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# Characterizing Phosphorus Forms in Saskatchewan Soils Using Solution $^{31}\text{P}$ NMR Spectroscopy

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## Abstract

In soils and other environmental samples, phosphorus (P) may be found in a range of inorganic and organic forms. Inorganic P forms include orthophosphate, which is readily available to plants, and complex inorganic P forms such as pyrophosphate and polyphosphate. Organic P forms can be divided into groups such as orthophosphate monoesters (e.g. sugar phosphates, phytic acid) and orthophosphate diesters (e.g. phospholipids, DNA) and phosphonates. Identifying P forms is important to enhance crop growth, with and without fertilization, and to minimize P loss to water, where it can cause harmful algal blooms. This presentation describes some of our recent research using solution  $^{31}\text{P}$  NMR spectroscopy to characterize P forms in a range of Saskatchewan soils, as well as swine manure.

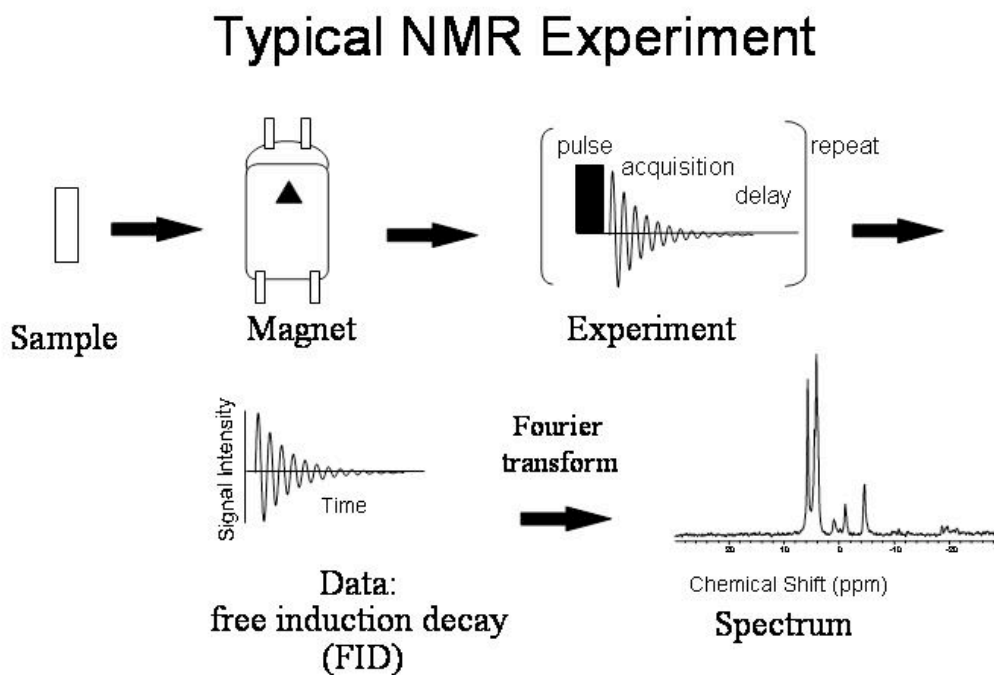
## Introduction

Phosphorus is an essential element for all organisms. Too little P is a concern for plant growth, so P fertilizers are used. However, excess P in aquatic systems can trigger blooms of harmful algae. Phosphorus has traditionally been difficult to study relative to other nutrients such as nitrogen (N), particularly with respect to characterizing P forms. However, advances have been made in our understanding of P forms by using Nuclear Magnetic Resonance (NMR) spectroscopy. For P, this is a quantitative technique, because the only natural P isotope is  $^{31}\text{P}$ , which is 100% NMR visible. Both solid-state and solution P-NMR are possible, but solution is preferred because it allows the sample to be concentrated and gives better spectral resolution, for better peak identification. Dr. Cade-Menun, an expert in  $^{31}\text{P}$  NMR analysis of environmental samples such as soils, manure, plants and water, began working at SPARC in March 2008, and recently acquired the necessary probe to analyze P in environmental samples by NMR spectroscopy at the Saskatchewan Structural Sciences Centre at the University of Saskatchewan. Presented here are some preliminary results of ongoing research projects.

## Methods

The equipment required for an NMR experiment is an NMR spectrometer, consisting of a magnet and console, with a probe for the magnet that can be tuned to the frequency of P. The solid sample (soil, manure, plant material, sediments, etc) is extracted with NaOH-EDTA and freeze-dried to concentrate the P. It is redissolved in a small volume of NaOH and placed in an NMR tube. In a typical NMR experiment (Fig. 1), the sample tube is placed in the probe in the magnet. A radio-frequency pulse is applied, which excites the P nuclei, followed by a delay to allow the nuclei to relax back to equilibrium. The energy emitted from the nuclei during relaxation is measured (termed "acquisition"). The shielding of the P nuclei (i.e. the molecules in

which the P nuclei are contained), will determine the degree of excitation and relaxation, and thus the amount of signal acquired for each P nucleus, and in turn each P form. This pulse-acquisition-delay experiment is repeated as many times as necessary to get enough signal to produce a clear spectrum, and usually ranges from 200 to 8000 times in a typical NMR experiment on environmental samples. The collected data is a frequency induction decay (FID), plotted as signal intensity over time. This is converted to a spectrum by Fourier transformation, using NMR software (Fig. 1).



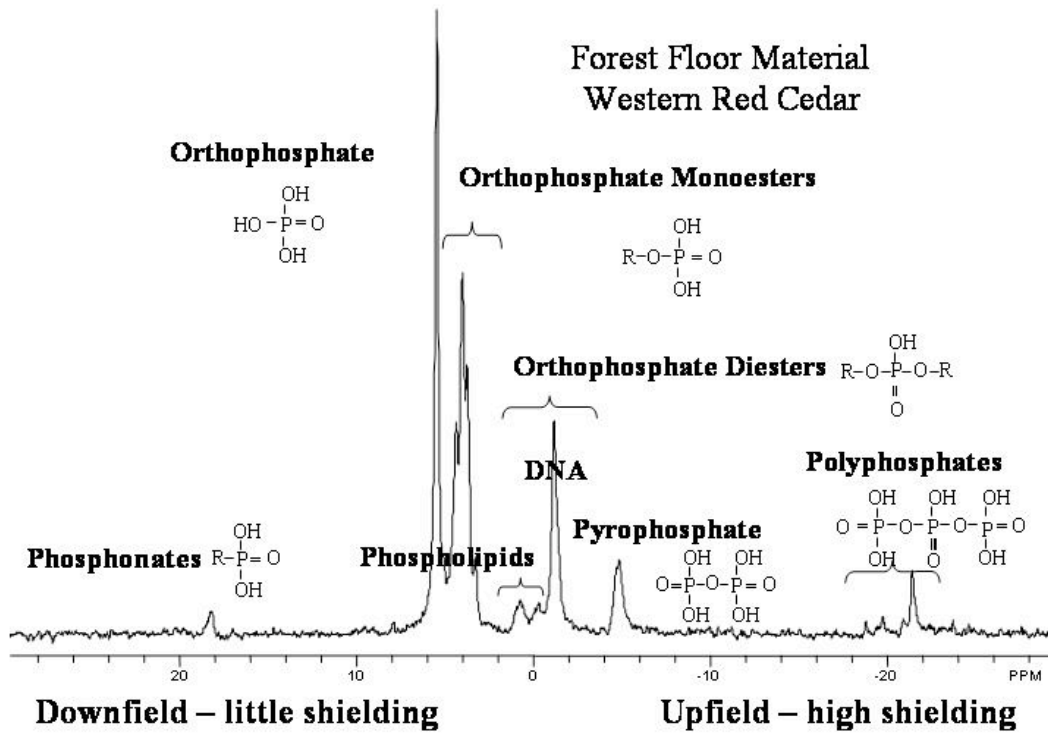
**Figure 1:** The steps of a typical solution  $^{31}\text{P}$  NMR experiment.

The  $^{31}\text{P}$  NMR spectrum of an environmental sample may contain some or all of the P forms of the spectrum shown in Figure 2, which is a spectrum for forest floor material under western red cedar. Peaks for P nuclei with little shielding (e.g. phosphonates) are found to the left (downfield), while those with more shielding, such as polyphosphates, are found to the right (upfield). The areas under each peak can be determined by integration using NMR software. Because all P is visible in an NMR spectrum, peak areas can then be converted to concentration if the total P concentration of the sample in the NMR tube is known. For more information on  $^{31}\text{P}$  NMR experiments for environmental samples, please see Cade-Menun (2005). For more information on P chemistry and P forms in environmental samples, please see Condon et al., (2005).

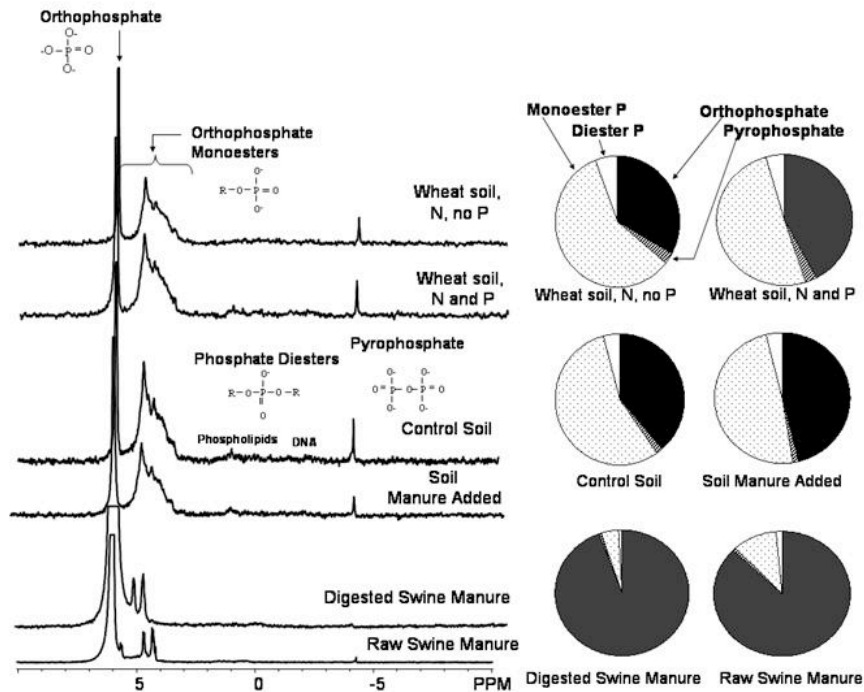
### Preliminary Results

Some of our on-going Saskatchewan projects include characterizing P forms in long-term wheat rotations and characterizing P forms in swine manure and soils to which it is applied.

Preliminary results show that all Saskatchewan soils examined thus far contain many P compounds and are high in organic P.



**Figure 2:** An example of a  $^{31}\text{P}$  NMR spectrum, showing the P forms that may be present in environmental samples.



**Figure 3:** Preliminary results for Saskatchewan soils and swine manure.

The long term wheat rotation plots were established in 1967, and have received N ( $41 \text{ kg N ha}^{-1} \text{ y}^{-1}$ ) and P ( $10 \text{ kg P ha}^{-1} \text{ y}^{-1}$ ) fertilizers annually. In 1993, a sub-plot of each field without P fertilization was established. We compared P forms in samples collected in 2005 from plots with and without P fertilization (Fig. 3 top). Samples receiving P fertilizers contained more inorganic P, particularly orthophosphate. In the samples from plots where P fertilization stopped in 1993 (the N, no P plots), orthophosphate had decreased 12 years after fertilization stopped, and organic P forms, particularly orthophosphate monoesters, were the dominant P forms.

Animal manures are an environmental concern because they contain P as phytic acid, a complex organic P form containing six phosphate groups. This is a component of animal feed, but cannot be digested by animals and thus is released in manures. When manures are applied to soil, phytic acid accumulates. It is not an available P form for terrestrial plants, so doesn't work as a fertilizer. However, aquatic organisms can use phytic acid as a P source, so if it is transferred to aquatic systems it can contribute to harmful algal blooms.

We examined swine manure with and without anaerobic digestion (used to capture methane for energy), as well as soil after application of this swine manure (Fig. 3, middle and bottom). The P in both digested and raw swine manure was predominantly orthophosphate. The remainder was phytic acid – 10% in raw manure and 5% in digested manure. This indicates that anaerobic digestion helps to convert phytic acid to orthophosphate in swine manure, thus increasing the plant-available P. Adding swine manure to soil increased the orthophosphate in soil, and lowered the relative percentage of organic P.

## Summary

In summary, we are now set up to use  $^{31}\text{P}$  NMR spectroscopy to characterize P forms in soils and environmental samples in Saskatchewan. Our preliminary results suggest that Saskatchewan soils contain a wide range of organic and inorganic P forms, and these will be altered by fertilization with manure and chemical fertilizers. We welcome the opportunity to develop collaborative research projects with others who are interested in using this technique.

## References

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