP analysis methods, other P analysis methods are outlined in the official protocol. However, the usefulness of ICP for P analysis in citrate extracts has been confirmed (Jones 1982; Hamalová et al. 1997). For procedure validation, we determined the available  $\text{P}_2\text{O}_5$  in three fertilizers [triple superphosphate (TSP), diammonium phosphate, and monoammonium phosphate] from the Magruder Check Sample Program obtained from the Pennsylvania Department of Agriculture. For all samples, available  $\text{P}_2\text{O}_5$  was within approximately 7% of the reported mean values (Womer 2001). The Al and Fe contents in the NAC extract solutions were also determined by ICP.

### Greenhouse Experiments

Greenhouse studies were conducted to correlate the amount of P extracted (NAC-P and other P tests) with the amount of P taken up by the plant (O'Connor et al. 2004). Seven of the survey biosolids were mixed with surface horizons of two acid, P-deficient, low organic Florida sands (Candler soil and Immokalee soil). Immokalee soil is a sandy, siliceous, hyperthermic Arenic Alaquods, and Candler soil is a sandy, siliceous, uncoated, hyperthermic Typic Quartzisamments. Masses of biosolids necessary to provide the equivalent of  $56 \text{ kg P ha}^{-1}$  and  $224 \text{ kg P ha}^{-1}$  were applied on the basis of total P concentrations of each P-source. Treated soils were packed to a bulk density of  $1.3 \text{ Mg m}^{-3}$  in columns made of PVC pipe, 15-cm diameter and 45-cm length. Variable N additions were made to equalize N across treatments, and blanket additions to each column of K, Mg, and S were also made. Bahiagrass (*Paspalum notatum* Flugge) seed was sown on each column and covered with about 3 mm of sand of negligible P retention capacity. The greenhouse experiment continued for 4 months with harvesting at 1-month intervals. Phosphorus uptake by bahiagrass in individual harvests was combined and regressed against P applied to assess the plant-available P (PAP) in biosolids relative to triple superphosphate (TSP). For details on the greenhouse study, consult O'Connor et al. (2004).

## RESULTS AND DISCUSSION

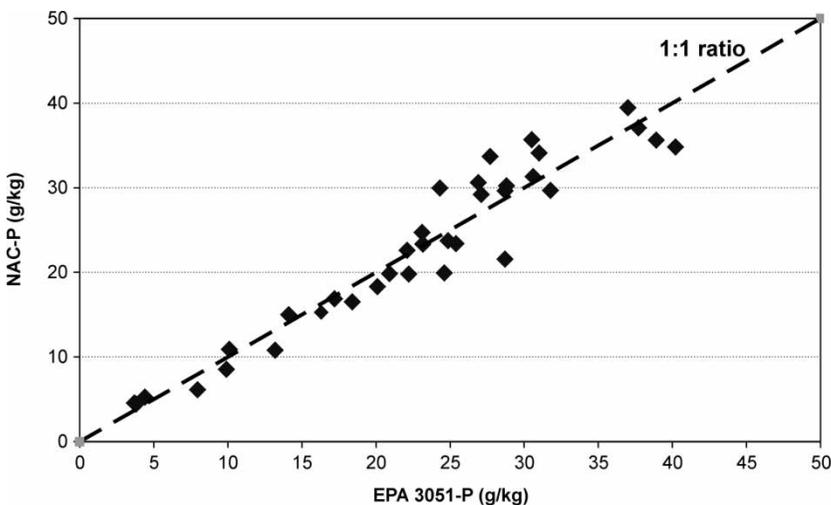
### NAC-Extractable Phosphorus in Biosolids

Table 1 shows selected properties of the biosolids tested. Total concentrations of major elements (Al, Fe, and Ca) were representative of biosolids produced nationally and reflected individual wastewater and sludge treatment processing. Thus,  $\text{Fe}_T$  and  $\text{Al}_T$  were generally less than  $10 \text{ g kg}^{-1}$ , unless

chemicals were added to the waste stream, [e.g., Fe (Baltimore) or Al (Quaker town) for P removal and Philadelphia where Fe-containing water treatment residuals (WTRs) are discharged to the sanitary sewers]. High  $\text{Ca}_T$  content reflects lime stabilization (Alcosan) or N-Viro processing (Syracuse). These biosolids also had markedly higher pH. Total P levels (average  $P_T = 22.6 \text{ g kg}^{-1}$ ) were typical of biosolids produced nationally (USEPA 1995), with higher levels exhibited by facilities using BPR (Ephrata, Largo cake) and lower values reflecting dilution by addition of alkaline materials for stabilization (Valley Forge, Syracuse N-Viro<sup>®</sup>).

The NAC-P values determined for the biosolids are also shown in Table 1. The values range from approximately 75% to 124% of the  $P_T$  as determined by EPA 3051 (EPA 3051-P). Notably, the mean NAC-P of all 35 biosolids ( $22.7 \text{ g kg}^{-1}$ ) was nearly identical to the mean  $P_T$  ( $22.6 \text{ g kg}^{-1}$ ) as determined by EPA 3051-P. Figure 1 shows the NAC-extractable P with respect to the total P content as determined by EPA 3051-P digestion. Comparison with the 1:1 slope line shows the NAC-P gives a good indication of total P content of biosolids. A paired *t*-test ( $p = 0.05$ ) also indicated NAC-P was not statistically different from 3051-P.

That NAC-P was comparable with and, for some samples, even exceeded “total P” by EPA 3051 digestion for some biosolids warrants comment. It is surmised that this behavior stems from the ability of polycarboxylic ligands such as citrate to dissolve hydrous oxide surfaces, which play a key role in immobilizing P in biosolids and other matrices such as soils and sediments. Van der Zee and Riemsdijk (1988) found that oxalate-extractable P was, on average, 93% of total P (determined by X-ray fluorescence) for soils both



**Figure 1.** NAC-extractable P content of biosolids as a function of total P measured by EPA 3051 digestion.

heavily manured and low in P. Other investigators (Maguire et al. 2001) have similarly reported oxalate-extractable P values that exceeded total P (EPA 3051) for biosolids. Except for high Ca biosolids, inorganic P is dominantly in Fe- and Al-associated forms (Elliott et al. 2002). Citrate is well known for its ability to attack Al and Fe oxides via ligand-promoted dissolution (Stumm and Morgan 1996). The rate of oxide dissolution is related to the number of carboxylate groups on the extractant molecule and is maximal for extractants with three carboxylate groups like citrate (Blesa et al. 1994). Even for the biosolids with the highest Fe content (Philadelphia compost), the citrate:Fe molar ratio was approximately 65, suggesting adequate citrate in the NAC extractant to form the surface and solution complexes that drive the ligand-promoted dissolution process.

Analysis of Al + Fe in the NAC extracting solution (Table 1) supported the notion that citrate was reasonably effective in dissolving P-binding hydrous oxides. The sum of Al + Fe dissolved by NAC was highly correlated ( $r^2 = 0.92$ ) with the "total" Al + Fe released by EPA 3051 digestion. On average, the NAC procedure extracted approximately 68% of the Al + Fe extracted in the EPA 3051 procedure. Differences between 3051-P and NAC-P for individual biosolids may be linked to the composition and crystallinity of P-binding solids phases and their differential kinetics of acid-promoted (EPA 3051) vs. ligand-promoted (NAC) dissolution. For example, the two methods may differ in their ability to release P associated with silicate or crystalline oxide phases. Although the NAC solution could not extract as much of the hydrous oxides from the biosolids, it apparently was effective in solubilizing surface oxide coatings and X-ray amorphous forms, which likely constitute most of the P-binding capacity of the total hydrous oxide pool.

The consistently high P extraction efficiency of the NAC procedure does not correspond with that of published growth experiments designed to assess biosolids-P plant availability. Reported biosolids-P bioavailability to plants ranges from less than 10% to approximately 100% compared with soluble fertilizer P (de Haan 1981; Gestring and Jarrell 1982; McCoy et al. 1986; O'Connor et al. 2004). A number of studies attribute low bioavailability of biosolids-P to chemical additions during sewage treatment. Wen et al. (1997) reported that application of composted sludge did not increase plant P uptake because Al and Fe were added during treatment. McCoy et al. (1986) found that for sludges treated with ferric chloride, plant uptake of P was 4% of the uptake of P from monocalcium phosphate (MCP) and 0% of MCP uptake for a sludge treated with both Fe and Al sulfates. O'Connor et al. (2004) reported significantly lower P availability to pasture grass when heat-dried biosolids contained high total Al + Fe. In the present study, biosolids NAC-P levels were not statistically different from EPA 3051-P. Based on data from the present study and the literature, NAC extraction appears to overestimate plant-available P for some types of biosolids.

### Greenhouse Experiments

Although comparison of extraction results with literature studies is useful, actual plant uptake studies are superior in gauging the ability of NAC-P to estimate the P fertilizer value of biosolids products. Concurrent with the laboratory studies, uptake studies (O'Connor et al. 2004) were conducted by using seven of the biosolids materials (Table 2). Bahiagrass was grown on two P-deficient soils of differing P-holding capacities (Candler and Immokalee soils). Statistical analysis of regressions of plant uptake against total P applied for the various P forms allowed relative P phytoavailability determinations. Figure 2 (solid line) shows that the percent PAP was poorly correlated ( $r^2 = 0.10$ ) with the NAC-extractable P of the biosolids-P source.

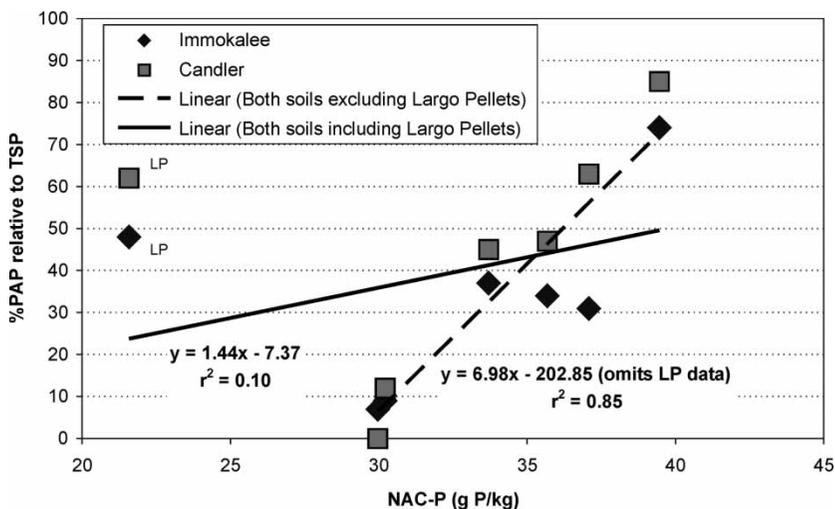
Previous studies have led to the conclusion that bioavailability of biosolids-P is influenced by treatment method and the levels of other elements (notably Fe and Al) in the biosolids. Several investigations have documented that biosolids-P phytoavailability is lower for biosolids containing elevated Al and Fe content (de Haan 1981; Häni et al. 1981; Corey 1992; Frossard et al. 1996; Wen et al. 1997). This conclusion is reinforced in Figure 3 where the %PAP is regressed against the total Al + Fe content of the biosolids. The %PAP for the biosolids was inversely correlated ( $r^2 = 0.66$ ) with total Al + Fe (EPA 3051) content.

Thus, any extraction method that purports to quantify the agronomically available P of a fertilizer material should extract incrementally smaller quantities of P as the content of Al + Fe in the material increases. Figure 4 shows that there

**Table 2.** Plant uptake results of P sources

Source	Treatment	pH	NAC-P (g P kg <sup>-1</sup> )	% PAP <sup>a</sup> Immokalee	% PAP <sup>a</sup> Candler
Philadelphia compost	Activated sludge, codisposal of WTRs	5.90	30.0	7	—
Philadelphia cake	Activated sludge, codisposal of water treatment residuals	8.32	33.7	37	42
Baltimore pellets	Activated sludge, Fe addition	6.84	30.2	9	12
Baltimore cake	Activated sludge, Fe addition	8.51	35.7	34	47
Tarpon Springs pellets	BPR plus alum addition	8.71	37.1	31	63
Largo cake	BPR	7.87	39.5	74	85
Largo pellets	BPR	6.42	21.6	48	62

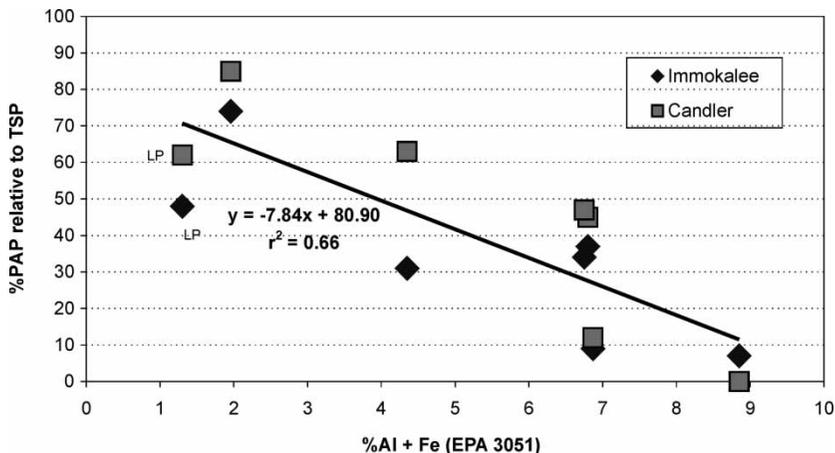
<sup>a</sup>Percent plant available P relative to TSP fertilizer treatment.



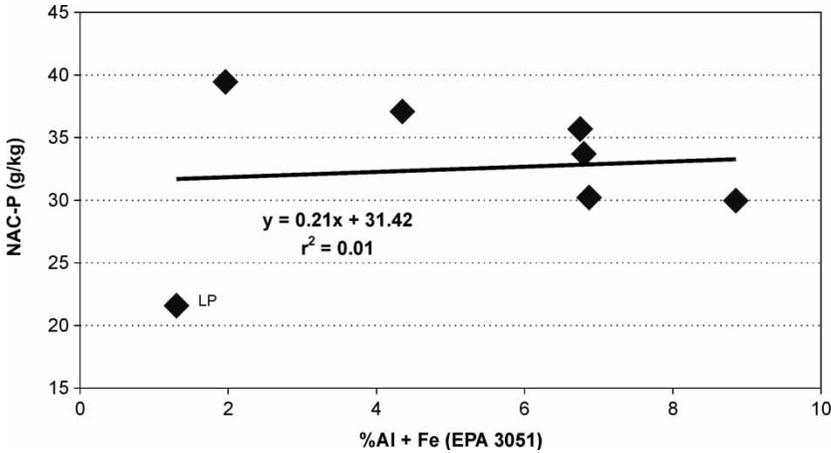
**Figure 2.** Relative phytoavailability of seven biosolids as a function of the biosolids NAC-P, with and without consideration of LP data.

was no correlation ( $r^2 = 0.01$ ) between NAC-P and the Al + Fe content (by EPA 3051) of the seven biosolids studied in the greenhouse. For NAC-P to be a reliable measure of bioavailable P, it should be inversely correlated with Al + Fe content (Figure 3). Because NAC approximates total P (Figure 1), the extractant tends to mask differences in P lability of various biosolids.

Inspection of Figures 2 and 4 suggests that the data from one P source [Largo pellets (LP)] exerts a disproportionate influence on the data regression



**Figure 3.** Relative phytoavailability of biosolids as a function of biosolids total Al + Fe contents.



**Figure 4.** Biosolids NAC-P as a function of biosolids total Al + Fe content.

coefficients. Although there is no scientific reason for considering the Largo pellets a unique material in the present context (e.g., see labeled data in Figure 3), deletion of the LP points dramatically improves the regression coefficients of Figures 2 and 4. For example, consider the dashed line in Figure 2 ( $r^2 = 0.85$ ). This regression implies that biosolids materials have no plant-available P unless NAC-P is greater than  $30 \text{ g kg}^{-1}$  and that when NAC-P is more than approximately  $40 \text{ g kg}^{-1}$ , essentially 100% of P is phytoavailable. Such behavior has no theoretical justification and implies, contrary to research findings (O'Connor et al. 2004), that greater than half of the biosolids surveyed have *no* P replacement value (see Table 1 and Figure 1). Moreover, the steepness of the response curve has little practical usefulness in determining agronomic biosolids application rates based on the NAC extraction. Small changes in NAC-P would translate into large changes in P phytoavailability and, in turn, biosolids loading rates.

In the NAC test, citrate effectively dissolved a significant fraction of the P-binding Fe/Al hydrous oxides, the very components that reduce phytoavailability when biosolids are land applied. Thus, the NAC extraction is too aggressive to mimic the P-extracting properties of plant roots; some weaker extracting solution may be more appropriate. Weaker extraction methods (Kang 2003) were better correlated with percent phytoavailability [water-soluble P ( $r^2 = 0.67$ ), Mehlich-P ( $r^2 = 0.51$ ), and 2% citric acid ( $r^2 = 0.48$ )]. Sarkar and O'Connor (2001) reported the Fe-impregnated paper strip method was reasonably correlated ( $r^2 = 0.64\text{--}0.70$ ) with available P in soils amended with biosolids. The selection of a reliable test for predicting biosolids-P bioavailability is outside the present scope, but it is clear that other extraction methods deserve investigation as predictors of the plant availability of P in land-recycled biosolids.

## CONCLUSIONS

The plant-available fraction of biosolids P is reported to be highly variable and often “much lower than that of equivalent amounts of P fertilizer” (Pastene 1981). Regulating land application under P-based nutrient management requires accounting for differences in the P phytoavailability of various biosolids. Neutral ammonium citrate (NAC) extraction of P, widely used for quantifying P content of marketed phosphate fertilizers, effects near complete release of P as measured by EPA 3051 digestion. Both methods appear to effectively dissolve noncrystalline Al/Fe oxides and control P sorption in biosolids. Data from greenhouse studies confirm the inability of NAC extraction to reliably predict biosolids-P uptake by plants. Better predictors of plant uptake are needed before P nutrient management planning is mandated for agricultural use of biosolids.

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