Development of nutrient NIRS models for the rapid characterisation of important manure types

Final Report (LK0988)

Introduction

The research objective was to develop a technique for the rapid, low cost analysis of solid and semi solid manures based on Near Infrared Reflectance Spectroscopy (NIRS). Current methods of laboratory analysis are slow, relatively expensive and can give inconsistent results. NIRS is a technique involving measurement of the spectrum of infrared energy reflected from a sample illuminated by white light. The spectral properties of the sample are related to chemical composition and need to be calibrated against classical "wet chemistry" data in order to predict the composition of subsequent samples. Accurate calibration is dependent on reliable reference data and routine wet analysis of manures has proven problematic due to the heterogeneous nature of manure samples. Homogenisation using mechanical mixers at room temperature is ineffective due to material being extracted from rather than incorporated into the final mix. Sample homogenisation in this research was ensured by sample brittleness after rapid freezing in liquid nitrogen and subsequent smashing, rather than reliance upon a cutting action at room temperature. This technique would be too expensive to use on routine samples and can only be realistically used for the accurate calibration of a secondary rapid measurement technique. NIRS technology allows multiple rapid scans with spectral averaging to be recorded covering more of the 'as is' material than would normally be taken for a wet method, thereby further reducing the error involved in analysis. In order to cover the range of likely analyses, sample and NIR spectral variability, a very large number of samples was scanned and a representative calibration set selected using Principal Component Analysis (PCA), over two years. Additionally samples scanned for the mineralisable N models, over three years, were also included in the nutrient model development. The research involved the development of calibration models for estimation of the content of dry matter (DM), total nitrogen (N), ammonium-N (NH_4-N), sulphur (SO_3), phosphate (P_2O_5), potash (K_2O), and magnesium (MgO).

Materials and method

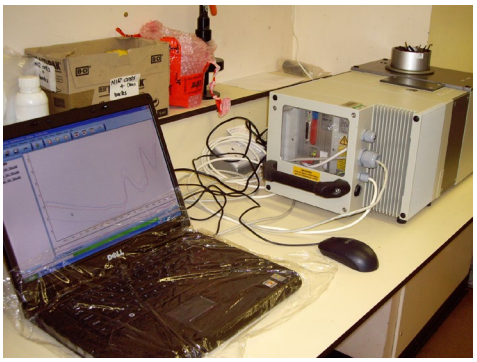
NIRS scanning

Within this research, the NIRS scanning was performed using a FT-NIR instrument, the Matrix-I (Bruker UK, Ltd). The samples are placed in a sample cup, with a clear quartz base which allows the sample to be irradiated from below and the reflected IR spectrum recorded, with the results of multiple scans integrated using the Opus software (version 6 © Bruker UK). The Matrix-I, which has been used previously for analysis of immature grain samples, was chosen due to its robustness, sensitivity and its potential for on-farm measurement. Samples are routinely scanned three times, though in the case of potentially heterogeneous manure samples, a 6x scanning procedure has been used to develop the calibration set. To establish the initial spectral database for selection of calibrants at a later date, samples were single scanned and frozen until the target sample numbers had been reached. The target sample numbers were designed to cover as much of the spectral variation as possible. PCA selection of a representative subset of these then allows for efficient use of laboratory resources with redundancy in the calibration set kept to a minimum. Once the selection process was complete the selected samples were thawed and 6x re-scanned before homogenisation of the sample and submission to the laboratory for wet analysis.

In each of the first two years of the research, a target total of 1050 samples of manures and biosolids were to be collected from across the UK, with the aim of covering the anticipated range in

variability in the manures, both in terms of chemical and physical characteristics, and NIRS spectral variability. The projected sample totals for individual manure types were 250 of both pig and cattle FYM, 150 of both pig and cattle slurries and 250 biosolids samples, reflecting the anticipated variability of these different materials. Robustness in NIRS models is achieved by adding to the database over time, thereby including seasonal and geographical sample changes in the model.

(a)



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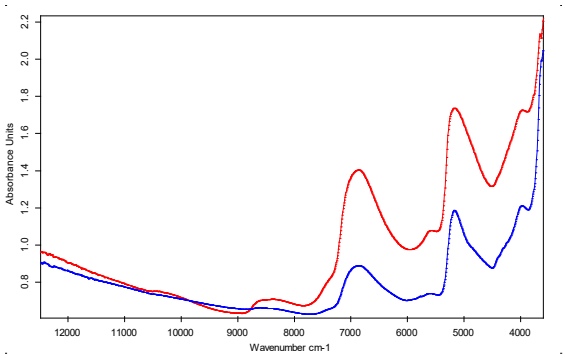


Figure 1: Scanning of manure samples for nutrient content: (a) using the Bruker Matrix-I NIRS and (b) NIR spectra of two cattle manure samples.

Homogenisation of samples.

The homogenisation procedure prior to this research involved using a rotary blender at room temperature. The main problem observed with this was the tendency for the wet fibrous content to wrap around the cutting blades of all homogenisers trialed. This extracted material from the sample that could not be broken down. Trialed devices ranged from food mixers to specialist laboratory homogenisers such as the Ultra Turrax machine supplied by Carl Stuart limited. All failed to produce an acceptable homogenate which is essential for consistent wet analysis.

The procedure developed in a previous project, NT2508 (Smith et al., 2005), involved cooling the material to very low temperatures using liquid nitrogen. The resulting brittle state then allowed the blades of a powerful homogeniser to break the whole sample down. Preparing the sample involved suspension of a representative aliquot in a Dewar flask containing liquid nitrogen. A suitable sample basket was constructed in the laboratory. This consisted of a chrome steel wire basket, 13cm x 13cm x 13cm, with the addition of a bent wire loop to act as a handle. This was further lined with 1mm steel meshing to prevent sample material dropping through into the liquid nitrogen. A powerful homogeniser was used to break up the hard frozen material (FOSS 2096).

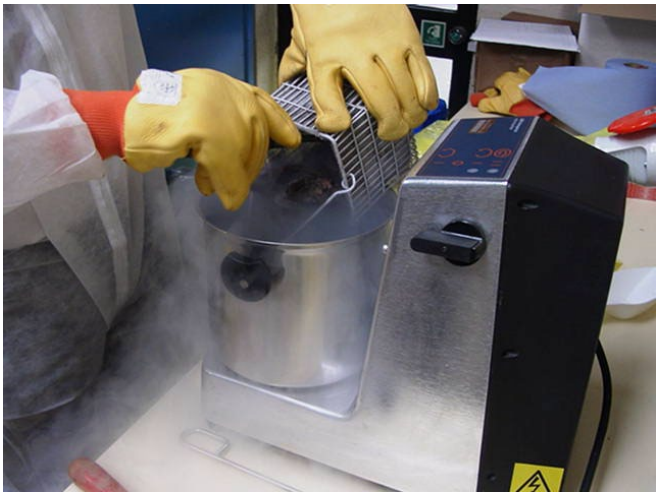
Initially 500g sub-samples were frozen at –18^{0}C before treatment with liquid nitrogen. The homogeniser could not cope with a single frozen block of this size. This was overcome by creating disc shaped sub-samples of the fresh unfrozen material of approximate dimensions 50mm diameter by 6mm thick. The addition of this step also removed stones which can damage the blades. These pre-frozen discs, when added to the basket, created airspace between the bulk sample which allowed easier homogenisation. The resulting product, while at -190 ^{0}C, resembled a powder. Attempts to homogenise material frozen at – 18^{o}C only resulted in an unacceptable coarse material and the lower temperature was required to achieve the needed brittleness. While using this procedure, experience showed that using material first frozen at – 18^{o}C reduced the amount of liquid nitrogen used per sample but still allowed the necessary break up of the sample bulk. Also a minimum amount of 400g was required, with amounts less than this producing an undesirable coarser particle size. Figure 1 illustrates the homogenisation procedure.

Figure1. Homogenisation procedure

a) Sample as received

b) Loading the sample cage

c) Lowering the sample into liquid nitrogen



d) Transferring the sample to the homogeniser

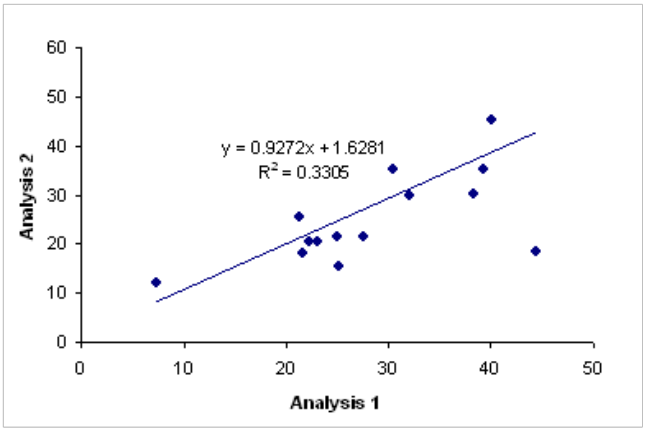
e) Typical sample after homogenisation, before thawing.

Results and discussion

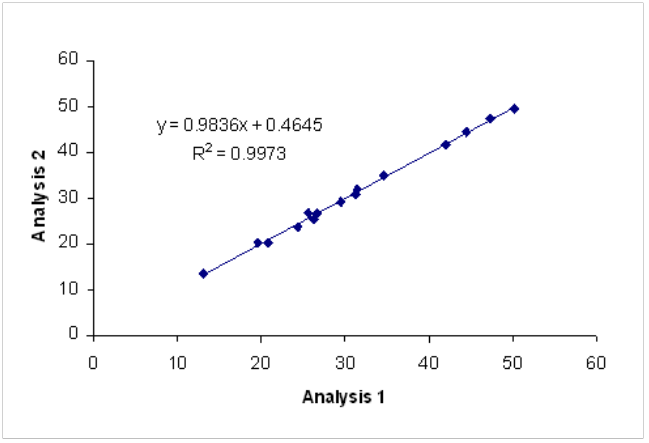
An earlier research project involved the development of a homogenisation procedure and a homogeneity study was carried out to demonstrate the effectiveness of the new technique. Samples of manures from various animal types were submitted for analysis as blind duplicates both before and after homogenisation.

The results demonstrate an improvement in the homogeneity of the sample using the liquid nitrogen process developed. An improvement in repeatability here benefits interpretation of data for field use and leads to an improved correlation when matching to spectral data for generating NIRS prediction models. The use of this technique for routine use however would prove prohibitively expensive and is only used here to generate reliable NIRS models. The plots below demonstrate the improvement the technique offers, in this case for N content of manures (poultry, pig and cattle solid manures).

Figure 2. Sample homogenisation procedure for analysis of manures - total N (g/kg DM) (Poultry, Pig & Dairy manures)



a) before sample homogenisation



b) after sample homogenisation

A total of 1019 samples were collected and scanned between January and June 2008, with the active support of the project partners. The total comprised 190 pig FYM, 134 pig slurry, 338 cattle FYM, 176 cattle slurry and 181 biosolids samples. Principal Component Analysis was used to select a total of 118 samples to represent the spectral variability and range of manure analysis, in order to assemble preliminary nutrient calibration models. In addition the 58 samples selected for the first year mineralisable N pot experiments were included in the nutrient model development. These samples, comprising 25 pig FYM, 49 pig slurry, 37 cattle FYM, 28 cattle slurry and 37 biosolids, were multi-scanned (6x) and analysed by wet chemistry, the solids following homogenisation. The multiple scans were spectrally averaged before performing model regression. In 2009, a total of 1158 samples were collected and scanned between January and July 2009; the total comprising 254 pig FYM, 135 pig slurry, 377 cattle FYM, 145 cattle slurry and 247 biosolids samples. PCA was again used in combination with outlier analysis from the preliminary models to select a further subset of 120 samples (24 of each manure type) for chemical analysis. The 60 samples selected for the second year mineralisable N pot experiments were again included. These samples, comprising 36 pig FYM, 26 pig slurry, 46 cattle FYM, 33 cattle slurry and 39 biosolids, were multi-scanned (6x) and analysed by wet chemistry, the solids following homogenisation. The final 62 samples of the third year mineralisable pot experiments were multi-scanned (6x) and analysed by wet chemistry, the solids following homogenisation, to expand the range of spectral variability and analyses and continue the development of robust nutrient calibration models. In total

this gave 418 samples for the final nutrient model calibration set comprising 70 pig FYM, 80 pig slurry, 101 cattle FYM, 75 cattle slurry and 92 biosolids samples.

A summary of the scanning activity:

2177 single scans to establish initial spectral database;

2508 replicate (x6) scans taken to develop calibration set comprising 418 samples.

A summary of the wet chemistry analysis results is presented in Table 1.

Table 1. Summary of wet analysis results (averages of 418 duplicate analyses).

ID	DM (g/Kg)	Total N (g/Kg)	P_2O_5 (mg/Kg)	SO_3 (mg/Kg)	K_2O (mg/Kg)	MgO (mg/Kg)	NH_4N (mg/Kg)
Bio-solids							
Min	169.00	3.05	1742	1360	253	648	7
Max	890.00	46.50	56299	26334	6005	12360	4353
Mean	331.52	12.65	14206	8277	1021	2296	1841
Pig slurry							
min	2.71	0.50	41	44	466	13	156
max	147.00	9.22	6652	3319	6652	2620	4839
mean	33.81	3.22	1083	807	2434	376	2195
Pig FYM							
min	164.50	1.65	771	690	992	421	9
max	857.00	20.70	22050	18457	16158	7891	5032
mean	259.57	7.98	5912	4061	6700	2058	1999
Cattle slurry							
min	1.77	0.14	33	29	299	27	105
max	157.50	17.80	2460	2650	6842	1633	2729
mean	62.73	2.51	904	753	2546	608	953
Cattle FYM							
min	162.74	2.63	885	917	1054	339	41
max	846.00	28.15	32147	49499	29025	6832	4808
mean	273.78	7.25	3571	3577	8359	1824	755

Upon completion of spectra collection and analysis, refinement of the calibration models was undertaken with expert chemometrics input from Bruker. Calibration optimization was performed with 50% of the data first used as a test set. The test and calibration sets were exchanged after the initial regression to test the robustness of the data to form a model. Regressions were run with this test set selected by both analyte value and by PCA. To create the final working versions regression models using cross validation were created as these use all the available data. The regression runs were limited to the bandwidth 9500cm^{-1} to 3800cm^{-1} in order to reduce interference due to noise. The removal of some samples from the calibration set was necessary and included effluent samples of very low solids content, in fact “dirty water” samples, and also samples with very low

nutrient content (at p.p.m. level). Some of these samples were almost translucent and, hence, unsuitable for reflectance-based analysis.

From the statistics associated with the validation procedure, Malley *et al.* (2005) proposed the following guideline for describing the performance of calibration models for environmental samples, based on the r^2 (correlation coefficient) and RPD statistic (standard deviation of the analyte set divided by standard error of prediction): r^2>0.95, RPD>4 - *excellent*; r^2= 0.9-0.95, RPD 3-4 – *successful*; r^2= 0.8-0.9, RPD 2.25-3 – *moderately successful*; r^2= 0.7-0.8, RPD 1.75-2.25 – *moderately useful*. It was considered that some calibrations with r^2<0.7 may be useful for screening purposes.

In selecting the final models, as well as the above, consideration was given to the number of ranks (terms) selected to describe the analyte and the magnitude of the model loadings. As a general rule-of-thumb, loadings greater than 10,000 indicate that noise within the spectra rather than analyte signal may be being modelled. Also the model with the lowest number of ranks is preferred so as to avoid over fitting of the model to the calibration set, however having said this some complex systems such as manures will require more ranks.

Model statistics obtained on test set validation and final cross validation model are presented below, with calibration models in Figure 3 (a-g):

Dry matter

- Model based on test set selection by lab value
- Set A R^2 94.94 RPD 4.44
- Set B R^2 96.66 RPD 5.48
- Model based on test set selection by PCA
- Set A R^2 95.10 RPD 4.52
- Set B R^2 96.19 RPD 5.12

Cross validation model - R^2 96.07 RPD 5.05 13 ranks, loadings ~ 7000

Total nitrogen

- Model based on test set selection by lab value
- Set A R^2 96.84 RPD 5.62
- Set B R^2 92.60 RPD 3.68
- Model based on test set selection by PCA
- Set A R^2 92.05 RPD 3.55
- Set B R^2 94.38 RPD 4.22

Cross validation model - R^2 94.78 RPD 4.38 14 ranks, loadings ~ 20

Ammonium nitrogen

- Model based on test set selection by lab value
- Set A R^2 93.74 RPD 4.00
- Set B R^2 94.99 RPD 4.47
- Model based on test set selection by PCA
- Set A R^2 91.18 RPD 3.37
- Set B R^2 93.33 RPD 3.87

Cross validation model - R^2 90.75 RPD 3.29 11 ranks, loadings ~15000 (reworked originally 80,000)

Phosphate as P_2O_5

- Model based on test set selection by lab value
- Set A R^2 93.50 RPD 3.92
- Set B R^2 93.61 RPD 3.96
- Model based on test set selection by PCA
- Set A R^2 91.92 RPD 3.52
- Set B R^2 90.96 RPD 3.33

Cross validation model - R^2 90.85 RPD 3.31 8 ranks, loadings ~20,000 (reworked originally 100,000)

Sulphate as SO_3

- Model based on test set selection by lab value
- Set A R^2 86.96 RPD 2.77
- Set B R^2 85.38 RPD 2.62
- Model based on test set selection by PCA
- Set A R^2 87.30 RPD 2.81
- Set B R^2 87.78 RPD 2.86

Cross validation model - R^2 88.11 RPD 2.90 7 ranks, loadings ~ 15,000

Potassium as K_2O

- Model based on test set selection by lab value
- Set A R^2 82.86 RPD 2.42
- Set B R^2 82.62 RPD 2.40
- Model based on test set selection by PCA
- Set A R^2 74.62 RPD 1.99
- Set B R^2 77.97 RPD 2.13

Cross validation model - R^2 83.54 RPD 2.46 12 ranks, loadings ~ 30,000 (reworked originally 6,000000)

Magnesium as MgO

- Model based on test set selection by lab value
- Set A R^2 76.82 RPD 2.08
- Set B R^2 73.77 RPD 1.95
- Model based on test set selection by PCA
- Set A R^2 67.60 RPD 1.76
- Set B R^2 81.48 RPD 2.32

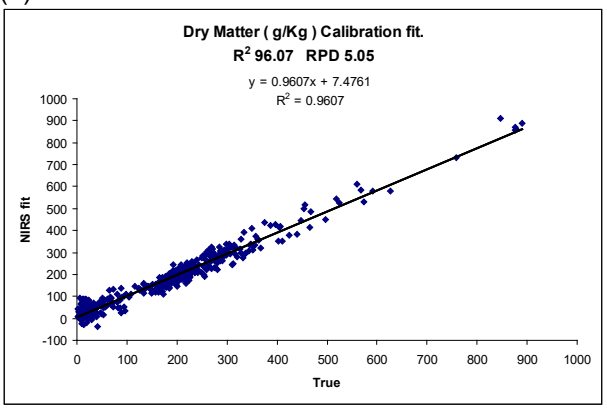
Cross validation model - R^2 69.94 RPD 1.82 4 ranks*, loadings ~ 800

* rank low may be correlating to some grand physical property

The pH models gave poor correlations, below 0.70, so were abandoned.

Figure 3: Calibration models for manures: (a) DM content; (b) total N content; (c) ammonium N content; (d) P_2O_5 content; (e) SO_3 content; (f) K_2O content; (g) MgO content.

(a)



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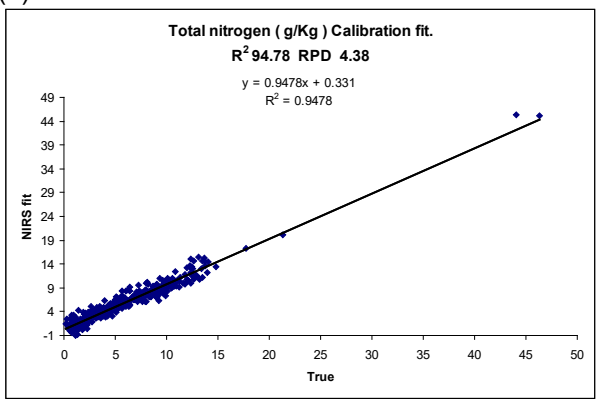
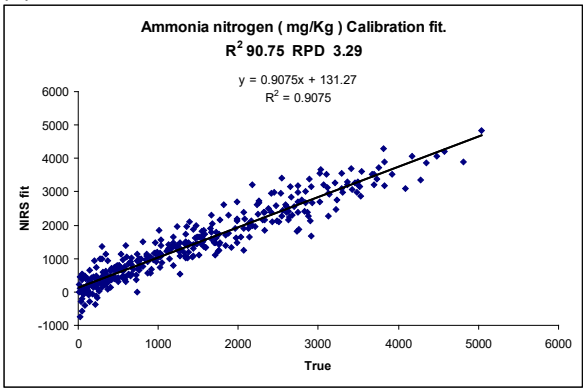
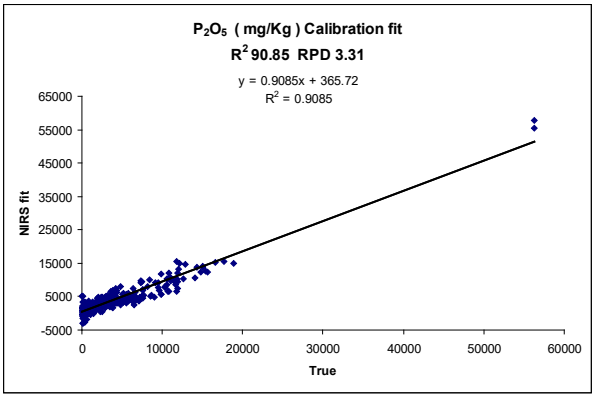


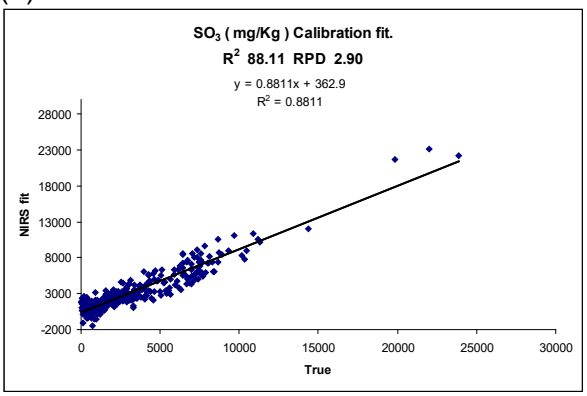
(c)



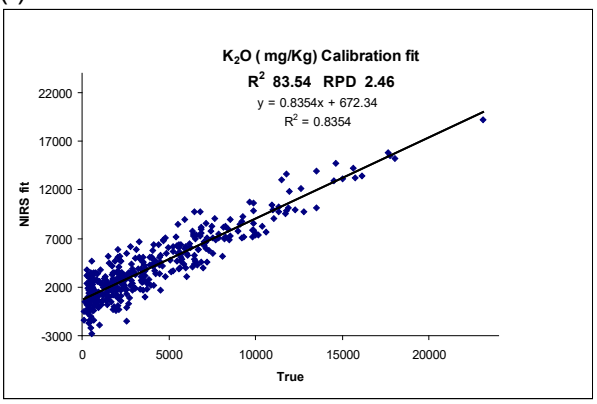
(d)



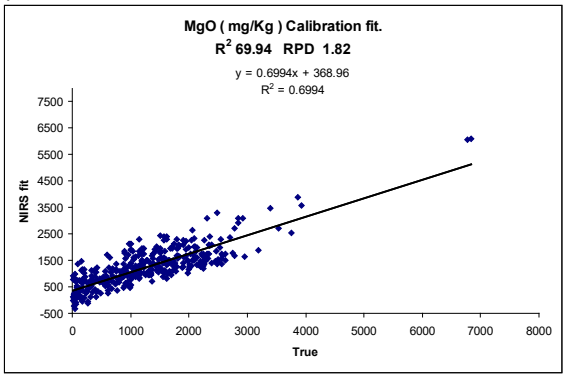
(e)



(f)



(g)



On this basis, performance of the calibrations for conventional analysis of manure and biosolids samples was as follows: *excellent* – DM, total N; *successful* – NH_4-N, P_2O_5; *moderately successful* – SO_3, K_2O; *moderately useful* – MgO.

Near infrared radiation (9000cm^{-1} – 4000cm^{-1}) is absorbed by various chemical bonds, mainly C-H, N-H, O-H, all of which are characteristic of organic matter. It is therefore no surprise that the calibrations have been particularly successful for dry matter (moisture), nitrogen, ammonium-N and phosphate content, but rather less so for minerals, potassium and magnesium (Figure 3 a-g). Performance for pH was unsatisfactory (r^2 0.6, RPD 1.63), which was not a surprise given the limited range in pH (almost all between pH 6.5 and 9.0), even within the very large range of samples scanned and analysed.

Conclusions

The research has confirmed the potential of NIRS to provide a rapid, reliable and reduced cost analysis capability for farm manures, slurries and biosolids. The calibration models for conventional analysis parameters were improved by the exclusion of very dilute effluents, where the low solids content and very low nutrient content appears to reduce the reliability of the reflectance assessment. It is therefore suggested that a threshold limit of between 2% and 5% solids content be set, below which NIRS should not be used for sample analysis. It is envisaged that the further development of a predictive capability for mineralization of manure organic N (Appendix 2) will enhance interest in NIRS as an analytical tool.

Highlights of the research, presented throughout the project within reports, press articles, conference papers and farming events (e.g. Grassland-MUCK2008 and 2011) have attracted significant industry interest. Key information and advice have been compiled within promotional material (posters, technical leaflets) used at events and in farmer leaflets (AHDB and Eurofins) produced in support of the recent launch of a NIRS-based commercial service for manure analysis. This service is to be provided initially by the research partner, Eurofins Laboratories, under the terms of a two-year licence agreed by the consortium.

References

Malley, DF, C McClure, P D Martin, K Buckley and W P McCaughey 2005. Compositional analysis of cattle manure during composting using a field-portable NIRS. Commun. Soil Sci Plant Anal, 36: 455-475.

Smith, K A, D Farrington, S Shelley, S Lister and P J Hobbs 2005. Rapid, reduced cost analysis of manures by near-infrared spectroscopy. CSG15 Final Project Report, Defra Research Contract NT2508 (available via Defra website: http://www.randd.defra.gsi.gov.uk/)