Chemosphere 78 (2010) 13–21

Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

# Estimation of phosphorus content and dynamics during composting: Use of near infrared spectroscopy

L. Galvez-Sola [a], J. Morales [b], A.M. Mayoral [b], F.C. Marhuenda-Egea [c], E. Martinez-Sabater [c], M.D. Perez-Murcia [a], M.A. Bustamante [a], C. Paredes [a], R. Moral [a,*]

[a] Department of Agrochemistry and Environment, Miguel Hernandez University, EPS-Orihuela, Orihuela (Alicante), Spain
[b] Applied Statistical Unit, Operations Research Center, Miguel Hernandez University, Elche (Alicante), Spain
[c] Department of Agrochemistry and Biochemistry, University of Alicante, Alicante, Spain

ARTICLE INFO

*Article history:*
Received 11 June 2009
Received in revised form 24 September 2009
Accepted 24 September 2009
Available online 28 October 2009

*Keywords:*
Compost
P forms
$^{31}$P NMR spectroscopy
NIR spectroscopy
Multivariate calibration

ABSTRACT

The content and chemical forms of P in compost are essential variables for its proper management with an agricultural purpose, especially considering the increasing P over-fertilization in agrosystems. In this study, the estimation of P content and dynamics in different composting scenarios was developed using near infrared reflectance spectroscopy (NIRS) coupled with a statistical tool for calibration, a penalized signal regression. Samples were analyzed on total P and partitioned using NaOH-solution $^{31}$P NMR spectroscopy quantifying pyrophosphate, orthophosphate, orthophosphate diesters, phospholipids, and orthophosphate monoesters pools. According to the results obtained, total P content ($r^2$ = 0.99 and root mean square error of cross-validation = 0.53) and P forms can be estimated during composting using NIRS, as well as in the mature product, orthophosphate and orthophosphate monoesters being the most abundant P forms throughout the experiment. Penalized signal regression allows detecting the significant wavenumbers in each composting period, and also with the different P pools in the composting pile depending on time.



## 1. Introduction

Build-up of P in excessively fertilized soils has become an environmental concern, since accumulation of P in surface soil can result in a transfer of P to groundwater producing concentrations that exceed groundwater quality standards. Thus, several countries have implemented protective measures in order to prevent an excessive P input. Amlinger et al. (2004) recommended a maximum application per year between 50 and 68 kg $P_2O_5$ equivalent ha$^{-1}$. The main input of P in agriculture came from fertilizers, but also from manures and organic amendments. Knowledge of P partitioning, solubility and, ultimately, bioavailability are essential to consider in balancing the use of compost as a fertilizer while protecting the environment (Toor et al., 2006). The use of only nitrogen based criterion to establish the organic application dose to soils could lead to an over-fertilization of nutrients such as P and K, especially when composts with very low mineralization rates are considered. As Shober and Sims (2003) reported, 24 states of the USA have regulations or guidelines related to the restriction of land application of biosolids based on the P criterion. However, in the future, P supply could be reduced, because mining phosphorus for fertilizer is consuming the mineral faster than geologic cycles can replenish it (Déry and Anderson, 2007). In this future scenario, organic wastes and, especially compost, could constitute a strategic resource to cover P fertilization in an environmentally friendly management.

On the other hand, the organic wastes used to elaborate compost are also affected by a high variability in their composition. In Spain, variations of 25% for N and 19% for P contents in sewage sludge, respectively, were detected during the 2001–2003 period. For this reason, a complete characterization of P during composting, including content and speciation, could be useful to understand the P dynamics and thus, its bioavailability in the amended soils. Nevertheless, most of the studies about compost only include P total contents or increments between initial and final total P concentrations, without consideration of the chemical speciation of P and how it changes with time. Chemical analysis is the usual way of studying the P speciation in organic materials, NaOH–EDTA being the main extractant for organic P in compost samples according to Bowman and Moir (1993) and Turner et al. (2005). However, other instrumental techniques, such as NMR could be also used for these purposes (Toor et al., 2006). Using solution $^{31}$P NMR spectroscopy, multiple P compounds can be quantified simultaneously in compost extracts with minimal sample preparation and handling (Condron et al., 1997).

* Corresponding author. Tel.: +34 966749652; fax: +34 966749711.
*E-mail address:* raul.moral@umh.es (R. Moral).

0045-6535/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.chemosphere.2009.09.059

Recent advances in methodology for solution $^{31}$P NMR spectroscopy of alkaline soil extracts include improvements in signal identification (Makarov et al., 2002; Turner et al., 2003; Turner and Richardson, 2004) and the development of a quantitative extraction procedure (Cade-Menun and Preston, 1996). In this speciation, inorganic forms of P, such as pyrophosphate or orthophosphate, and organic P forms, such as orthophosphate diesters, phospholipids, orthophosphate monoesters could be elucidated. Therefore, organic P can be now quantified and partitioned more accurately than using the classical analytical methods. However, chemical speciation coupled with NMR analyses is time-consuming and expensive. A promising alternative could be the estimation of the P content in compost based on the use of near infrared reflectance spectroscopy (NIRS), a non-destructive analytical technique that is pollution-free and cost-effective, capable of analyzing organic substances rapidly (<1 min). NIRS is frequently applied in environmental analyses including soil organic matter (Ludwig et al., 2002; Cozzolino and Morón, 2006); soil carbon (Reeves et al., 2006); humic and fulvic acids (Polak et al., 2005); organic wastes (Malley et al., 2005; Grube et al., 2006); mineral concentration in plants (Halgerson et al., 2004) and small compounds present in animal cells, such as ammonium, glucose, or lactate (Riley and Crider, 2000). In several studies, estimations of the total content of P in an organic matrix were obtained with more or less accuracy. Malley et al. (2005) obtained a coefficient of correlation of 0.71 and a standard error of prediction of 0.70 mg g$^{-1}$ in manure compost, useful for screening, while Halgerson et al. (2004) observed a coefficient of determination of 0.91 and a standard error of cross-validation of 175 mg kg$^{-1}$ in alfalfa leaves. However, other authors have reported the use of NIRS in the estimation of P in soil with different results, as Chang et al. (2001) that obtained a regression coefficient of 0.40 and a root mean standard error of cross-validation of 32.28 mg kg$^{-1}$ and He et al. (2007), which showed a correlation coefficient between measured and predicted P of 0.47, values not suitable to use this technique for P estimation.

Several mathematical approaches have been used for modeling the signal obtained from NIRS and optimizing the output of information, such as the use of multivariate calibration. Some of the challenges posed in this framework are non-trivial and include the following aspects: (a) the number of regressors ($p$ wavenumbers) may greatly exceed training observations ($n$); (b) the regressors are highly correlated, so we have a very ill-conditioned statistical problem (Frank and Friedman, 1993). Two general approaches have been used to solve these issues: (1) reduction of the regression bases, including partial least squares, principal components, or projection onto bases of splines (Marx and Eilers, 1999) and (2) penalized regression, like ridge regression or penalized signal regression (Eilers and Marx, 2003). With smooth spectra, penalized signal regression is a very promising approach because it forces the vector of regressor coefficients to vary smoothly with wavenumber.

Therefore, the main objectives of this study were to establish the effectiveness and accuracy of NIRS in the evaluation of the P total content and P forms during composting of winery and distillery residues. For this purpose, the development of a modern mathematical approach through penalized signal regression and the use of solution $^{31}$P NMR spectroscopy procedure were also used and validated.

## 2. Materials and methods

### 2.1. Compost samples

Two hundred and four samples were used in this study from four different composting piles elaborated using several residues, but including in all the cases wastes from the winery and distillery industry. Four different piles (P1, P2, P3 and P4) were prepared with mixtures of grape stalk (GS), grape marc (GM), exhausted grape marc (EGM), sewage sludge (SS), cow manure (CM) and poultry manure (PM), using the Rutgers static pile composting system. The composition of the composting piles and the characteristics of the raw materials used are detailed in previous works (Bustamante et al., 2007, 2008b, 2009). In P1 and P2, GS, GM, EGM and SS were used. Initially, GS, GM and EGM were mixed in both piles and after 17 d, SS was added to both piles as a source of nitrogen and microorganisms. On the other hand, P3 and P4 were elaborated by mixing EGM and two different animal manures, CM and PM, respectively. The moisture of the piles was controlled weekly by adding the necessary amount of water to obtain a moisture content not less than 40%. In P1, fresh collected vinasse (V) was used for watering; 0.4 L V kg$^{-1}$ was added on the first day, and the remaining volume, up to 0.95 L kg$^{-1}$, was added gradually up to 25 d of composting. The mixtures (about 1700 kg each for P1 and P2 and 1800 kg each for P3 and P4) were composted in a pilot plant, in trapezoidal piles (1.5 m high with a 2 × 3 m base) with temperature control and supplied with forced aeration. The timer was set for 30 s ventilation every 30 min, and the ceiling temperature for continuous air blowing was 55 °C. Forced aeration was used during the bio-oxidative phase, which lasted 130 d for P1 and P2, respectively, 134 d for P3 and 157 for P4. The maximum temperature values reached by the composting piles throughout the thermophilic phase were 47 and 42 °C for P1 and P2, and 54 and 50 °C for P3 and P4, respectively (the temperature evolution of the piles is described in detail by Bustamante et al. (2007) and Bustamante et al. (2008b)). The bio-oxidative phase of composting was considered finished when the temperature of the pile was stable and near to that of the surrounding atmosphere. Then, the piles were allowed to mature for two months. Samples were obtained by mixing seven sub-samples coming from seven different zones of the piles, from the whole profile (from the top to the bottom of the pile) and they were collected approximately every 2 weeks.

### 2.2. Sample preparation and solution NMR spectroscopy

Total P content was measured colorimetrically after $HNO_3$/$HClO_4$ digestion (Kitson and Mellon, 1944) in all the samples. For P speciation, P was extracted by shaking 1 g of air-dried compost samples with 20 mL of a solution containing 0.25 M NaOH and 0.05 M $Na_2EDTA$ for 16 h at 22 °C (Cade-Menun and Preston, 1996). It is assumed that NaOH–EDTA quantitatively recovers organic P from compost samples (Bowman and Moir, 1993; Turner et al., 2005), although there is no direct method that confirms this. Extracts were centrifuged at 10 000$g$ for 30 min and an aliquot was taken for determination of total P. The remaining solution was concentrated with speedvac Eppendorf Concentrator 5301. Each concentrate was re-dissolved in 0.1 mL of deuterium oxide and 0.9 mL of a solution containing 1.0 M NaOH and 0.1 M EDTA, and then transferred to a 5 mm NMR tube. Solution $^{31}$P NMR spectra were obtained using a Bruker Avance DRX 400 MHz spectrometer operating at 121.5 MHz for $^{31}$P. Samples were analyzed using a 6.5 μs pulse (30°), a delay time of 2.0 s, an acquisition time of 0.67 s, and broadband proton decoupling. The delay time used here allows sufficient spin–lattice relaxation between scans for P compounds in NaOH–EDTA (Cade-Menun et al., 2002). Approximately 512 scans were acquired for all samples. Chemical shifts of signals were determined in ppm relative to an external standard of 85% $H_3PO_4$. The values can vary slightly among spectra due to slight differences in pH, viscosity, and ionic strength, although this is minimized by analyzing samples at a strongly alkaline pH (>13). Signals were assigned to P compounds or functional groups based on literature reports (Turner et al., 2003) and signal areas calcu-

lated by integration. Sample analyses were carried out in triplicate and the error of the NMR method determined for each constituent was lower than 5%. Spectra were plotted with a line broadening of 1 Hz to preserve resolution in the phosphate monoester region.

### 2.3. NIRS analysis

NIRS analysis was performed using a FT-NIR spectrometer (MPA, Bruker Optik GmbH, Germany) in the range of wavenumber from 12 000 to 3800 $cm^{-1}$ (equivalent to wavelength 830–2630 nm) with a step of 8 $cm^{-1}$. Compost samples were dried at 105 °C, grinded (⌀ < 1 mm) and dried again after storing. Samples were divided in two sets (2/3 for the calibration and 1/3 for the validation), the mean, range, and standard deviation of these two sample sets being very similar. About 20 g of dried sample were scanned in a glass plate. Individual sample were remixed and rescanned three times, being averaged after using Opus software (version 6, © Bruker Optik), recording absorbance, as log 1/*R*, where *R* is reflectance, for a total of 64 scans per spectrum. No outliers were found.

### 2.4. Mathematical model for P total content

A penalized signal regression procedure was used to establish a prediction model for the P concentration from the information given by the spectra, measured at different time points in four similar compost piles, similar to that proposed by Eilers and Marx (2003):

Notation of the model:

$Y_i$, $i = 1, \ldots, n$, the P concentration as measured by the analytic procedure, in every sample. Each measure is related to certain time point during the observation period.

$X = [x_{ij}]$, $i = 1, \ldots, n$ and $j = 1, \ldots, p$ is the absorbance matrix observed from the spectra, where $p$ denotes the number of wavelengths considered and $n$ the number of time points measured.

Then, the model can be formulated in terms of the expected P concentration, as:

$$E(Y_i) = \alpha_0 + \sum_{j=1}^{p} x_{ij} * \alpha_{ji}, \quad (1)$$

where $\alpha_0$ represents intercept and $\alpha_{ji}$ gives the weight of the wavenumber $w_j$ to predict P at time point $t_i$. However, this formulation encounters a conditioning problem as the number of observations $n$ is quite fewer than the number of predictors $p$. In fact, they propose to project $\alpha$ onto a known basis of smooth functions, so making use of the ordered feature of the wavenumbers.

The number of initial predictors $p$ given by the number of wavelenghts considered was reduced to the number of nodes established on the range of wavenumbers, $v_w$, and throughout the observed period of time, $v_t$. These numbers must be small enough to make viable the estimation problem, but large enough to capture the spectrum behaviour, especially on ranges where it varies a lot. The proposed modelization states that the coefficients $\alpha$ of the prediction model (1) can be written in terms of a B-spline basis conformed from wavenumbers and the time points as:

$$\alpha = \sum_{m=1}^{V_K} \sum_{l=1}^{v_t} B_m B_l' \gamma_{ml}, = \mathbf{B}\Gamma, \quad (2)$$

where $\Gamma = [\gamma_{ml}]$ is the matrix of unknown tensor product B-spline coefficients, $\gamma = \mathrm{vec}(\Gamma)$ and $\mathbf{B} = (B \otimes 1_{v_l}) \odot (1_{v_k} \otimes B')$. The symbols $\otimes$ and $\odot$ denote, respectively, Kronecker product and element-wise multiplication of matrices. Then, it can be rewritten (1) as:

$$E(Y_i) = \alpha_0 + \mathbf{XB}\alpha = \alpha_0 + \mathbf{U}\gamma, \quad (3)$$

with $\mathbf{U} = (\mathbf{XB} \otimes \mathbf{1}_{v_l}) \odot (\mathbf{1}_{v_k} \otimes \mathbf{B}')$ and dim $\mathbf{U} = n \times v_k v_l$. If $v_k$ and $v_l$ take small values (generally less than 60) the estimation problem reduces to a multiple linear regression one, where the number of observations n is greater than the number of coefficients to estimate, $1 + v_k v_l$.

Finally, an optimal solution for $v_k$ and $v_l$ was obtained by the predictive cross-validation criteria given by Eilers and Marx (2003). It consists of dividing the sample into two subsets, one used to validate the model and the other one to study its capability for prediction, always comparing the observed values to the estimated ones (in our case, P concentration).

### 2.5. Mathematical model for P forms

When considering the organic P pools in compost (P forms), measures for everyday under study were not available, but just quantified at four different time periods. So, the model proposed above it was not valid here, as data cannot be fitted by the day of analysis. Then, an individual analysis was performed at each period and for each P form.

The proposed model was quite similar to the previous one, but here the coefficients related to the absorbances were just modelled in terms of the wavenumber. Then, for every P form and time period, the model was given by:

$$E(Y_i) = \alpha_0 + \sum_{j=1}^{p} x_{ij}\alpha_{ji},$$

with

$$\alpha = \sum_{m=1}^{v_k} B_m \gamma_m = \mathbf{B}\Gamma,$$

where $\Gamma = [\gamma_m]$ is the vector of unknown of B-spline coefficients and $v_k$ is giving the same value that the optimum reached at the model fitted above for the P, in order to preserve a similar structure in the modelling process. So, we can write:

$$E(Y_i) = \alpha_0 + \mathbf{XB}\alpha = \alpha_0 + \mathbf{U}\gamma,$$

with $\mathbf{U} = \mathbf{XB}$, where $\mathbf{B}$ is a univariate (cubic) B-spline basis presented along the axis defined by the wavenumber.

The analysis has been resolved with the R package, version 2.7.0 (R Development Core Team, 2008). The functions used were programmed by Brian D. Marx and are available at http://www.stat.lsu.edu/bmarx/.

## 3. Results

### 3.1. Total P content and P partition according to $^{31}$P NMR

Total P analysis is shown in Table 1, with the range and mean value of total P contents during the composting process. A significant increase in total P concentration during the process was observed in all the piles. Compost elaborated with PM reached the

**Table 1**
Total P contents in all compost piles (dry weight basis).

| Compost pile | P range (g $kg^{-1}$ d.m.) | Mean value ± SD (g $kg^{-1}$ d.m.) |
|---|---|---|
| P1 | 0.86–5.67 | 4.16 ± 1.41 |
| P2 | 0.13–5.72 | 4.29 ± 1.70 |
| P3 | 3.19–5.81 | 4.39 ± 0.72 |
| P4 | 5.38–10.76 | 8.28 ± 1.57 |

P1 [grape stalk + grape marc + exhausted grape marc + sewage sludge + vinasse]; P2 [grape stalk + grape marc + exhausted grape marc + sewage sludge]; P3 [exhausted grape marc + cattle manure]; P4 [exhausted grape marc + poultry manure].

highest value (more than 10 g $kg^{-1}$ dry weight basis); SS and CM ingredients induced similar P concentration in mature compost. Ferrer et al. (2001) obtained total contents of P in winery composts around 7 g $kg^{-1}$ dry weight basis, especially when manures are used as co-composting material. The total P contents in the winery-distillery wastes show significant differences, wine lee and winery sludge being the wastes that showed the greatest values compared to grape stalk and grape pomace or marc (Bustamante et al., 2008a). Solution $^{31}P$ NMR spectra of compost sample extracts from the piles 2, 3 and 4 are shown in Fig. 1. Pile 1 spectrum is not included due to the high similarity with pile 2 spectrum, since the only difference between these piles was the addition of vinasse in pile 1. Orthophosphate monoesters appeared as an envelope between 4.0 and 6.0 ppm, with major signals at 5.89, 5.19, 4.93, 4.86, 4.71, 4.45 and 4.14 ppm. Well-resolved signals in this region of the spectra allowed the obtaining of detailed information on individual compounds. The latter signal probably represented *scyllo*-inositol hexakisphosphate (Turner and Richardson, 2004). *Myo*-inositol hexakisphosphate appeared as a signal at 5.89, originating from the C-2 phosphate on the inositol ring. Strong signals at 5.19 and 4.86 ppm almost certainly represented phosphatidic acid and β-glycerophosphate, respectively. These compounds are originated from the degradation of phosphatidyl choline in alkaline solution (Turner et al., 2003), so they are probably artifacts of the analytical procedure rather than being present in significant quantities in compost samples.

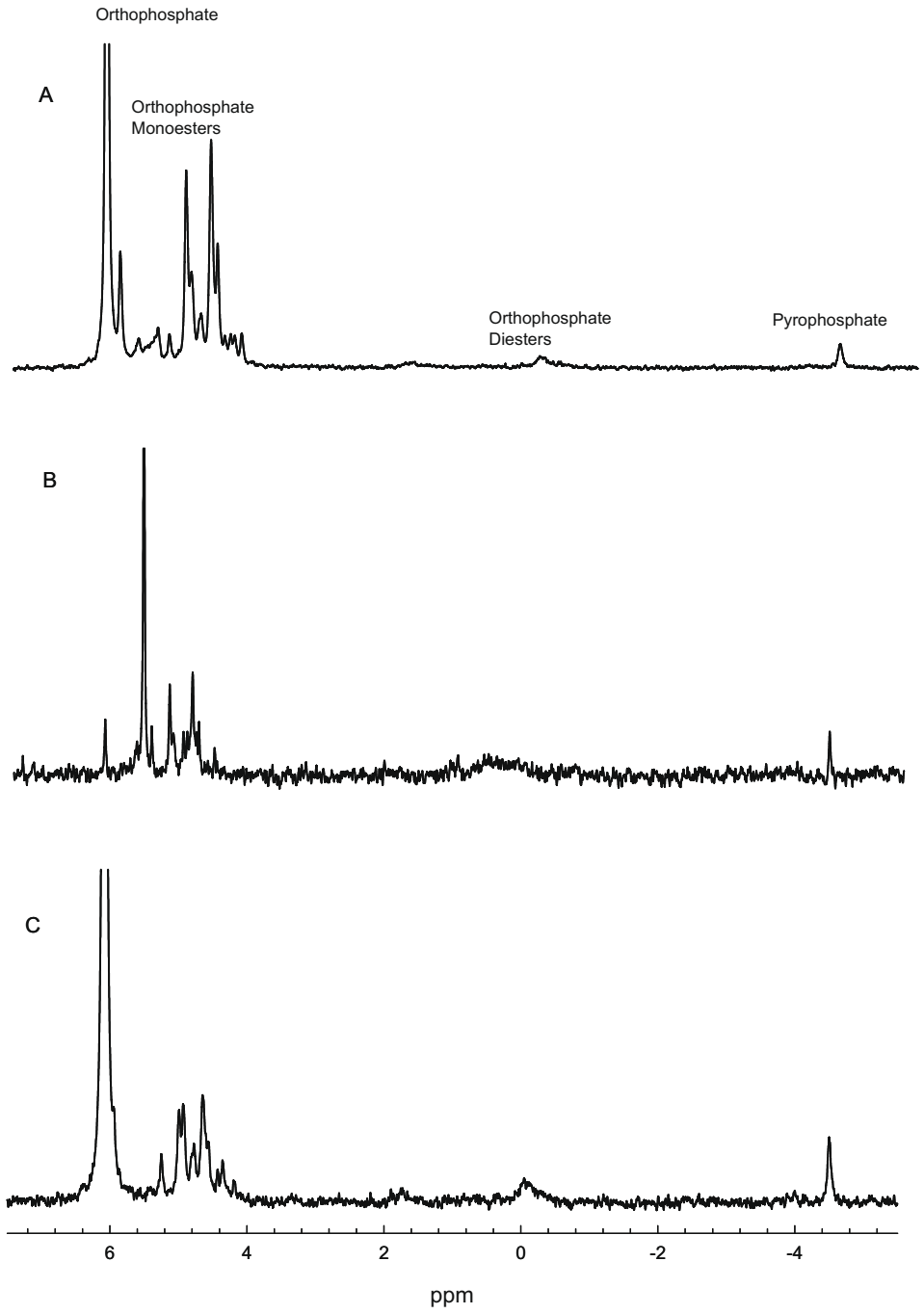


**Fig. 1.** Solution $^{31}P$ NMR spectra of NaOH–EDTA extracts of compost samples: (A) pile 4 (exhausted grape marc + poultry manure); (B) pile 2 (grape stalk + grape marc + exhausted grape marc) at 0 d; (C) pile 2 (grape stalk + grape marc + exhausted grape marc + sewage sludge) after 17 d.

Signals from inorganic orthophosphate appeared at approximately 6.08 ppm in the extracts of all compost samples. The inorganic orthophosphate was present in only small amounts, since the signal at 6.08 was weak in the spectra (Fig. 1). Pyrophosphate signals appeared at −4.46 ppm. Signals around −20 ppm, from penultimate and mid-chain polyphosphate groups from organic condensed phosphates (e.g. ADP or ATP), were absent in the spectra.

Clear signals from DNA appeared in compost sample extracts at approximately −0.2 ppm, with other signal at 1.7 ppm corresponding to phospholipids from microbes (phosphatidyl ethanolamine). As it was previously commented, the presence of strong signals in the orthophosphate monoester region from degradation products of phosphatidyl choline suggests that the phospholipids were considerably under-estimated in most of the extracts.

Table 2 shows the evolution of the P composition determined in NAOH–EDTA extracts of compost samples by solution $^{31}$P NMR spectroscopy. In all the composting procedures, inorganic P forms were dominant in mature compost. On the other hand, only in the composts with SS (piles 1 and 2), the proportion of pyrophosphate (a short chained inorganic polyphosphate) was significant in relation to the total amount of inorganic P at the beginning of the composting process. However, the proportion of pyrophosphate represented less than 1% of the total P in mature compost. Net transformation of organic P to inorganic P was observed during all the composting process. This fact was noticeable in the first stages of the thermophilic period (2–3 first week), especially for the SS and PM derived compost. In addition, a change in the ratio organic P vs. inorganic P from 9,4; 0.78 and 1.4 at the beginning of composting to 0.09; 0.18 and 0.25 at the end of maturing period was observed for pile 2, 3 and 4 respectively. About organic P form evolution, orthophosphate monoesters presented higher contents compared to orthophosphate diesters and phospholipids, the latest being the most labile in the organic pool, with negligible presence in mature compost. However, orthophosphate diesters still represents around 3% of the total organic P in matured compost elaborated with manure.

### 3.2. Mathematical model for P content

To fit the model proposed in the Section 2, 136 samples (66.6%) were used for estimation and 68 (33.3%) for predictive validation. All point times (d) used at the experiment were included in the estimation data. The range of values considered for $v_k$ was 20–60, and 5–20 for $v_l$. Optimal values obtained were $v_k = 40$ and $v_l = 14$.

Through the confidence interval estimated of the $\gamma_{ml}$ coefficients, it can be concluded on which wavenumbers are significantly related to prediction of P: those that did not contain zero. Fig. 2 shows the point estimates of these coefficients (continuous line) and their 95% confidence intervals (dotted lines). Those ranges where all lines stay above or below the zero, do actually resemble ranges of wavenumbers that significantly contribute to predict P concentration. It can be also appreciated the effect of time on these estimates. For the early phase of the experiment, confidence intervals resulted thinner than those for last phases because the observed variability on the P concentration increased with time. The model actually captures this behaviour.

The use of a penalized statistical model could be used as the best way to estimate P using NIRS due to the high significance obtained in all the time points (data not shown), because this approach can incorporate the variation in the contribution of each wavenumber range during the composting process. This fact could be also in concordance with the P forms variation detected in the previous section. Fig. 3 shows the prediction results. On the left, the predicted values of total P for the validation sample versus the point time at which measurements were made. On the right, observed values of P versus predicted ones with the model for the validation sample. Predicted and observed values are quite similar (in fact a diagonal line is appreciated) along all the time range, even at the last time points, when the variability of P increases. The correlation coefficient for observed and predicted values is 0.9937, much better than any other result published so far.

### 3.3. Mathematical model for P forms

In Figs. 4 and 5 are shown the results obtained for the different P forms. These figures also include the correlation coefficients between observed and predicted values, which are always greater or equal to 0.99, so implying that the fitted model is quite efficient for prediction. Right graphs show the wavenumber ranges that result significant at each period. We can observe clear differences on the significant ranges for the different P forms at each period, so giving support to the individualized modelling. In Table 3, partial linear square regression (PLS) modeling was compared with the proposed penalized signal regression procedure for total P and P forms in compost samples. With penalized signal regression procedure, we obtained a nearly perfect adjustment in total and the rest of P pools, and only for the Orthophosphate form, the value of the root mean square error of cross-validation was relatively high compared with the other forms. However, PLS adjustment was lower in all the parameters, especially for the P forms in compost.

**Table 2**
The chemical speciation of phosphorus over time determined in NaOH–EDTA.

| Composting time (d) | Pyrophosphate | Orthophosphate diesters | Phospholipids | Orthophosphate monoesters | Orthophosphate |
|---|---|---|---|---|---|
| *Pile 2: grape stalk + grape marc + exhausted grape marc + sewage sludge* | | | | | |
| 0 | 2.1 | 16.3 | 5.1 | 47.5 | 3.4 |
| 16 | 2.4 | 2.6 | 0.3 | 22.7 | 77.1 |
| 86 | 1.1 | 1.2 | 0.1 | 11.9 | 87.7 |
| 234 | 0.5 | >0.1 | 0.4 | 8.2 | 92.5 |
| *Pile 3: exhausted grape marc + cow manure* | | | | | |
| 0 | 3.8 | 2.8 | 0.8 | 39.7 | 50.1 |
| 29 | 1.4 | 2.7 | 1.2 | 25.9 | 56.5 |
| 91 | 1.6 | 2.0 | 2.0 | 21.9 | 63.4 |
| 192 | 0.8 | 0.6 | >0.1 | 14.5 | 87.1 |
| *Pile 4: exhausted grape marc + poultry manure* | | | | | |
| 0 | 1.5 | 2.2 | 1.1 | 54.6 | 39.1 |
| 28 | 0.5 | 1.7 | 0.3 | 26.8 | 69.6 |
| 90 | 0.2 | 0.3 | >0.1 | 21.9 | 84.8 |
| 140 | 0.5 | 1.0 | >0.1 | 19.1 | 80.3 |
| 212 | 0.4 | 0.4 | 0.2 | 15.3 | 86.6 |

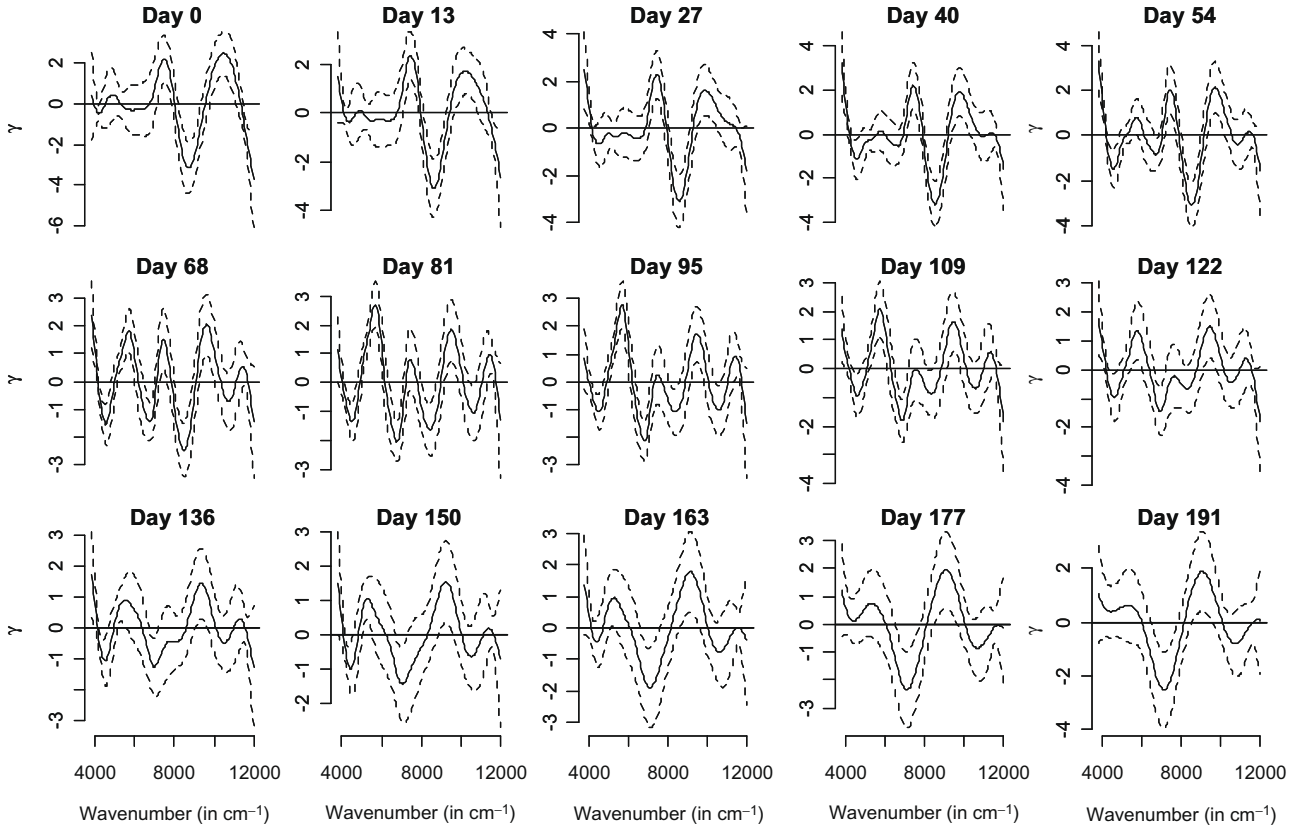


**Fig. 2.** Estimation coefficients evolution (continuous line) and 95% confidence intervals (dotted lines).

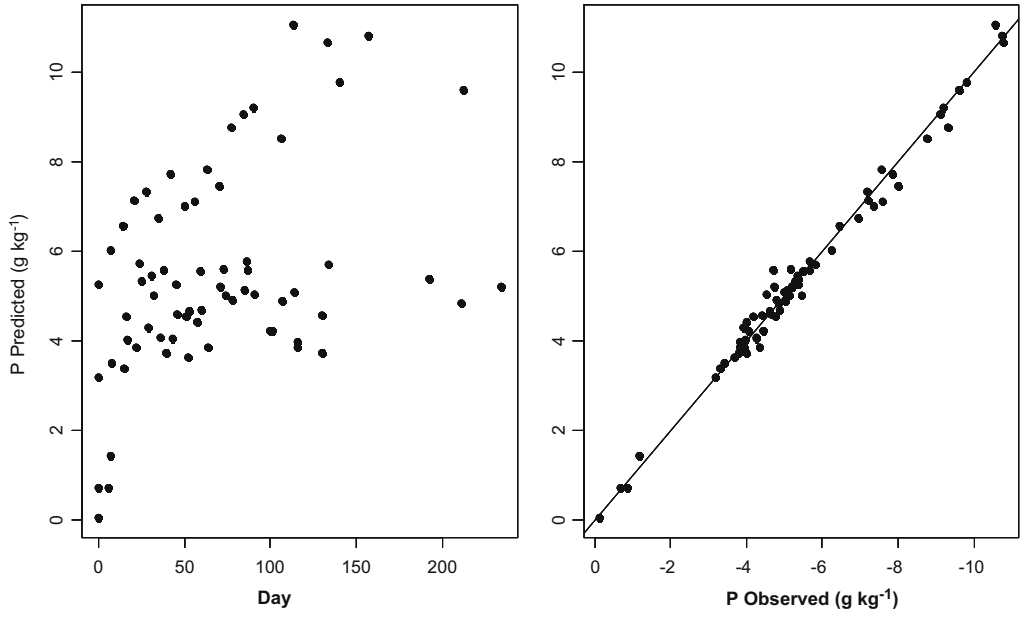


**Fig. 3.** Prediction results using penalized signal regression. On the left, the predicted values of phosphorus for the validation sample versus the point in time at which measurements were made. On the right, the observed values of P versus predicted ones with the model for the validation sample.

## 4. Discussion

P content and dynamics in compost mainly depends on the ingredient composition of the pile and the biotic degradation of organic matter. Shober and Sims (2007) reported that P solubility in organic sources can be influenced by several aspects, such as animal diet, biological treatment, composting or storage practices. Due to the nature of the P forms and their transformation into

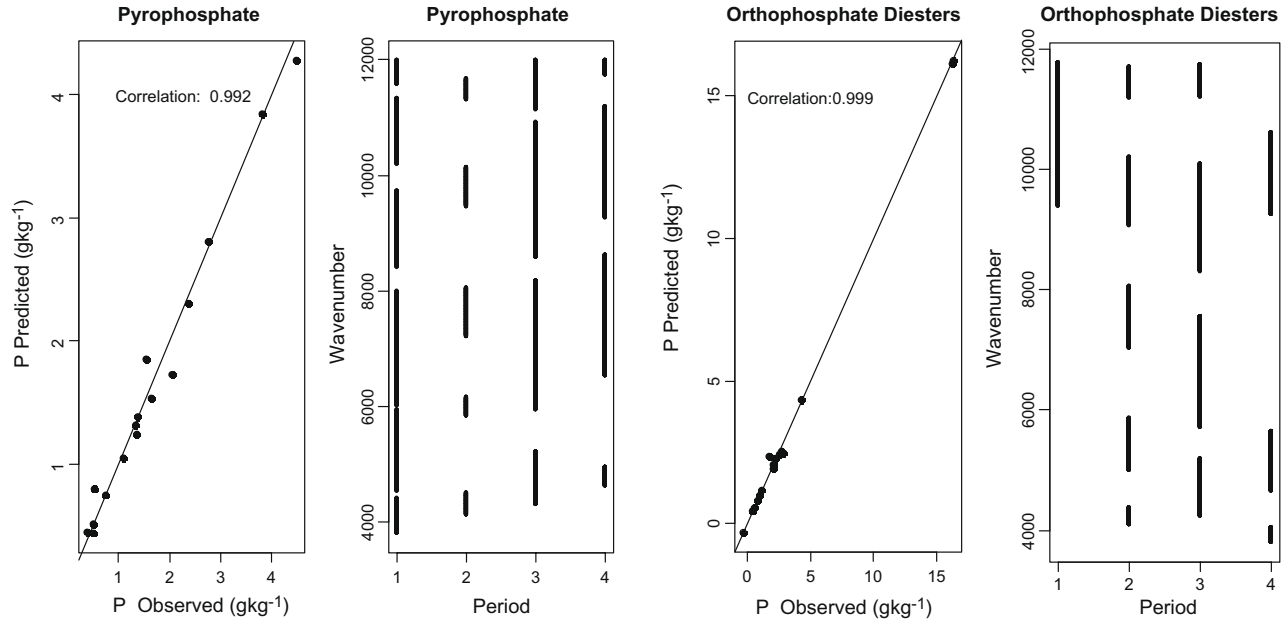


**Fig. 4.** Prediction results using penalized signal regression. Predicted values for the validation sample versus the observed values and the significant region of NIR spectra for each period for pyrophosphate (on the left) and for orthophosphate diesters (on the right).

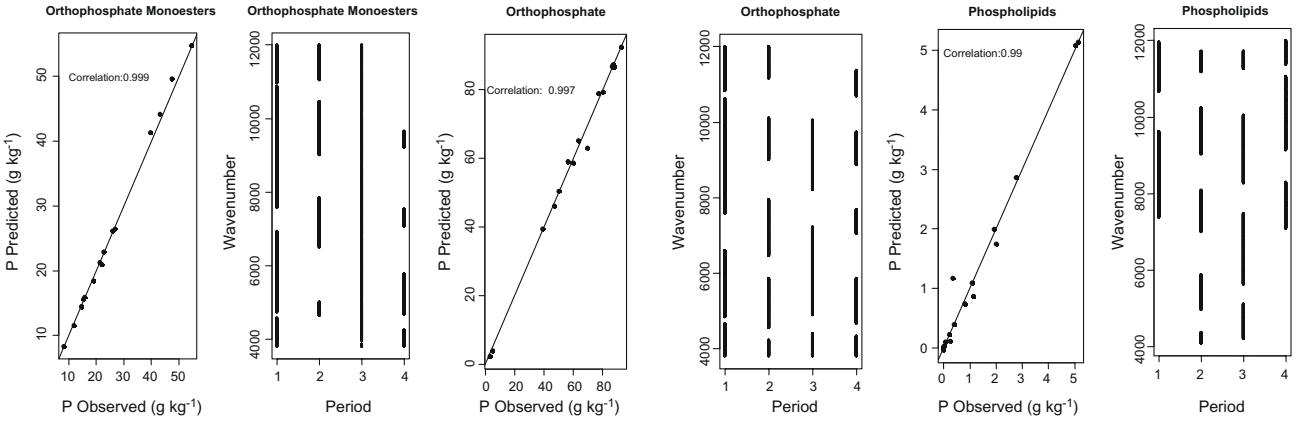


**Fig. 5.** Prediction results using penalized signal regression. Predicted values for the validation sample versus the observed values and the significant region of NIR spectra for each period for orthophosphate monoesters, orthophosphate and for phospholipids.

**Table 3**
NIRS validation results for total P and P forms in compost samples.

| Parameter | Penalized signal regression | | Partial linear square regression | |
|---|---|---|---|---|
| | $r^2$ | RMSECV | $r^2$ | RMSECV |
| P (g kg$^{-1}$) | 0.99 | 0.53 | 0.93 | 0.59 |
| Pyrophosphate | 0.98 | 0.02 | 0.79 | 0.45 |
| Orthophosphate diesters | 0.99 | 0.04 | 0.98 | 0.54 |
| Phospholipids | 0.99 | 0.06 | 0.82 | 0.59 |
| Orthophosphate monoesters | 0.99 | 0.62 | 0.88 | 4.75 |
| Orthophosphate | 0.99 | 3.97 | 0.93 | 6.47 |

P: phosphorous; $r^2$: coefficient of determination for validation; RMSECV: root mean square error of cross-validation.

the composting process, a significant increase in P total concentration during the process is usually observed (Cooperbrand and Middleton, 1996; Felton et al., 2004). The use of SS or manures, especially CM and PM could increase significantly the P content in piles based on agricultural crop wastes with relatively low values of this element compared to N and K, obtaining a balanced compost (Moral et al., 2009).

However, there are some difficulties in the interpretation of solution $^{31}$P NMR (Ajiboye et al., 2007), since this technique give us direct information about molecular and structural characterization of inorganic and organic P in NaOH extracts. It is possible to distinguish more labile organic species e.g. phospholipids from the less labile, such as phytic acid, the major orthophosphate monoester (Makarov et al., 2002). In this experiment, the most important feature was the increase of the inorganic orthophosphate from organic P forms during the compost process, ranging the inorganic P from 80% to 91.5% of the total P. Ajiboye et al. (2007) reported values of inorganic orthophosphate in biosolids and hog manure NaOH extracts around 95% and 79%, respectively. The differential behaviour of P in compost elaborated with SS or manure could be associated to the wastewater treatment and the

role of chemicals added in the treatment plants. Hinedi et al. (1989) reported that total P in anaerobically digested municipal biosolids was almost entirely orthophosphate. Huang et al. (2008) reported a different fractionation pattern of P in biosolids depending on the use of ferrous sulphate, calcium oxide and aluminium sulphate. So, the chemical forms of P in organic amendments are essential variables that must be considered prior to the use of these amendments in agriculture in order to carry out a suitable management of these materials. Ajiboye et al. (2007) observed that most of the total P analyzed in organic amendments in the sequential extracts of organic amendments was orthophosphate from readily soluble calcium and some aluminium phosphates, except in poultry samples, which was dominated by organic P.

Total P estimation using commercial NIRS mathematical approaches obtained low accuracy, especially in compost samples (Shepherd et al., 2003; Malley et al., 2005). In the majority of experiments, time evolution is not considered in the mathematical model. In this experiment, total P estimation and P form evolution throughout the compost process were very well described using penalized signal regression. In our study, penalized signal regression was more powerful in identifying significant NIR region for P estimation compared with PLS procedure. Dao et al. (2001) observed significant changes in P availability in composting procedures by addition of aluminium- and iron by-products, with a decrease of dissolved reactive P. Due to the heterogeneous nature of the wastes used in commercial composting, the proposed quick estimation could contribute to a better understanding of P dynamics in composting and therefore, improve the managing of P fertilization due to the different availability of P pools in amended soils. In addition, the monitoring of the transformation of organic P forms to orthophosphate during the composting could be a quality parameter for this biotic process.

## 5. Conclusions

The proposed combination between NIR analysis and penalized signal regression for estimate total contents and the changes in P forms during this process is a promising and easy way to improve the management of P input with agricultural purposes, especially in industrialized composting plants in order to achieve availability partners of P in organic materials. P speciation using a standardized NaOH–EDTA extraction and solution $^{31}$P NMR spectroscopy procedure increased significantly the output capabilities of prediction of the NIR-penalized signal regression.

## Acknowledgments

This work has been supported by the Ministerio de Educación y Ciencia of Spain (CICYT CTM2006-01363/TECNO and MTM2007-61554 Projects), University of Alicante (UAUSTI07-02), and Generalitat Valenciana (GVPRE/2008/208).

## References

Ajiboye, B., Akinremi, A.O., Hu, Y., Flaten, D.N., 2007. Phosphorus speciation of sequential extracts of organic amendments using nuclear magnetic resonance and X-ray absorption near-edge structure spectroscopies. J. Environ. Qual. 36, 1563–1576.

Amlinger, F., Pollak, M., Favoino, E., 2004. Heavy Metals and Organic Compounds from Wastes Used as Organic Fertilizers. EU Report ENV.A.2/etu/2001/0024, Brussels, Belgium.

Bowman, R.A., Moir, J.O., 1993. Basic EDTA as an extractant for soil organic phosphorus. Soil Sci. Soc. Am. J. 57, 1516–1518.

Bustamante, M.A., Moral, R., Paredes, C., Pérez-Espinosa, A., Moreno-Caselles, J., Pérez-Murcia, M.D., 2008a. Agrochemical characterisation of the solid by-products and residues from the winery and distillery industry. Waste Manage. 28, 372–380.

Bustamante, M.A., Paredes, C., Marhuenda-Egea, F.C., Pérez-Espinosa, A., Bernal, M.P., Moral, R., 2008b. Co-composting distillery wastes with animal manure: carbon and nitrogen transformations and evaluation of compost stability. Chemosphere 72, 551–557.

Bustamante, M.A., Paredes, C., Moral, R., Moreno-Caselles, J., Pérez-Murcia, M.D., Pérez-Espinosa, A., Bernal, M.P., 2007. Co-composting of distillery and winery wastes with sewage sludge. Water Sci. Technol. 56 (2), 187–192.

Bustamante, M.A., Paredes, C., Morales, J., Mayoral, A.M., Moral, R., 2009. Study of the composting process of winery and distillery wastes using multivariate techniques. Bioresource Technol. 100, 4766–4772.

Cade-Menun, B.J., Liu, C.W., Nunlist, R., McColl, J.G., 2002. Soil and litter phosphorus-31 nuclear magnetic resonance spectroscopy: extractants, metals, and phosphorus relaxation times. J. Environ. Qual. 31, 457–465.

Cade-Menun, B.J., Preston, C.M., 1996. A comparison of soil extraction procedures for $^{31}$P NMR spectroscopy. Soil Sci. 161, 770–785.

Chang, C.W., Laird, D.A., Mausbach, M.J., Hurburgh Jr., C.R., 2001. Near-infrared reflectance spectroscopy-principal component regression analyses of soil properties. Soil Sci. Soc. Am. J. 65, 480–490.

Condron, L.M., Frossard, E., Newman, R.H., Tekely, P., Morel, J.L., 1997. Use of $^{31}$P NMR in the study of soils and the environment. In: Nanny, M.A., Minear, R.A., Leenheer, J.A. (Eds.), Nuclear Magnetic Resonance Spectroscopy in Environmental Chemistry. Oxford University Press, Oxford.

Cooperbrand, L.R., Middleton, L.H., 1996. Changes in chemical, physical and biological properties of passively-aerated co-composted poultry litter and municipal solid waste compost. Compost Sci. Util. 4, 24–34.

Cozzolino, D., Morón, A., 2006. Potential of near-infrared reflectance spectroscopy and chemometrics to predict soil organic carbon fractions. Soil Till. Res. 85, 78–85.

Dao, T.H., Sikora, L.J., Hamasaki, A., Chaney, R.L., 2001. Manure phosphorus extractability as affected by aluminium- and iron by-products and aerobic composting. J. Environ. Qual. 30, 1693–1698.

Déry, P., Anderson, B., 2007. Peak phosphorus. Energy Bull., <http://energybulletin.net/node/33164>.

Eilers, P.H.C., Marx, B.D., 2003. Multivariate calibration with temperature interaction using two-dimensional penalized signal regression. Chemometr. Intell. Lab. 66, 159–174.

Felton, G.K., Carr, L.E., Prigge, C.E., Bouwkamp, J.C., 2004. Nitrogen and phosphorus dynamics in co-composted yard trimmings and broiler litter. Compost Sci. Util. 12, 349–355.

Ferrer, J., Paéz, G., Mármol, Z., Ramones, E., Chandler, C., Marín, M., Ferrer, A., 2001. Agronomic use of biotechnologically processed grape wastes. Bioresource Technol. 76, 39–44.

Frank, I.E., Friedman, J.H., 1993. A statistical view of some chemometric regression tools. Technometrics 35, 109–148.

Grube, M., Lin, J.G., Lee, P.H., Kokorevicha, S., 2006. Evaluation of sewage sludge-based compost by FT-IR spectroscopy. Geoderma 130, 324–333.

Halgerson, J.L., Sheaffer, C.C., Martin, N.P., Peterson, P.R., Weston, S.J., 2004. Near-infrared reflectance spectroscopy prediction of leaf and mineral concentrations in alfalfa. Agron. J. 96, 344–351.

He, Y., Huang, M., Garcia, A., Hernández, A., Song, H., 2007. Prediction of soil macronutrients content using near-infrared spectroscopy. Comput. Electron. Agr. 58, 144–153.

Hinedi, Z.R., Chang, A.C., Lee, R.W.K., 1989. Characterization of phosphorus in sludge extracts using phosphorus-31 nuclear magnetic resonance spectroscopy. J. Environ. Qual. 18, 323–329.

Huang, X.L., Chen, Y., Shenker, M., 2008. Chemical fractionation of phosphorus in stabilized biosolids. J. Environ. Qual. 37, 1949–1958.

Kitson, R.E., Mellon, M.G., 1944. Colorimetric determination of P as molybdovanadate phosphoric acid. Ind. Eng. Chem. Anal. Ed. 16, 379–383.

Ludwig, B., Khanna, P.K., Bauhus, J., Hopmans, P., 2002. Near infrared spectroscopy of forest soils to determine chemical and biological properties related to soil sustainability. Forest Ecol. Manage. 171, 121–132.

Makarov, M.I., Haumaier, L., Zech, W., 2002. The nature and origins of diester phosphates in soils: a P-31-NMR study. Biol. Fert. Soils 35, 136–146.

Malley, D.F., McClure, C., Martin, P.D., Buckley, K., McCaughey, W.P., 2005. Compositional analysis of cattle manure during composting using a field-portable near-infrared spectrometer. Commun. Soil Sci. Plant 36, 455–475.

Marx, B.D., Eilers, P.H.C., 1999. Generalized linear regression on sampled signals and curves: a P-spline approach. Technometrics 41, 1–13.

Moral, R., Paredes, C., Bustamante, M.A., Marhuenda-Egea, F., Bernal, M.P., 2009. Utilisation of manure composts by high-value crops: safety and environmental challenges. Bioresource Technol. 100, 5454–5460.

Polak, J., Sulkowski, W.W., Bartoszek, M., Papiez, W., 2005. Spectroscopic studies of the progress of humification processes in humic acid extracted from sewage sludge. J. Mol. Struct. 744–747, 983–989.

R Development Core Team, 2008. R: A Language and Environment for Statistical Computing, R Foundation for Statistical Computing, Vienna, Austria. <http://www.R-project.org>.

Reeves III, J.B., Follet, R.F., McCarty, W.C., Kimble, J.M., 2006. Can near or mid reflectance spectroscopy be used to determine soil carbon pools? Commun. Soil Sci. Plant 37, 2307–2325.

Riley, M.R., Crider, H.M., 2000. The effect of analyte concentration range on measurement error obtained by NIR spectroscopy. Talanta 52, 473–484.

Shepherd, K.D., Palm, C.A., Gachengo, C.N., Vanlauwe, B., 2003. Rapid characterization of organic resources quality for soil and livestock

management in tropical agroecosystems usin near-infrared spectroscopy. Agron. J. 95, 1314–1322.
Shober, A.L., Sims, J.T., 2003. Phosphorus restrictions for land application of biosolids: current status and future trends. J. Environ. Qual. 32, 1955–1964.
Shober, A.L., Sims, J.T., 2007. Integrating phosphorus source and soil properties into risk assessments for phosphorus loss. Soil Sci. Soc. Am. J. 71, 551–560.
Toor, G.S., Hunger, S., Peak, J.D., Sims, J.T., Sparks, D.L., 2006. Advances in the characterization of phosphorus in organic wastes: environmental and agronomic applications. Adv. Agron. 89, 1–72.
Turner, B.L., Cade-Menun, B.J., Condron, L.M., Newman, S., 2005. Extraction of soil organic phosphorus. Talanta 66, 294–306.
Turner, B.L., Mahieu, N., Condron, L.M., 2003. Phosphorus-31 nuclear magnetic resonance spectral assignments of phosphorus compounds in soil NaOH–EDTA extracts. Soil Sci. Soc. Am. J. 67, 497–510.
Turner, B.L., Richardson, A.E., 2004. Identification of scyllo-inositol phosphates in soil by solution phosphorus-31 nuclear magnetic resonance spectroscopy. Soil Sci. Soc. Am. J. 68, 802–808.