

# **Analysis of Nutrients in Hog Manure by Field-portable Near-infrared Spectroscopy: Results for the Zeiss Corona® Spectrometer**



**Final Report 3 of 3 to CETAC-West on Manure Demo  
Project**

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## Executive Summary

The overall purpose of this project was to develop a field protocol for testing two field-portable near-infrared (NIR) spectrophotometers as on-site, free-standing (not in-line), measurement tools for nutrients in hog manure at the time of application of manure to agricultural land. This project was a developmental step between the analysis of hog manure in the laboratory using near-infrared spectroscopy (NIRS) and the deployment of NIR instruments in the manure stream for real-time measurement of composition of manure during application to land. The field-portable instruments included the Textron Systems Corp (USA)/Case NH (USA) ProSpectra™ Spectrometer and the Carl Zeiss (Germany) Corona® Spectrometer.

The first step in this project was the design and construction of a mobile laboratory mounted on the bed of a 1/4-ton pick-up truck. Considerations in designing and building this system included reliable power supply for the instruments and laptop computers, security of the equipment against movement during transit and against theft, protection from the elements, and worker safety.

Secondly, the project involved sampling of manure (n = 121) at 13 application operations in the vicinity of Winnipeg MB during between 28 September and 4 November 2000. All but two of these operations held the manure in earthen stores, one had a slurry store and a secondary lagoon, and one had above ground storage. Six of the operations were visited once and seven were visited from two to seven times during pump-out from agitated lagoons. Using conventional physical and chemical methods, the samples were analyzed by the Freshwater Institute Analytical Laboratory for pH, conductivity, density, ammonium-nitrogen (NH<sub>4</sub>-N), total dissolved nitrogen (TDN), particulate nitrogen, dissolved phosphorus (dissolved P), particulate P, and particulate carbon (particulate C, i.e., a measure of the particular organic matter). Eighty of the 121 samples of manure were analyzed for 31 minor elements and metals by inductively coupled plasma spectrometry and for moisture by drying by Norwest Labs.

The samples ranged in pH from 6.7 - 8.1, conductivity from 6.8 - 27.2 mS/cm, density from 1.002 - 1.049 g/mL, ammonium-N from 0.56 - 5.54 g/L, total dissolved N from 0.59 - 6.11 g/L, suspended N from 0.01 - 4.08 g/L, total N from 0.61 - 10.14 g/L, soluble reactive P (phosphate-P) from 0.05 - 3.81 g/L, total dissolved P from 0.06 - 3.86 g/L, suspended P from 0.003 - 2.65 g/L, total P from 0.06 - 6.51 g/L, and suspended C from 0.09 - 54.60 g/L.

Thirdly, the project involved operating the two field-portable NIR instruments on the mobile laboratory for the scanning of the manure samples. As well, all of the samples were scanned with a laboratory instrument, the Foss NIRSystems Inc. (USA) model 6500 visible/near-infrared scanning spectrophotometer. The NIR spectral data from the 6500 and field-portable instruments were statistically correlated with the chemical data on the same samples to develop calibrations, or statistical models, for each constituent on each instrument. The success of calibrations was evaluated statistically as a measure of the performance of the instruments and

their suitability for on-site manure analysis. Successful calibrations can be used with the respective instruments in the field or laboratory to predict composition of future manure samples.

This is the third in a series of six reports on the analysis of nutrients, metals and minor elements in hog manure by the two field-portable near-infrared instruments. This report describes the success of the Zeiss Corona® 45 NIR spectrometer for the measurement of nutrients in hog manure samples collected in the fall of 2000.

The Corona® is a diode array instrument with rapid scanning times. The instrument used had a polychromator head containing a light source and a number of diode array detectors. The Corona® 45 NIR spectrometer collected spectral data from 938 to 1700 nm wavelengths.

Calibrations were developed with The Unscrambler® multivariate analysis software using Principal Component Analysis/Partial Least Squares regression. Calibrations were developed on two-thirds of the samples and validated using the test set method on the remaining third.

Calibrations achieved with the Corona were successful for total dissolved P and suspended P. They were moderately successful for ammonium-N, total dissolved N, suspended N, suspended C and pH. They were useful for soluble reactive P. The calibration for conductivity was marginal.

The calibrations developed for pH, several forms of N and P, and for suspended C from spectra recorded by the Corona® on hog manure indicate that the instrument has promise for the rapid, on-site measurement of these constituents.

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The photo on the cover shows the Zeiss Corona®. Photo is property of Carl Zeiss, Germany.

# Table of Contents

Executive Summary.....	2
Acknowledgments.....	3
Introduction.....	4
Methods.....	6
Development of the Truck Lab and Sampling of Manure in Fall 2000.....	6
Zeiss Corona® 45 NIR Spectrometer.....	6
Recording Spectra with the Zeiss Corona® Spectrometer.....	7
Principal Component Analysis of the Spectral Data.....	7
Calibration Procedure Using The Unscrambler®.....	8
Results.....	11
Spectra.....	11
NIR Prediction of pH, Conductivity, and Nutrients in Hog Manure.....	12
Discussion.....	16
References.....	16

## Introduction

Hog manure has become a recognized and valued fertilizer resource, largely because the N it contains is mostly in the form of  $\text{NH}_4\text{-N}$  that binds to soil and is less prone to leaching to groundwater than  $\text{NO}_3\text{-N}$ . Furthermore, as inorganic fertilizer costs rise, the low costs and the availability of manure in agricultural areas make the use of manure increasingly attractive. Nevertheless, management of manure from hog production is a particular challenge because of, among other things, the variable nutrient content (Malley, Martin and Woods 2001).

The nutrients in manure have implications not only for agronomy and water pollution, but also for the emission of greenhouse gases. Agriculture and land use are important sources of the major greenhouse gases,  $\text{CO}_2$ ,  $\text{CH}_4$ , and  $\text{N}_2\text{O}$ . Agricultural practices, particularly including the use and overuse of fertilizers, influence emissions of the most potent  $\text{N}_2\text{O}$  (310 times the global-warming potential of  $\text{CO}_2$ , compared with  $\text{CH}_4$  that has 21 times the potential of  $\text{CO}_2$ ). The agricultural emission of these gases is mediated by microbial communities in the soil, influenced by amounts of fertilizer added, land use practices (such as cultivation), soil type, and weather.

A method of analytical testing that is rapid, cost effective, and, if possible, field-portable and in-stream that has the capability to analyze N and P and salts in liquids and slurries would have wide use for monitoring and guiding manure application to land. This would be useful not only for maximizing the agronomic use of the nutrients but also for providing loading data useful for the calculation of greenhouse gas emissions from manured lands.

Near-infrared spectroscopy (NIRS) is a 30-year old rapid, analytical technology that has the capability of determining quantities of organic constituents in liquids, slurries, and solids. It is described in more detail by Malley, Martin and Woods (2001). PDK Projects, Inc. has demonstrated that near-infrared spectroscopy, a rapid, non-destructive analytical technology based on the measurement of the absorption of near-infrared light, is useful in the laboratory for the analysis of nutrients and minor elements in hog manure (Malley 1999; Malley and Currie 1999; Malley and Vandenbyllaardt 1999; Malley et al. 1999; Malley et al. submitted for publication). Millmier et al. (2000) demonstrated the use of NIRS in the laboratory for the analysis of hog and dairy manure. Moreover, NIRS is a useful method for measuring C quantity and quality and total N in the soil.

The development of field portable NIR instruments for field and on-the-go analysis of agricultural crops provides instruments that have the potential for analyzing manure composition as it is applied to agricultural land. One such instrument is the Corona® manufactured by Carl Zeiss, Germany. The Corona® is an industrial reflection/transmission head for measuring applications in the UV/VIS/NIR range in which the spectral sensor and micro-computer are already integrated. Using a combination of modern diode array technology, high-precision optics, fast and high-resolution electronics, the Corona® is employed in process monitoring, quality control, and in the laboratory in food, agricultural, plastics and pharmaceutical industries. There are no moving mechanical components resulting in a high degree of reliability and accuracy of spectral wavelengths. Scanning time is in the milliseconds range with simultaneous detection of a wavelength range from as low as 200 nm to over 2000 nm depending on the

instrument model. The Corona® has been tested in Europe on a Haldrup harvester for the analysis of grain and forage quality during the harvesting process (Rode 2001).

In a field demonstration study, the Corona® was mounted and operated on a truck lab to evaluate its performance for the analysis of hog manure. Six reports describe the results from this entire study. The overall goals of the study, the design and construction of the truck lab, sampling of hog manure and results for nutrients from the use of a laboratory visible/near-infrared spectrophotometer, the Foss NIRSystems Model 6500 are described by Malley, Martin and Woods (2001). The second report describes the results for the nutrients from the operation of the Textron/Case NH ProSpectra™ NIR spectrometer (Malley, Martin, and Moffatt 2001a). This is the third report. The fourth report by Malley, Martin, Woods and Dettman (2001) describes the results with the 6500 for metals and minor nutrients. The fifth report is a companion report to Malley, Martin and Moffatt (2001a) and describes the results for the ProSpectra™ for metals and minor elements (Malley, Martin and Moffatt 2001b). Finally, the sixth report is a companion report to this one and describes the results with the Corona for metals and minor elements (Malley, Martin and Dettman 2001).

This report describes the spectral data obtained with the Corona® and the success of calibrations developed for several chemical parameters and nutrients in the manure.

## Methods

### *Development of the Truck Lab and Sampling of Manure in Fall 2000*

The design and construction of the truck lab are described by Malley, Martin and Woods (2001).

The sampling and analysis of hog manure samples from 13 hog operations in the vicinity of Winnipeg from September to November 2000 are described in Malley, Martin and Woods (2001).

The samples were analyzed by the Freshwater Institute Analytical Laboratory, Winnipeg, (Stainton et al. (1977) for pH, conductivity, density, ammonium-nitrogen (NH<sub>4</sub>-N), total dissolved nitrogen (TDN), particulate or suspended nitrogen (susp N), soluble reactive phosphorus (SRP, i.e., phosphate or inorganic P), total dissolved phosphorus (TDP, dissolved inorganic and organic P), particulate or suspended P (susp P), and suspended carbon (susp C, i.e., a measure of the particulate organic matter). The compositional data are given in Malley, Martin and Woods (2001).

### *Zeiss Corona® 45 NIR Spectrometer*

The instrument used in this study was a Zeiss Corona® 45 NIR 1.7 Spectrometer consisting of a spectral measuring body, bonded with a concave grating that corrects aberrations, a fibre cross-section converter as an optical input and a diode array detector. It was compact, permanently aligned, robust, thermally stable, small, and possessed a high degree of light sensitivity

Table 1. Technical specifications of the Zeiss Corona® 45 NIR 1.7 Spectrometer

<b>Parameter</b>	<b>Specification</b>
Wavelength Range	950 - 1680 nm
Wavelength Resolution	6 nm/diode
Wavelength Accuracy	< 0.6 nm
Wavelength Reproducibility	< 0.1 nm
Noise	< 0.0002 AU rms
Minimum integration time	2 ms
Amplitude resolution	15/16 bit
Light source	Halogen lamp, 10W

Receiver sensor	Multiplexed InGaAs-Array 1.7 $\mu\text{m}$
Number of diodes	128 diodes
PC Interface	RS 422
Operating Temperature	0° - 40° C

### ***Recording Spectra with the Zeiss Corona® Spectrometer***

During scanning the Corona® was mounted on a moveable deck on the bed of a pick-up truck (Fig. 1). The Corona was not set up and operating on the truck until late in the sampling period. All the samples were scanned at one time during mid-November 2000, at which time the truck was housed in a partially-heated garage.

Initially the sample was presented in a watertight circular cell with a path length of 1 cm fashioned from a Foss/NIRSystems standard sample transport cell. An opaque ceramic was attached to the upmost side of the cell to prevent light passing through the sample and being lost. Using this transfectance cell, absorbance by the instrument was higher than desirable. Therefore, the cell was re-designed to have a path length of approximately 2.5 mm that decreased absorbance values to a useful range.

The 2.5-mm pathlength cell was used to obtain spectra from all samples. A ceramic reference provided by Zeiss was placed inside a duplicate cell and used as the system reference. The cells were centered on top of a spacer ring mounted on the upward-facing sensor head. The sample cell was washed and dried between each manure sample. Samples were scanned three times, with the cell being turned 120 ° between each scan. This resulted in 3 spectral files for each sample.

The Corona 45 NIR was operated using Aspect Plus software provided with the instrument. This recorded spectra as absorbance from 938 to 1700 nm at approximately 6 nm intervals. Unlike NSAS, Aspect software saves each spectrum in a separate file.

### ***Principal Component Analysis of the Spectral Data***

Using multivariate analysis software, The Unscrambler® (CAMO ASA Oslo, Norway), principal component analysis (PCA) was performed on the spectral data. Scores for individual samples on the first two PCs explaining the variance in the spectral data were plotted. This plot can show sample patterns, groupings, similarities or differences. For example, the closer samples are in the score plot, the more similar their spectra are. Conversely, samples far away from each other have more different spectra.



Fig. 1. The Corona® fastened to the bench of the truck lab with Velcro straps with the head, containing the light source and the polychromator (sensors) facing upwards. It was powered by 600-amp/hour bank of batteries and operated from the notebook computer using Aspect Plus software. The black tower for obtaining the dark reference (noise) is behind the head.

### ***Calibration Procedure Using The Unscrambler®***

The ability of NIRS to provide rapid analyses depends on the prior preparation of mathematical calibrations used to predict constituents, parameters or functionality in unknown samples. A calibration is a statistical correlation model relating the spectral data for a set of samples to the constituent data determined by conventional methods.

It was planned to develop all calibrations in this study with Near-infrared Spectral Analysis Software (NSAS) so that the software package used for calibration was not a variable in evaluating instrument performance (Malley, Martin and Woods 2001). Nevertheless, it was found that spectral data were not readily exportable from Aspect into NSAS. In Aspect, each spectrum was saved in a separate file and the wavelength interval between absorbance values was approximately 6 nm. In Aspect, spectral were files were bundled, and transformed through the "interpolation" function to 2 nm intervals. From Aspect, the spectra were imported into Grams 32 software and exported in NSAS format. Unfortunately, the NSAS format files exported from Grams 32 were not functional in NSAS, presumably because the wavelength intervals were expressed as 2.00000 nm instead of the integer format that NSAS requires.

The bundled files from Aspect, interpolated to 2 nm wavelength point intervals, were readily imported into The Unscrambler® 7.6 and calibrations were developed using this software. Replicate spectra were averaged and the constituent data were added to the Unscrambler® file with the spectral data.

Calibrations were developed in The Unscrambler® using principal component analysis/partial least squares regression (PCA/PLS). Using the test set method, calibrations were developed on two-thirds of the samples and validated on the remaining third of the samples (every third sample was selected) using PLS1. In this algorithm, calibrations were developed for constituents individually. Calibrations were developed and validated on untransformed spectral data and on the spectral data smoothed over 5, 11, 21, or 41 wavelength points (where wavelength points were at 2 nm intervals). Calibrations were also performed on data smoothed over each interval and then transformed to first or second derivative where the derivative interval, or gap, was 5, 11, 21, or 41 wavelength points. This gave a total of 37 attempted calibrations. The calibration that resulted in the highest  $r^2$  between the NIR-predicted values in the validation set and the chemically-measured values, and in the lowest SEP was selected as the best. Using the smoothing and derivative combination of this best calibration, calibration and validation were repeated twice more so that each sample was predicted in a validation set. In this way, all of the samples were predicted. NIR-predicted values for all of the samples from the validation sets were graphed as a function of the chemically-measured values. The regression statistics,  $r^2$ , RMSEP, RPD, and RER were tabulated. The calibration models were not slope and bias corrected, to bring the y-intercept to 0 and the slope to 1, as they were after NSAS calibration.

There were several important differences between calibrations developed in NSAS with multiple linear regression (MLR) and those developed in The Unscrambler with PCA/PLS. MLR selects a few individual wavelengths, up to 8 in our calibrations, at which the constituent values best correlate with absorbance values. In PCA/PLS, absorbance values at all of the wavelengths are projected onto a smaller number of underlying variables, the principal components. Therefore, areas of noise in the spectra, unless removed, may reduce the quality of calibrations more in PLS than in MLR. The difference between the predicted and the measured values in PLS is termed the root mean square error of prediction (RMSEP) rather than the SEP. The RMSEP contains error due to bias, the systematic difference along the y-axis between the measured and predicted values, as well as error in the calibration model.

# Results

## *Spectra*

Spectra recorded using the Corona from representative manure samples in this study are graphed in Fig. 2. The absorbance due to the OH<sup>-</sup> group in water around 1400 nm is clearly seen in all spectra. The thin samples with little particulate matter had lowest absorbance below 1400 nm and highest above. Spectra with high particulate matter content were general higher in absorbance below 1400 nm and lower in absorbance above 1400 nm than the thin samples.

Figure 2 shows that despite reducing the path length of the sample presentation cell to 2.5 mm from the original 1 cm, the absorbance of some samples was greater than 2.0, the upper desirable limit for high moisture material. The samples with highest absorbances above 1400 nm and some samples with maximum absorbances between 1.5 and 2.0 showed noise.

Figure 3 shows the scores of all the manure samples on the first two principal components explaining the variance in the spectral data. This figure shows the locations of the samples along the first two PC's explaining the variability in the spectral data. Although for simplicity the samples are not labeled, it can be seen that there are different

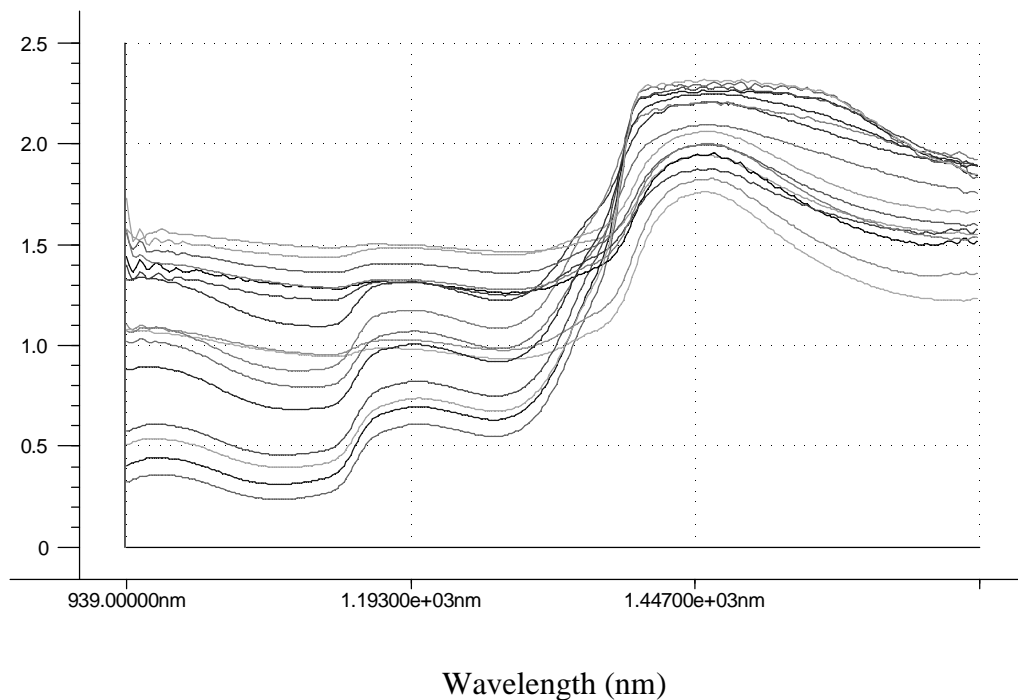


Fig. 2. Representative spectra of hog manure in this study recorded with the Corona between 938 and 1700 nm. Y-axis is absorbance (log 1/R where R is reflectance). Each spectrum is the average of three replicate spectra.

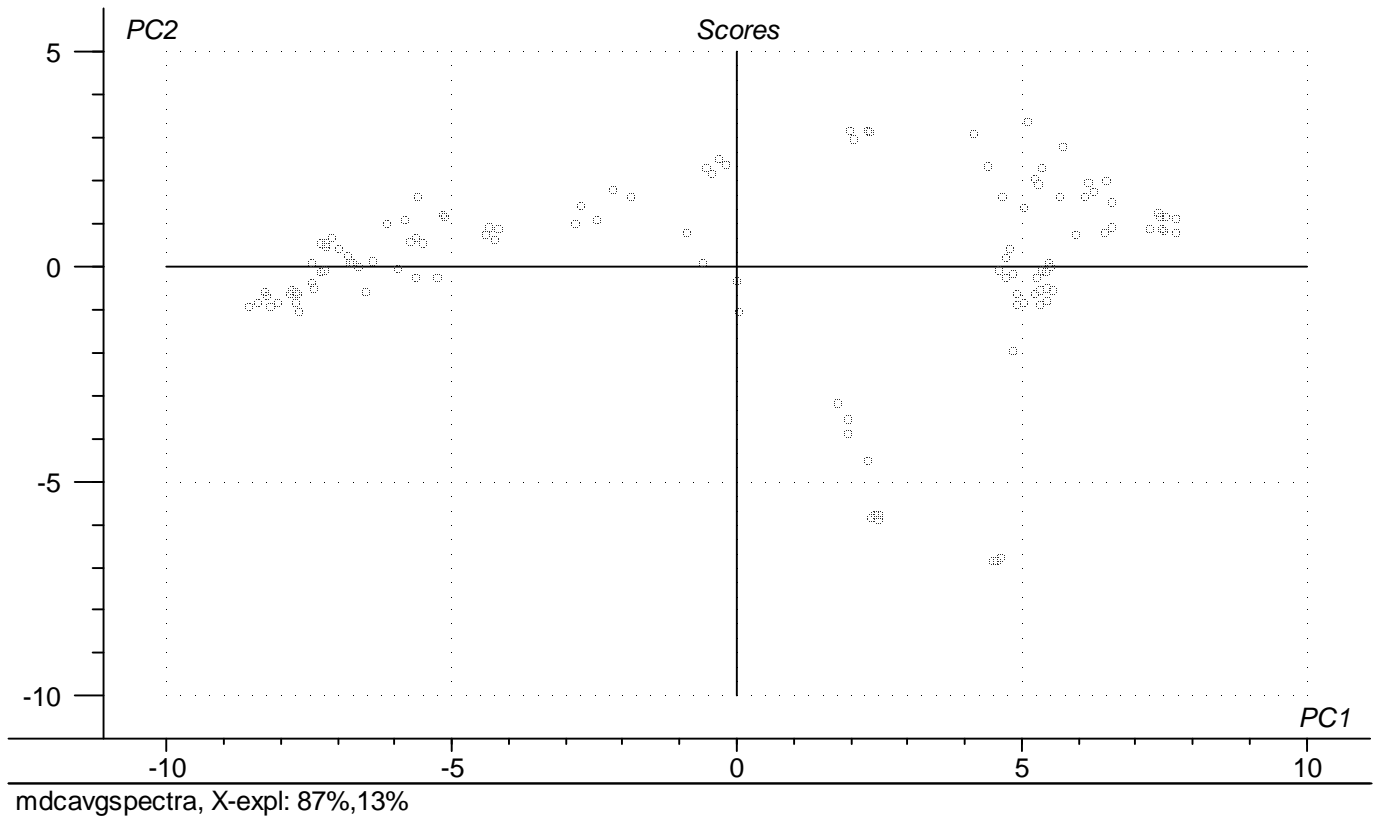


Fig. 3. Scores plot for the hog manure samples collected in year 2000 on the first two principal components explaining the variance in spectral data.

groupings of samples. Also, it is evident that there is a favourable distribution of samples with relatively uniform separation of samples and little aggregation.

### ***NIR Prediction of pH, Conductivity, and Nutrients in Hog Manure***

Table 2 and Fig. 4 show that calibrations achieved with the Corona were successful ( $r^2 \sim >0.9$ , RPD  $> 3.0$ ) for total dissolved P and suspended P. They were moderately successful ( $r^2 = 0.8 - 0.9$ , RPD 2.5 - 3.0)  $\text{NH}_4\text{-N}$ , total dissolved N, suspended N, suspended C and pH. Calibrations were useful ( $r^2 = 0.7 - 0.8$ , RPD = 1.75 - 2.5) for and soluble reactive P. Conductivity was not well predicted, although the calibration may be useful for screening samples with low vs high conductivity.

Table 2. Accuracy of prediction for NIR calibrations for several parameters and nutrients in 121 samples of hog manure collected in fall 2000 and scanned using the Zeiss Corona® spectrometer. Wavelength range was 938 - 1680 nm. Abbreviations are described in the Methods. Calibrations were developed using PCA/PLS in The Unscrambler®.

<b>Statistic</b>	<b>pH</b>	<b>Conductivity mS/cm</b>	<b>NH<sub>4</sub>-N g/L</b>
<b>r<sup>2</sup></b>	0.882	0.607	0.830
<b>RMSEP</b>	0.123	2.86	0.45
<b>RPD</b>	2.91	1.65	2.47
<b>RER</b>	11.71	7.12	11.07

<b>Statistic</b>	<b>Total Dissolved N g/L</b>	<b>Suspended N g/L</b>	<b>Soluble ReactiveP g/L</b>
<b>r<sup>2</sup></b>	0.875	0.874	0.769
<b>RMSEP</b>	0.44	0.27	0.35
<b>RPD</b>	2.75	2.96	2.01
<b>RER</b>	12.66	15.07	10.76

<b>Statistic</b>	<b>Total Dissolved P g/L</b>	<b>Suspended P g/L</b>	<b>Suspended C g/L</b>
<b>r<sup>2</sup></b>	0.898	0.920	0.866
<b>RMSEP</b>	0.23	0.16	3.69
<b>RPD</b>	3.11	3.58	2.71
<b>RER</b>	16.54	16.54	14.77

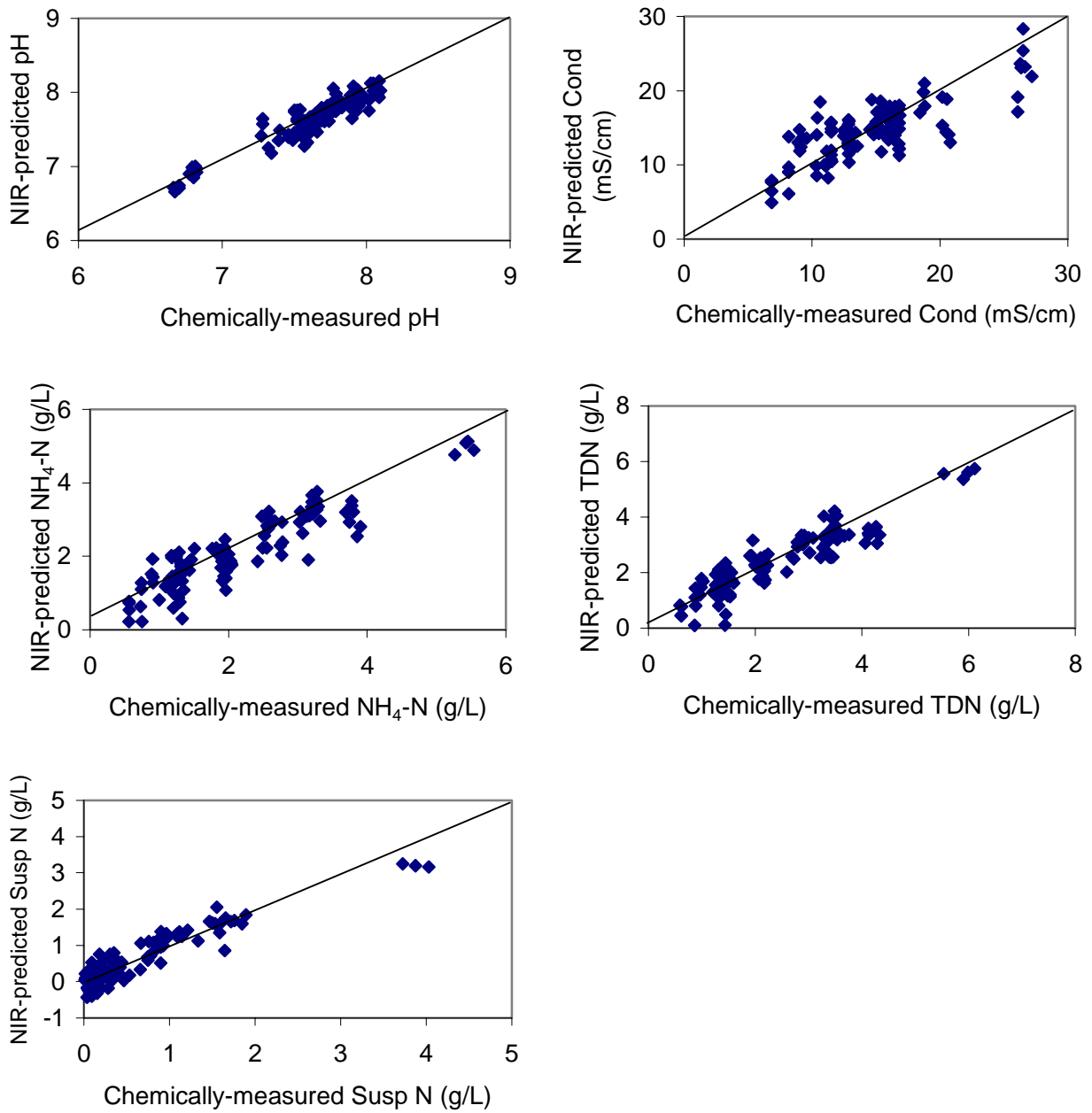


Fig. 4. Linear regression relationships for NIR-predicted vs chemically-measured reference values for pH, conductivity, and nutrients. The line is 1:1, not the best fit through the points.

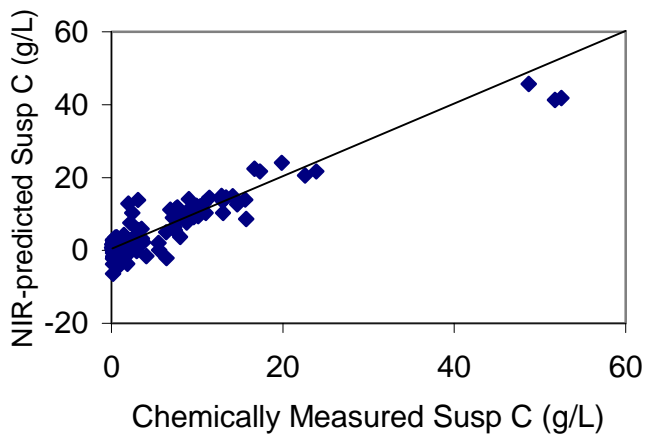
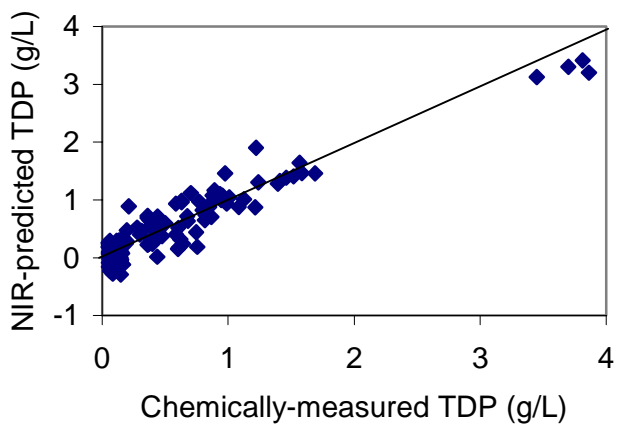
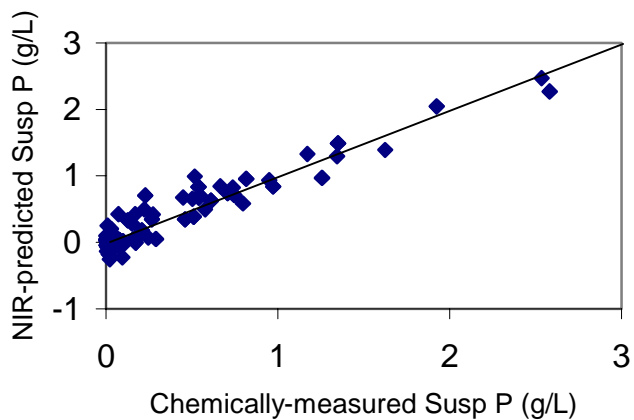
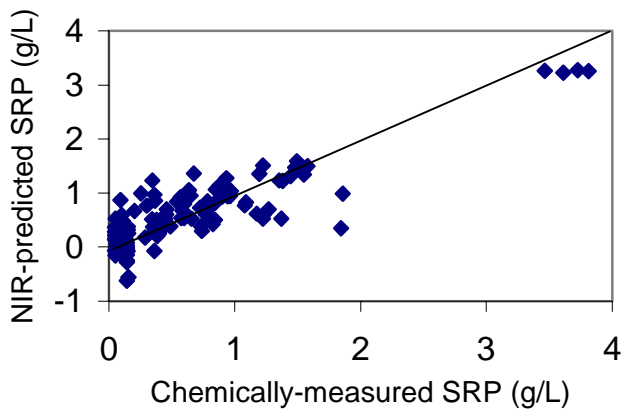


Fig. 4 cont'd

## Discussion

The calibrations developed for pH, several forms of N and P, and for suspended C from spectra recorded by the Corona on hog manure indicate that the instrument has promise for the rapid, on-site measurement of these constituents. There are several steps to be taken in further evaluating the performance of the instrument. Further work with The Unscrambler may improve the calibration success. For example, Fig. 2 showed that some of the spectra were noisy at wavelengths between 938 and 1000 nm and above 1400 nm. Restricting the wavelength region over which calibrations are developed may improve the results by eliminating areas of noise. Ultimately, calibrations to run the instrument must be developed in the operating software of the instrument or be importable into it. Other methods of sample presentation, such as the use of fibre optic probes may lead to improved calibration success. The calibrations based on the fall 2000 samples need to be validated using the future samples scanned with this instrument.

The evaluation of an instrument for an new application and new operating conditions, such as in the field, is complex (Malley, Martin, and Woods (2001)). The choice among instruments and presentation methods will be guided in part by the accuracy that is required for various nutrients in various application situations. Ultimately, the decision to utilize NIRS for rapid, on-site analysis of hog manure, and the choice of instrument, will depend on the expectation of achieving economic, environmental, and/or regulatory benefits that exceed the cost of employing the technology.

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