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**Organic P Transformation Reactions, and
Transport in Soils monitored by ^{31}P NMR
Spectroscopy**

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C. ABSTRACT

Organic-P mineralization and sorption in soils play a major role in plant P nutrition, P mobility in soil and P transport in run-off water. Despite their considerable importance to agriculture and to the environment organic-P transformations and retention reactions in soil are poorly understood, presumably due to unsatisfactory analytical techniques to determine concentration of various organic-P compounds in soil and soil extracts.

The objective of the cooperative research was to study and improve high resolution NMR techniques in order to facilitate simultaneous analyses of important organic and inorganic-P compounds extracted from soils, and develop techniques for the direct determination of solid phase organic and inorganic-P. The P compounds that were studied are orthophosphate, pyrophosphate, phytic acid (inositol hexaphosphate), glycerophosphate and RNA.

The effectiveness of various organic and inorganic-P extraction methods, and the NMR analysis of P species extracted from different soils were studied in UC Riverside. With the most advanced equipment, ^{31}P NMR peaks could be detected for concentrations of 45 mg P/L solution, or higher. With current methods it was impossible to distinguish between the resonances of orthophosphate and phytic acid when found in the same solution. The combined incubation, extraction and NMR experiments proved that soil organic-P mineralization was best characterized by the decomposition of phytic acid.

Work on MAS NMR analysis of solid phase-P was carried out in Israel. Peaks of the above mentioned organic and inorganic-P compounds, which were added to the Gilat soil and to gibbsite ($\text{Al}(\text{OH})_3$), were detected and characterized in terms of optimal apparatus set up. It was possible to measure signals from single compounds at a concentration of 1 g P/kg soil (or gibbsite), or higher, in reasonable time. It was impossible to distinguish between signals of the studied P compounds when they were found in a mixture on the solid phase. Cross polarization could not help with this respect.

d. OBJECTIVES OF THE ORIGINAL RESEARCH PROPOSAL

The original research proposal IS-1610-89, consisted of four objectives aimed to quantify the extent and rate of organic-P mineralization and transport, and inorganic-P immobilization in soil as affected by soil and environmental factors.

BARD decided to support our proposal for one year as a feasibility study based on objective 4 of the original proposal. This objective was to refine and improve high-resolution NMR techniques in order to facilitate simultaneous analyses of major organic-P compounds extracted from soils and develop techniques for the direct determination of solid phase organic and inorganic P in soils.

e. BODY OF THE REPORT

EXTRACTABILITY AND ^{31}P MAS NMR OF ORGANIC PHOSPHORUS IN SOILS

A.C. Chang, B. Bar-Yosef, and M.E. Bishop

Introduction

Soil-P is characterized by several organic and inorganic compounds and pools. The organic-P (Po) exists at concentrations which vary from 20 to 650 mg P/kg soil (Thompson et al, 1954; Dalal, 1977; Tate and Newman, 1982), depending on soil parent materials, climatic conditions and cropping sequence. Soil Po consists mainly of esters of o-phosphoric acid. The most important esters are those with inositol ($\text{C}_6\text{H}_{12}\text{O}_6$). The most common ester in soils is myo-inositol hexaphosphoric acid (IHP, or phytic acid, $\text{C}_6\text{H}_6(\text{OPO}_3\text{H}_2)_6$). Together with inositol pentaphosphate, a somewhat less abundant compound, it comprises about 60% of soil Po (Tiesen et al, 1984). IHP is very stable in alkaline solutions but gradually hydrolyses in acid media, at an optimum rate around pH 4. It is also hydrolyzed by phytase enzymes (Anderson, 1980; Frolich et al, 1988). The ester forms a number of very insoluble salts (K_{sp} data unavailable) and strong complexes with proteins (Anderson, 1980). Due to its reactive o-phosphate groups $[(\text{RO})\text{PO}_3\text{H}_2]$, IHP is prone to strong adsorption (Goring and Bartholomew, 1952; Anderson et al, 1974). The dependency of IHP adsorption on pH, ionic strength and solution-to-soil ratio, and the competition between IHP and

o-phosphate on common adsorption sites (Evans, 1985) have yet to be quantified.

Choline phosphate is another o-phosphate monoester found in soils. Due to difficulties in its separation, extraction and identification, very little is known about its abundance and reactivity in soils (Condon et al, 1985).

Another family of Po compounds in soils is o-phosphate diesters, characterized by the form $(RO)PO_2(OR)$. The compounds include nucleotides (mainly RNA and DNA) and phospholipids (predominantly phosphoglycerides), which usually constitute 0.5-2% and 5% of total Po in soil, respectively (Anderson, 1980). The diesters have a lower charge density per molecule than IHP, and their P-groups are shielded from ionic interaction (Jenkinson and Ladd, 1981). This renders them more soluble than o-phosphate monoesters, and enhances their mobility in soil and accessibility to soil enzymes.

Organic-P compounds other than P-mono- or diesters comprise 20- to 40% of total Po in soils, and have yet to be identified (Anderson, 1980; Condon et al, 1985).

Although the o-phosphate diester content in soil is relatively low, it may contribute to P uptake by plants more than o-phosphate monoesters, owing to its higher water solubility and mobility. The relative mobility of the various Po compounds in soils cannot be assessed yet, due to insufficient information on their partitioning between solid and liquid phases of soils (Goring and Bartholomew, 1952).

Various sequential extraction procedures to determine P fractions in soils have been suggested (Olsen and Somner, 1982; Hinedi et al, 1988;

Bowman, 1989). The most comprehensive procedure is a combination of Tiessen et al (1983) and Condon et al (1985) extraction which includes 0.5 M, NaHCO_3 , 0.1 M NaOH, 0.5 M HCl, 0.2 M acetylacetone, 0.5 M HCl, and 0.5 M NaOH, consecutively. This combined procedure is analytically simple and does not require drastic extractions as did previous methods (Mehta et al, 1954). It also minimizes Po hydrolysis during the acid extractions due to intermediate NaOH extractions. Hinedi et al. (1988) reported that high recovery of organic-P from sludge-treated soils was obtained by a single 0.5 M NaOH extract and by a two steps trichloroacetic acid/KOH extract.

The sequential extractions yield various Po and Pi compounds in each extract. Current colorimetric and chromatographic methods (Anderson and Malcolm, 1974; Masella and Cantafora, 1988) to determine the solution concentration of these species are very tedious and imprecise. Consequently, most reports on P extraction from soils were expressed as total-P and total inorganic-P. Total-P in extracts was obtained by oxidizing the Po, and analysing the o-phosphate by routine colorimetric procedures. Total-Po was estimated as (total-P) - (total-Pi).

The colorimetric method of o-phosphate analysis introduces a serious problem, since up to 90% of Po in the solution can be hydrolyzed in the presence of the acidic molybdate reagent (Ringler, 1964). This means that the Pi values obtained in the presence of Po are doubtful.

NMR techniques, which have but recently been introduced in the study of P in soils (Tate and Newman, 1982; Condon et al, 1985; Hinedi et al, 1988), are free of the above-mentioned analytical limitations. The technique enables one to examine the chemical nature of a substance by

focusing on the micro-environment surrounding one of the nucleus of a molecule, thus allowing to quantitatively determine concentration of different P species in solution and in solid phases. Tate and Newman (1982) reported very good agreement between total Po determined by routine procedures and the sum of Po as determined by NMR analysis of soil extracts. Those authors, and later, Condon et al. (1985), successfully determined known concentrations of o-phosphate mono- and di-esters, and polyphosphate in aqueous solutions.

Despite the encouraging reports and general knowledge in ^{31}P NMR spectroscopy (Gorensteine, 1984), some problems with the NMR methodology have yet to be resolved: (i) overlaps in the chemical shift between P-lipids, Pi, ATP and pyrophosphate; (ii) low sensitivity for various P species. The low sensitivity stems from instrument limitations, and from (iii) the presence of paramagnetic cations in the soil extracts. Available high-resolution NMR spectrometers can readily detect 0.1 mM of a specific P species in solution, but in some steps of the sequential extraction, this is insufficient, and test solutions must be concentrated by ultrafiltration (Tate and Newman, 1982) or by liophilization (Hinedi et al, 1988).

Cross polarization (CP) magic angle spinning (MAS) NMR allows a direct analysis of sorbed P in minerals and soils (solid state NMR) (Williams et al, 1981; Aue et al, 1984; Hinedi and Chang, 1989; Hinedi et al, 1992). This method may open new avenues in the study of soil P, since it saves soil extractions and reflects the nature of soil-P, since it saves soil extractions and reflects the nature of soil-P chemical bonds. However, the presence of Fe and Mn in the soil matrix may hinder such a direct analysis.

Objectives

The overall goal of this study is to explore the usage of NMR spectroscopy for determining chemical forms of P in soils. Specific objectives are outlined as follows:

1. To evaluate the ability of two extraction procedures (KOH and TCA/KOH) in extracting P from soils and identifying the P species by ^{31}P NMR.
2. To examine the enzymatic hydrolysis of organic P in the soil.

Methods and Procedures

The experiments of this study were divided into several phases. The experimental methods of each phase are summarized as follows:

1. Comparing Soil P Extractions by TAC/KOH and KOH Procedures:

After a preliminary screening, 8 soils were selected to test the P extracting procedures. Depner and Mounthat soils were two virgin andic soils from the forests of northeastern California. Osorno and Kaiwika were and soils obtained from agricultural fields of Chile and Hawaii, respectively. Greenfield and Domino represented two municipal sludge-treated soils whose P solid phases were identified by solid-state ^{31}P NMR (Hinedi and Chang, 1989). Lost Hills was an agricultural soil found in San Joaquin Valley of California. Greenfield, Domino, and Lost Hills were calcareous soils. We also included a high P soil from Washington in this experiment. The procedures outlined in Olsen and Somers (1982) were followed to determine the soil P. Total P of these soils was determined by

the fusion method and the inorganic P was determined according to the extraction method of Mehta et al (1957). The organic P was calculated as the balance of total P and inorganic P.

For the TCA/KOH extraction, 5 g of soil with 25 ml of 10% TCA was sonicated for 3 min. and centrifuged. The supernatant was treated with Na-saturated Chelex-100 and then IR-120 exchange resins and neutralized to pH 7 for the NMR experiment. The residue was washed with sodium acetate saturated ethanol and then extracted with 25 ml of 1.0 M KOH (equilibrated for 15 h at 37 °C) and centrifuged. The supernatant was neutralized and used for the NMR experiment.

In addition to the TCA/KOH extraction, three variations of the one step KOH extraction method were tested:

- * 5 g of soil incubated with 25 ml of 0.5 M KOH for 15 h at 37°C.
- * 5 g of soil incubated with 0.5 M KOH for 15 h at room temperature (21°C) and then incubated for additional 15 h at 90°C.
- * 5 g of soil extracted twice with 25 ml of 0.5 M KOH at 15 h incubation time and room temperature.

The extracts were neutralized to pH 7 for NMR experiments.

2. Effects of pH on ^{31}P NMR Chemical Shifts of Selected Organic and Inorganic Compounds.

In this experiment, we used potassium dihydrogen phosphate (KH_2PO_4), phytic acid (PA), tetrasodium pyrophosphate (PP), and glycerophosphate (GP) as the testing compounds. Reagent grade compounds were used to make solutions with P concentrations at 2.25, 22.5, and 225 mg P/L. Mixtures of

several compounds at these P concentrations were also prepared. The solutions were then adjusted to pH 2, 7, 9 and 12 with NaOH or HNO₃.

The ³¹P NMR spectra of the solutions were obtained with 5 ml of samples in 12 mm NMR tubes using wide bore NT-300 MHz superconducting spectrometer at 121.468 MHz and 22-24°C. A single pulse sequence (with a 45° pulse for 20 τ sec followed by 2 sec of relaxation time) was used. The gated proton decoupler was used in some cases to increase resolution of the spectra. the number of accumulations varied from 16 to 2300 scans.

3. P Mineralization of Soils Amended with Selected Organic and Inorganic P Compounds

Experiment A - Incubation of P Amended Soil

A loess soil from Gilat (Israel) was treated with 0.4, 4, 40 mg P per g of soil in the form of KH₂PO₄, PA, PP, and GP. The treated soils along with blanks were equilibrated to 80% of the field capacity and incubated at 30 °C for 1, 4, and 8 weeks. The soil samples were dried and extracted according to the TCA/KOH procedure outlined in part 1 of this section. Extracts obtained from soils containing 0.4 and 4 mg P per g soil were further concentrated by freeze drying and redissolving in smaller volume of water. The ³¹P NMR spectra of the extracts were determined on a General Electric 500 MHz superconducting spectrometer at 202.457 MHz. A single pulse sequence with 100 τ sec delay was used. The number of accumulations varied from 1000 to 31000 scans. Known amounts of 2-aminoethylphosphonic acid was added into the extract at the time of the experiment and used as an external standard in all the NMR runs.

The TCA/KOH extraction was compared with the combined and modified Tiessen et al. (1983) - Condron et al (1985) procedures. The combined procedure included the following steps: (1) 0.5 g soil was extracted with 25 ml of 1 M NH_4Cl for 30 min. (2) The soil residue was extracted with 25 ml 1 M NaHCO_3 for 1 h. (3) Soil residue extracted with 25 ml of 0.1 M NaOH + 1 M NaCl for 20 h. (4) Soil residue extracted with 25 ml of 0.5 M HCl for 4 h. (5) Soil residue extracted with 25 ml of 0.5 M NaOH for 20 h. (6) a. heating the remaining soil for 1 h at 550C then adding 1 M HCl for 15 h. b. wet digest of soil residue with HClO_4 .

Experiment B - Enzymatic Hydrolysis of Soil Organic P

The Osorno soil was sterilized by gamma ray irradiated, brought to 80% of the soil's field capacity, and treated with P enzymes: alkaline phosphatase, acid phosphatase, phosphodiesterase, and phytase. The enzyme treated soils were then incubated overnight at 37 °C. At the end of the incubation, soils were extracted by the KOH extraction and the P in extracts were analyzed by ^{31}P NMR. The same experimental conditions described in Experiment A were used for the NMR assays. As each enzyme catalyze a specific biochemical reaction, the result of this experiment would permit us to determine components of the soil organic P that was susceptible to biological decomposition.

Results and Discussion

In the course of this experiment, a large number of ^{31}P NMR spectra was generated. They were carefully examined and catalogued. The discussions presented hereafter are based on the analysis of the entire data set. But

it is not possible to present all of them and we selected the pertinent ones to illustrate the results. The complete data set may be obtained upon request.

1. Comparison of the Soil P extraction Procedures

The total P concentration of the soils selected for testing the extraction procedures, measured on the dry weight basis, ranged from 926 to 3860 mg P kg⁻¹ and the organic P accounted for 22-65% of the total P (Tables 1 and 2). In the TCA/KOH extraction procedure, the TCA extractant was expected to remove the inorganic P that was associated with Ca and a portion of the P monoesters. The second reagent, 1.0 M KOH, would extract the remainder of the organic P and any Al- and Fe-related inorganic P present in the soil. For the four andic soils (Depner, Mounthat, Osorno, and Kaiwika), TCA extracted merely 2-8% of the total soil P. Although the KOH was far more effective in extracting the soil P, no more than 50% of the total P in these 4 soils was recovered by the TCA/KOH two-step extraction. The NMR spectra indicated that majority of the P in the KOH extract of these four soils were the Al- and Fe-related inorganic orthophosphate. Smaller amounts of organic P in the form P monoesters were also detected.

In the calcareous soils (Greenfield, Domino and Lost Hills), over 70% of the extracted P was in the TCA fraction (Table 1) and the NMR spectra of these extracts revealed only the orthophosphate peak (spectra may be obtained upon request). The KOH extracted <10% of the total P from these three calcareous soils indicating the organic P was not effectively extracted or the extracted organic P was hydrolyzed into the

orthophosphate. Because the P concentrations were low, no ^{31}P NMR signal was detected in the KOH extracts. The actual P recoveries of these 8 soils varies from 19% for Kaiwiki soil to 57% for the Lost Hills soil (Table 2).

Three variations of the KOH one-step extraction procedures were tested to find ways of increasing soil P recovery and reducing hydrolysis of organic P during the extraction (Tables 3 and 4). In this case, only the four andic soil were evaluated. The result of the testing indicated that reducing the strength of KOH from 1.0 M to 0.5 M did not reduce the P recovery. Temperature and duration of equilibration did not significantly affect the outcome of extraction. Extracting the soils twice with 0.5 M KOH slightly improved the P recovery. In no case, however, more than 35% of soil P was recovered by the KOH one-step extraction. Although it was far from perfect, the TCA/KOH extraction was a better procedure than the KOH extraction for recovering soil P.

2. Effects of pH on the ^{31}P NMR Chemical Shifts of P Compounds

The chemical shifts of ^{31}P NMR are sensitive to pH changes and, relative to 85% phosphoric acid external standard, the pH dependent chemical shifts of inorganic orthophosphate may vary from 5.5 to 0 ppm (Crutchfield et al, 1962; Gadian et al, 1979). Its effect was illustrated by the chemical shifts KH_2PO_4 measured at pH 2, 7, 9 and 12 (Table 5). Increasing the acidity of orthophosphate ion increases the shielding of P nucleus resulting in corresponding downfield shift of the chemical shifts from -0.23 ppm to 3.32 ppm, as pH of the 225 mg P L^{-1} solution changed from 2 to 12. For the same pH changes, the ^{31}P NMR chemical shifts of pyrophosphate

ranged from -10.82 ppm to -6.61 ppm. Both orthophosphate and pyrophosphate ions produced well resolved single resonance NMR signals.

The NMR spectra of glycerophosphate and phytic acid, however, exhibited multiple resonances and these signals were also affected by pH of the solutions. We are unable to locate any information on the ^{31}P NMR spectrum of glycerophosphate and are not sure of the reasons why no signal was detected from spectra of glycerophosphate solutions at pH 7 and 9. As the glycerophosphate we used for the experiment was a reagent grade compound, there was little reason to expect multiple-resonance spectra. Zuiderweg et al (1979) and Emsley and Niazi (1982) investigated the ^{31}P NMR of inositol hexaphosphate over a wide range of pH. They showed that the NMR spectra of this compound consisted of four resonances. They also noted broadening of the signals as the pH changed. The spectra of phytic acid solutions we tested also consisted of a group of 3-4 signals located around or downfield from 0 ppm (Table 5). This group of resonances corresponded to those reported by Zuiderweg et al (1979) and Emsley and Niazi (1982). A signal at -16.2 ppm appeared consistently in almost all the spectra we collected and it probably represented a product of degradation or a contaminant in the phytic acid specimen. As the phytic acid is stable and not likely to degrade under acidic conditions, the presence of the -16.2 ppm signal at pH = 2 appeared to indicate that this signal represented the presence of a contaminant. At the present, we are unable to assign it. Based on the chemical shift positions, it is apparent that the resonances of phytic acid and orthophosphate would overlap on a spectrum. If a soil containing all four of the above P compounds is examined by the ^{31}P NMR, it may be difficult to definitely identify them on the basis of their chemical

shift only (Figure 1). Methods need to be developed to provide independent confirmation of the assignments.

A primary constraint of using ^{31}P NMR for quantitative determination of P relies probably on the method's inherent lack of sensitivity relative to other analytical techniques. Because the small magnitude of the energy changes involved in NMR transition is not always distinguishable from the noise. As a result, the outcomes of NMR experiment of samples with low P concentrations and complex matrix are not always satisfactory. Experimentally, the sensitivity of NMR determination may be increased by increasing the strength of the applied magnetic field and the number of spectra accumulated. In this experiment, we were unable to obtain any ^{31}P NMR signal for solutions containing 2.25 and 22.5 mg P L⁻¹ even after we accumulated 2300 scans (Figure 2). Therefore, we determined the ^{31}P NMR of a set of phytic acid solutions with 135, 90, and 45 mg P L⁻¹. It appeared that ^{31}P NMR resonances were detectable for P concentration as low as 45 mg L⁻¹ (Figure 3). The signal to noise ratio of the spectra, however, continued to deteriorate as the concentration of P decreased.

3. Organic P mineralization in soils

The amounts of TCA/KOH extracted P of the Gilat soil are summarized in Table 6. With a few exceptions, between 60 to 80% of the added P were recovered. The extraction efficiency, in this case, was considerably better than the outcome of an earlier experiment where indigenous soil P was extracted using the same procedure (Table 2). Majority of the P recovered from the P amended Gilat soil was found in the TCA fraction.

These results can be compared with the sequential modified Tiessen-Condron extractions procedure. The comparison was made between samples 3B, 4D in Table 6, and the corresponding "control" and "4 x 0.1 mg P" treatments in Table 6a.

The TCA extracted-P in sample 3B (0.81 mg/g soil) was somewhat greater than the amount of P extracted by HCl (0.62 mg P/g soil, extractions 4 and 6, Table 6a). The KOH extracted-P (0.16 mg P/g) was similar to the amount of P extracted by NaOH (0.17 mg P/g, extractions 3 and 5, Table 6a). In the untreated (control) soil, the HCl extracted-P (Table 6a) exceeded the TCA extracted-P (Table 6), 0.61 vs 0.54 mg P/g soil, while the NaOH and KOH extracts gave very similar results (0.12 vs 0.13 mg P/g soil).

The extraction efficiency in the TCA/KOH method, defined as $[(\text{Extracted P in treated soil}) - (\text{Extracted P in untreated soil})]/(\text{Added P to treated soil})$, declined as the P enrichment level increased (116%, 70% and 66% at the 0.4, 4 and 40 mg P/g enrichment levels, Table 6). A similar decline in the extraction efficiency was obtained in another experiment in which the Gilat soil was loaded with a mixture of five P compounds at different P levels, and extracted by the same sequential extraction method (untreated soil, 2, 5 and 50 mg P/g, Table 6b). Here, the sequential extraction efficiency declined from 75% to 60% and 48% as P enrichment level increased. The decline in both cases was more pronounced in the acid than in the base extraction.

Because of their low P concentrations, the TCA/KOH extracts of soil containing 4 and 0.4 mg g⁻¹ P addition did not produce any resolved NMR signal, even when the solutions were concentrated. Our analysis of the results therefore was based on NMR spectra of the soil extracts of soils

treated with 40 mg P g⁻¹. They showed that the P in TCA fractions were almost entirely orthophosphate as indicated by the peak at 0 to -0.5 ppm region (Figure 4). This observation is supported by the data in Table 6a which shows that the acid extraction (number 4) released predominantly inorganic-P.

The two small peaks, located around -9.5 ppm and -14.5 ppm, were probably associated with pyrophosphate and glycerophosphate. In the spectra of KOH fractions, resonances due to phytic acid (at 4.76 ppm), pyrophosphate (at -5.46 ppm), and inorganic P (at 5.74 ppm) were well resolved and a signal at 19.65 ppm was caused by the phosphoric acid standard (Fig. 5).

In the KOH extracts, approximately 30% of the recovered P was in the inorganic P form (Figure 5). When the P was added, it was equally divided among the four P compounds (KH₂PO₄, Phytic Acid, Pyrophosphate, and Glycerophosphate) on the basis of their P contents. If the P extraction was complete and the extractants did not cause any chemical degradation of P, the amount of inorganic P in the soil should not exceed 25% of the total. The amount of inorganic P found in the extracts we examined, however, exceeded this upper threshold indicating significant organic P hydrolysis had taken place during the course of extraction. On the contrary, there was little evidence that organic P compounds were hydrolyzed when pH of the P containing solutions were adjusted by HNO₃ and NaOH (Table 5). Perhaps, 10% trichloroacetic acid (TCA) was too strong for the extraction.

Based on the ³¹P NMR spectra of the KOH extracts, it was apparent that pyrophosphate was very stable in the soil and the intensity of its NMR resonance remained unchanged throughout 8 weeks of incubation. The two

minor peaks (at -9.5 ppm and -14.5 ppm) persisted in the TCA extracts of soils incubated for all the time intervals. It appeared that the glycerophosphate added into the soil probably did not decompose during this period of time. The phytic acid added into the soil, however, decreased with time of incubation. At the end of the 4th week, it was no longer detected in the soil extract. The disappearance of phytic acid corresponded in proportion to increase of inorganic P in the extracts.

An enzyme hydrolysis experiment was used to illustrate that the mineralization of organic P might readily take place in the soil. In this experiment, the gamma radiation treated Osorno soil was treated with phytase, phosphodiesterase, acid phosphatase, and alkaline phosphatase and incubated overnight. At the end of incubation, the enzyme-treated soils were extracted by KOH and extracts were assayed by ^{31}P NMR. Before enzyme treatments, the KOH extract of Osorno soil showed resonances of both inorganic and organic P (Figure 6). Only under the phytase treatment, the organic P signal disappeared indicating the organic P of the soil was inositol hexaphosphate and it could be mineralized by biological decomposition. It appeared that mineralization of soil organic P would be best characterized by biological decomposition of inositol hexaphosphate.

Summaries and Conclusions

1. Neither the TCA/KOH nor the KOH extraction was effective to extract soil P for ^{31}P NMR analysis in untreated soils because the recovery of indigenous soil P was low and TCA might have caused the soil organic P to be hydrolyzed into inorganic P.

The extraction efficiency of the Gilat treated soil, declined as P concentration in the soil increased. That was true in the TCA/KOH and in the sequential extraction methods.

2. Chemical shifts of ^{31}P NMR resonances of orthophosphate, phytic acid, glycerophosphate, and pyrophosphate were affected by the pH of the solution. Using orthophosphate and pyrophosphate as examples, the signals shifted downfield as the pH changed from 2 to 12. Because the NMR resonances of orthophosphate and phytic acid overlapped over the entire pH range, it would be difficult to assign these two compounds in a mixture. Other methods of assigning the NMR peaks of these two compounds need to be investigated.

3. The lack of sensitivity of ^{31}P NMR is a primary constraint of using this technique for quantitative determination of P. No ^{31}P NMR signals could be obtained for solutions containing 22 mg P/L even after 2300 scans were accumulated. ^{31}P NMR resonances were detected for P concentration of 45 mg P/L or higher.

4. The pyrophosphate and glycerophosphate appeared to be biologically stable in the soil. In the P amended soil, their amounts remained unchanged after 8 weeks of incubation. Phytic acid added into the soil disappeared after 4 weeks of incubation and its disappearance corresponded with an increase in the inorganic P.

5. Soil organic P mineralization was best characterized by the biological decomposition of inositol hexaphosphate.

References

Anderson, G. and R.E. Malcolm. 1974. The nature of alkali-soluble soil organic phosphates. J. Soil Sci. 25:292-297.

Anderson, G., E.G. Williams and J.O. Moir. 1974. A comparison of the sorption of the sorption of inorganic orthophosphate and inositol hexaphosphate by six acid soils. J. Soil Sci. 25:51-62.

Anderson, G. 1980. Assessing organic phosphorus in soils. In: Khasawneh, F.E., E.C. Sample and E.J. Kamprath (Eds. 1984. The role of phosphorus in Agriculture. Amer. Soc. of Agronomy, Madison, WI, pp.

Aue, W.P., A.H. Roufosse, M.J. Glimcher and R.G. Griffin. 1984. Solid state ^{31}P NMR studies for synthetic solid phases of calcium phosphate: potential models of bone mineral. Biochem. 23:6110-6114.

Bowman, R.A. 1989. A sequential extraction procedure with concentrated sulfuric acid and dilute base for soil organic phosphorus. Soil sci. Soc. Am. J. 53:362-366.

Condon, L.M., K.M. Goh and J.H. Newman. 1985. Nature and distribution of soil phosphorus as revealed by sequential extraction method followed by ^{31}P nuclear magnetic resonance analysis. J. Soil Sci. 36:199-207.

Crutchfield, M.M., C.F. Callis, R.R. Orai, and G.C. Roth. 1962. Phosphorus nuclear magnetic resonance studies of ortho and condensed phosphates. Inorganic Chem. 1: 813-817.

Dalal, R.C. 1977. Soil organic phosphorus. Adv. Agron. 29:83-117.

Emsley, J. and S.B. Niazi. 1981. The structure of myo-inositol hexaphosphate in solution: ^{31}P NMR investigation. Phosphorus and Sulfur. 10: 401-407.

Evans Jr, A. 1985. The adsorption of inorganic phosphate by a sandy soil as influenced by dissolved organic compounds. Soil Sci. 140:251-255.

Frolich, W., M. Wahlgren and T. Drakenberg. 1988. Studies on phytase activity in oats and wheat using ^{31}P NMR spectroscopy. J. Cereal Sci. 847-53.

Gadian, D.G., G.K. Radda, R.E. Richards, and P.J. Seeley. 1979. ³¹P NMR in living tissue: The road from a promising to an important tool in Biology. In: R.E. Schuman (ed.) Biological Applications of Magnetic Resonance. Academic Press, NY.

Gorenstein, D.G. 1984. ³¹P NMR. Academic Press, New York.

Goring, C.A. and W.V. Bartholomew. 1952. Adsorption of mononucleotides, nucleic acids and nucleoproteins by clays. Soil Sci. 74:149-164.

Hinedi, Z.R., A.C. Chang and W.K. Lee. 1988. Mineralization of phosphorus in sludge-amended soils monitored by phosphorus-31 nuclear magnetic resonance spectroscopy. Soil Sci. Soc Am. J. 52:1593-1596.

Hinedi, Z.R. and A.C. Chang. 1989. Solubility and ³¹P MAS NMR of phosphorus in sludge amended soils. Soil Sci. Soc. Am. J. (accepted).

Hinedi, Z.R., S. Goldberg, A.C. Chang, and J.P. Yesinowski. 1992. A ³¹P and ¹H MAS NMR study of phosphate sorption onto calcium carbonate. J. Colloid Interface Sci. 152: 141-160.

Jenkinson, D.S. and J.N. Ladd. 1981. Microbial biomass in soil: measurement and turnover. In: Soil Biochemistry Vol 5. Eds E.A Paul and J.N. Ladd. pp415-471. Marcel Dekker, New-York.

Masella, R. and A. Cantafora. 1988. Determination of phospholipids in biological samples by an improved densiometer method on thin layer chromatograms. Clinic Chimica Acta 176:63-70.

Mehta, N.C., J.O. Legg, C.A.I. Goring and C.A. Black. 1954. Determination of organic phosphorus in soils. I. Extraction methods. Soil Sci. Soc. Am. Proc. 18:443-449.

Olsen, S.R. and L.E. Somner. 1982. Phosphorus. In: A.L. Page et al (Ed). 1982. Methods of soil analysis. Part 2, 2nd edition. Agronomy 9:403-440. Am. Soc. of Agronomy, Madison, USA.

Rigler, F.H. 1964. The phosphorus fractions and the turnover times of inorganic phosphorus in different types of lakes. Limnol Oceanogr. 9:511-518.

Tate, K.R. and R.H. Newman. 1982. Phosphorus fractions of a climosequence of soils in New Zealand Tussock grassland. Soil Biol. Biochem. 14:191-196.

Thompson, L.M., C.A. Black and J.. Zoellner. 1954. Occurrence and mineralization of organic phosphorus in soils, with particular reference to association with nitrogen, carbon and pH. Soil Sci. 77:185-196.

Tiessen, H., J.W.B. Stewart and J.O. Moir. 1983. Changes in organic and inorganic phosphorus composition of two grassland soils and their particle size fractions during 60-90 years of cultivation. J. Soil Sci. 34:815-823.

Tiessen, H., J.W.R. Stewart and C.V. Cole. 1984. Pathways and transformations of P in soils of different pedogenesis. Soil Sci. Soc. Am. J. 48:853-858.

Williams, R.J.P., R.G.F. Giles and A.M. Posner. 1981. Solid state phosphorus NMR spectroscopy of minerals and soils J.C.S. Chem. Comm. 20:1051-1052.

Zuiderweg, E.R.P., G.G. M. van Beek and S.H. de Bruin. 1979. The influence of electrostatic interaction on the proton-binary behavior of myo-inositol hexakisphosphate. Eur. J. Biochem. 94: 297-306.

Table 1: Phosphorus contents of experimental soils.

Soil	Total P	Inorganic P	Organic P
Depner	2571	2001	571
Mounthat	2175	1665	510
Osorno	2004	703	1302
Kaiwiki	1207	479	729
Greenfield	3543	2513	1031
Domino	3864	2925	940
Lost Hills	926	724	202
Washington	1954	1354	600

Table 2: TCA/KOH extractable P of experimental soils.

Soil	TCA/KOH		Total P Extracted (mg kg ⁻¹)	Recovery	
	TCA	1.0 M KOH (mg kg ⁻¹)		Total P (%)	Organic P
Depner	111	721	832	32	126
Mounthat	134	162	796	37	130
Osorno	43	683	726	36	52
Kaiwiki	11	205	216	19	28
Greenfield	1593	405	1998	56	39
Domino	1291	499	1790	46	53
Lost Hills	462	70	532	57	35
Washington	684	285	969	50	48

Table 3: 0.5 M KOH extractable P of experimental soils.

	Extraction A	Extraction B	Extraction C
Description of extraction procedure	5 g of soil + 25 ml KOH, 15 h at 12 ⁰ C	5 g soil + 25 ml KOH, 15 h at 21 ⁰ C and followed by 15 h at 90 ⁰ C	5 g soil + 2 x (25 ml KOH, 15 h at 21 ⁰ C)
Soil	----- mg P kg ⁻¹ -----		
Depner	552	423	899
Mounthat	362	386	720
Osorno	431	340	499
Kaiwiki	174	330	295

Table 4: P recovery by the 0.5 M KOH extraction procedures

	Extraction A	Extraction B	Extraction C
Description of extraction procedure	5 g of soil + 25 ml KOH, 15 h at 12 ⁰ C	5 g soil + 25 ml KOH, 15 h at 21 ⁰ C and followed by 15 h at 90 ⁰ C	5 g soil + 2 x (25 ml KOH, 15 h at 21 ⁰ C)
Soil	----- % -----		
Depner	21	16	35
Mounthat	17	18	33
Osorno	22	17	25
Kaiwiki	14	27	24

Table 5: Chemical shifts of orthophosphate, pyrophosphate, glycerophosphate and phytic acid.

Compound	pH	Chemical shift (ppm)
KH_2PO_4	2	-0.234
	7	1.275
	9	2.305
	12	3.321
Tetra sodium pyrophosphate	2	-10.821
	7	-7.884
	9	-6.948
	12	-6.605
Glycerophosphate	2	9.474, -8.099
	7	No NMR signal obtained
	9	No NMR signal obtained
	12	4.357, 4.015, -8.122, -19.323
Phytic acid	2	-0.894-0.797, -16.227
	7	0.7-2.045
	9	1.403-2.363
	12	3.582-4.975, -16.2

Table 6: Recovery of the amended P in Gilat soil.

Sample*	P Treatment (mg P g ⁻¹)	P Extracted		
		TCA	KOH	Total
1A	40	25.29	2.45	28.10
1B	40	27.99	3.02	31.01
1C	40	14.54	2.87	17.41
1D	40	28.76	2.52	31.71
2A	4	3.11	0.51	3.62
2B	4	3.06	0.40	3.46
2C	4	3.25	0.19	3.44
2D	4	3.19	0.15	3.34
3A	0.4	0.85	0.12	0.97
3B	0.4	0.81	0.16	0.97
3C	0.4	0.89	0.57	1.47
3D	0.4	0.89	0.16	1.05
4A	0	0.54	0.13	0.67
4D	0	0.51	0.13	0.63

* Letter designations following the numerals A, B, C and D denote incubation time of 0, 1, 4, and 8 weeks, respectively.

Table 6a: Sequential extractions of treated and untreated Gilat soil* for organic- and inorganic-P. Treatments included mixtures of (i) phytic acid; (ii) glycerol phosphate; (iii) pyrophosphate, and (iv) monopotassium phosphate, each added at 0.1 mg P/g.

Extraction	Treatment					
	Control			4x0.1 mg P		
	Inorg	Org	Total	Inorg	Org	Total
----- Extracted P (mg P/g soil) -----						
1. 1M NH ₄ Cl	0.06	0.03	0.09	0.22	0.03	0.25
2. 0.5M NaHCO ₃	0.06	0.04	0.10	0.09	0.07	0.16
3. 0.1M NaOH+1N NaCl	0.02	0.05	0.07	0.04	0.07	0.11
4. 0.5M HCl	0.49	0.02	0.51	0.49	0.02	0.51
5. 0.5M NaOH	0.03	0.02	0.05	0.03	0.03	0.06
6. 1M HCl 500°C/HClO ₄	0.05	0.05	0.10	0.06	0.05	0.11
Total extracted	0.71	0.21	0.92	0.93	0.27	1.20
Total added (mg P/g soil)			0			0.40

* All results are means of two independent extractions, and of manured and unmanured soils. Means only are presented because differences between replicates and between manured and unmanured soils were less than 10%. Incubation time was 1 week.

Table 6b. Sequential extractions of treated and untreated Gilat soil* for organic- and inorganic-P. Treatments included mixtures of (i) phytic acid; (ii) RNA; (iii) Glycerol phosphate; (iv) pyrophosphate, and (v) monopotassium phosphate, each added at 0.4, 1 or 10 mg P/g soil.

Extraction	Treatment							
	Control		5x10 mg P		5x1 mg P		5x0.4 mg P	
	Inorg	Org	Inorg	Org	Inorg	Org	Inorg	Org
----- Extracted P (mg P/g soil) -----								
1 1M NH ₄ Cl	0.05	0.03	11.71	4.89	1.52	0.36	0.82	0.09
2 0.5M NaHCO ₃	0.06	0.06	1.33	1.91	0.42	0.35	0.21	0.10
3 0.1M NaOH1N NaCl	0.03	0.03	0.52	1.19	0.17	0.19	0.10	0.12
4 0.5M HCl	0.50	0.03	2.44	0.61	0.63	0.04	0.61	0.04
5 0.5M NaOH	0.03	0.03	0.08	0.13	0.04	0.04	0.04	0.05
6 1M HCl 500 °C/HClO ₄	0.14	0.03	0.15	0.06	0.14	0.05	0.14	0.05
Total extracted	0.82	0.23	16.23	8.78	2.93	1.02	1.96	0.55
	1.05		25.0		3.95		2.50	
Total added	0		50.0		5.0		2.0	

* All results are means of two independent extractions, and of chloroform fumigated and unfumigated soils. Means only are presented because difference among replicates and between fumigated and unfumigated soils were less than 10%.

List of Figures

- Figure 1: ^{31}P NMR spectrum of a solution mixture consisting of KH_2PO_4 , phytic acid, glycerophosphate and pyrophosphate at 225 mg P L^{-1} each.
- Figure 2: ^{31}P NMR spectrum of a phytic acid solution containing 22.5 mg P L^{-1} .
- Figure 3: ^{31}P NMR spectra of pH=2 phytic acid solutions containing 225, 135, 90, and 45 mg P L^{-1} , respectively.
- Figure 4: ^{31}P NMR spectra of trichloroacetic acid extractable P of Gilat soil amended with KH_2PO_4 , phytic acid, glycerophosphate, and pyrophosphate at 10 mg P g^{-1} and incubated at 30°C for 0 (1a), 1 (1b), 4 (1c), and 8 (1d) weeks.
- Figure 5: ^{31}P NMR spectra of KOH extractable P of Gilat soil amended with KH_2PO_4 , phytic acid, glycerophosphate, and pyrophosphate at 10 mg P g^{-1} each and incubated at 30°C for 9 (1a), 1 (1b), 4 (1c), and 8 (1d) weeks.
- Figure 6: Enzymatic hydrolysis of soil organic P by phytase.

MIXTURE: 225 PPM. PH 9

Figure 1. ^{31}P NMR spectrum of a solution mixture consisting of KH_2PO_4 , phytic acid, glycerophosphate, and pyrophosphate at 225 mg P L^{-1} each.

PHYTIC ACID 30PPM PH2

ONE-PULSE, DECOUPLER ON
DURING ACQUISITION
D2 = 20.00 USEC
D5 = 2.00 SEC

NA = 2300
SIZE = 8192
AQ = 512.00 MSEC
QPD ON = 1
ARC ON
BUTTERWORTH FILTER ON
DS ATT. = 3
AOC = 12 BITS
Q1 = 0
Q4 = 47- 4000.00
QW = 125
AQ = 10 USEC
OF = 125 USEC
IL HIGH POWER ON
F2 = 300.067201
BB MODULATION ON
CF = 995.39
QF = 121.460000
FM = 10.00

SCALE 400.00 HZ/CM
3.3330 PPM/CM

Figure 2. ^{31}P NMR spectrum of a phytic acid solution containing 22.5 mg P L⁻¹.

Figure 3. ^{31}P NMR spectra of pH=2 phytic acid solutions containing 225, 135, 90, and 45 mg P L $^{-1}$, respectively.

Obs Freq 202.4870475
 ObsPreq 500.1351376
 SW 4000
 FILTER 5000
 No. Points 4096
 No. Scans 2500
 Dwell Time 1.0000e-04
 Delay 6.00e-05
 Field 11.747
 Temp K 0
 Line Broad 10.00
 DEC Att 0
 Nucleus P31
 Sequence 1PULS
 Filename 8Y1A1.FPT
 Acq. Time 4.10e-01
 Scale 17
 Amplitude 1.30

peak 1 9337.64 3819.79 18.87

DOB 08JUL82
 Sample 1a, TCA extract, #129
 1PULS ONE PULSE SEQUENCE

Obs Freq 202.4870475
 ObsPreq 500.1351376
 SW 4000
 FILTER 5000
 No. Points 4096
 No. Scans 2500
 Dwell Time 1.0000e-04
 Delay 6.00e-05
 Field 11.747
 Temp K 0
 Line Broad 10.00
 DEC Att 0
 Nucleus P31
 Sequence 1PULS
 Filename 8Y1B.FPT
 Acq. Time 4.10e-01
 Scale 17
 Amplitude 1.30

peak 2 2419.26 3817.35 18.86
 142066.53 -18.10 -0.09

DOB 08JUL82
 Sample 1b, TCA extract, #131
 1PULS ONE PULSE SEQUENCE

Obs Freq 202.4870475
 ObsPreq 500.1351376
 SW 4000
 FILTER 5000
 No. Points 4096
 No. Scans 2500
 Dwell Time 1.0000e-04
 Delay 6.00e-05
 Field 11.747
 Temp K 0
 Line Broad 10.00
 DEC Att 0
 Nucleus P31
 Sequence 1PULS
 Filename 8Y1C.FPT
 Acq. Time 4.10e-01
 Scale 18
 Amplitude 1.30

DOB 09JUL82
 Sample 1c, TCA extract, #132
 1PULS ONE PULSE SEQUENCE

Obs Freq 202.4870475
 ObsPreq 500.1351376
 SW 4000
 FILTER 5000
 No. Points 4096
 No. Scans 2500
 Dwell Time 1.0000e-04
 Delay 6.00e-05
 Field 11.747
 Temp K 0
 Line Broad 10.00
 DEC Att 0
 Nucleus P31
 Sequence 1PULS
 Filename 8Y1D.FPT
 Acq. Time 4.10e-01
 Scale 17
 Amplitude 1.30

DOB 09JUL82
 Sample 1d, TCA extract, #134
 1PULS ONE PULSE SEQUENCE

Figure 4. ^{31}P NMR spectra of trichloroacetic acid extractable P of Gilat soil amended with KH_2PO_4 , phytic acid, glycerophosphate, and pyrophosphate at 10 mg P g^{-1} and incubated at 30 C for 0 (1a), 1 (1b), 4 (1c), and 8 (1d) weeks.

Obs Freq	202.4570475	peak	amp	Hz	ppm
acqPreq	500.1351378	1	295619.91	3993.13	19.72
SW	5000.0	2	136231.23	1173.31	5.80
FILTER	5000	3	88621.79	973.11	4.81
No. Points	4096	4	178944.66	-1089.87	-5.38
No. Scans	3700				
Dwell Time	1.00000e-04				
Delay	6.000e-05				
Field	11.747				
Temp K	0				
Line Broad	10.00				
DEC Att	0				
Nucleus	P31				
Sequence	1PULS				
Filename	SSP1A1.FFT				
Acq. Time	4.10e-01				
Scale	18				
Amplitude	1.30				

Obs Freq	202.4570475	peak	amp	Hz	ppm
acqPreq	500.1351378	1	320027.50	3978.49	19.55
SW	5000.0	2	118013.80	1561.10	5.74
FILTER	5000	3	117316.01	263.33	4.76
No. Points	4096	4	179500.86	-1104.32	-5.46
No. Scans	3648				
Dwell Time	1.00000e-04				
Delay	6.000e-05				
Field	11.747				
Temp K	0				
Line Broad	10.00				
DEC Att	0				
Nucleus	P31				
Sequence	1PULS				
Filename	SSP1B.FFT				
Acq. Time	4.10e-01				
Scale	18				
Amplitude	1.30

Obs Freq	202.4570475	peak	amp	Hz	ppm
acqPreq	500.1351378	1	302375.91	3978.04	19.54
SW	5000.0	2	194363.64	1138.64	5.72
FILTER	5000	3	188331.39	-1104.32	-5.46
No. Points	4096				
No. Scans	3700				
Dwell Time	1.00000e-04				
Delay	6.000e-05				
Field	11.747				
Temp K	0				
Line Broad	10.00				
DEC Att	0				
Nucleus	P31				
Sequence	1PULS				
Filename	SSP1C.FFT				
Acq. Time	4.10e-01				
Scale	18				
Amplitude	1.30				

Obs Freq	202.4570475	peak	amp	Hz	ppm
acqPreq	500.1351378	1	200653.55	3978.04	19.54
SW	5000.0	2	150273.67	1161.10	5.74
FILTER	5000	3	146064.41	-1102.08	-5.44
No. Points	4096				
No. Scans	3700				
Dwell Time	1.00000e-04				
Delay	6.000e-05				
Field	11.747				
Temp K	0				
Line Broad	10.00				
DEC Att	0				
Nucleus	P31				
Sequence	1PULS				
Filename	SSP1D1.FFT				
Acq. Time	4.10e-01				
Scale	18				
Amplitude	1.30

Figure 5. ^{31}P NMR spectra of KOH extractable P of Gilat soil amended with KH_2PO_4 , phytic acid, glycerophosphate, and pyrophosphate at 10 mgP g^{-1} each and incubated at 30°C for 0 (1a), 1 (1b), 4 (1c), and 8 (1d) weeks.

Obs Freq 202.4570475 LEE 10FEB91
 dcplFreq 500.1355009 OSORINO BLANK
 SW +/- 5000.0 1PULS ONE PULSE SEQUENCE
 FILTER 3333
 No. Points 4096
 No. Scans 1500
 Dwell Time 1.0000e-04
 Delay 6.00e-05
 Field 11.747
 Temp K 0
 Line Brdng 5.00
 DEC Attn 0
 P31
 Nucleus 17PULS
 Sequence 47.1-12.FFT
 Filename 4.10e-01
 Acq. Time 18
 Scale 1.30
 Amplitude

Obs Freq 202.4570475 LEE 10FEB92
 dcplFreq 500.1351376 S4A 2/18/92
 SW +/- 5000.0 1PULS ONE PULSE SEQUENCE
 FILTER 3333
 No. Points 4096
 No. Scans 1500
 Dwell Time 1.0000e-04
 Delay 6.00e-05
 Field 11.747
 Temp K 0
 Line Brdng 5.00
 DEC Attn 0
 P31
 Nucleus 17PULS
 Sequence 52.1-12.FFT
 Filename 4.10e-01
 Acq. Time 20
 Scale 1.30
 Amplitude

Figure 6. Enzymatic hydrolysis of soil organic P by phytase.

APPENDIX
31P NMR SPECTRA

Spectra can be obtained
upon request

Index of NMR Runs

Standards:

ppt = precipitate formed upon pH adjustment

Run Number	File Name	Sample	Concentration (mg /L)	Total P pH	Number of Scans	Instrument
		Phytic Acid	2.25	2		
		Phytic Acid	2.25	7		
		Phytic Acid	2.25	9		
		Phytic Acid	2.25	12		
56	5PA.001	Phytic Acid	22.50	2		NMC 1280
		Phytic Acid	22.50	7		
		Phytic Acid	22.50	9		
		Phytic Acid	22.50	12		
57	9PA.001	Phytic Acid	225.00	2		NMC 1280
58	10PA.001	Phytic Acid	225.00	7		NMC 1280
59	11PA.001	Phytic Acid	225.00	9		NMC 1280
60	12PA.001	Phytic Acid	225.00	12		NMC 1280
		Pyrophosphate (Tetrasodium)	2.25	2		
		Pyrophosphate (Tetrasodium)	2.25	7		
		Pyrophosphate (Tetrasodium)	2.25	9		
		Pyrophosphate (Tetrasodium)	2.25	12		
		Pyrophosphate (Tetrasodium)	22.50	2		
		Pyrophosphate (Tetrasodium)	22.50	7		
		Pyrophosphate (Tetrasodium)	22.50	9		
		Pyrophosphate (Tetrasodium)	22.50	12		
61	21PP.001	Pyrophosphate (Tetrasodium)	225.00	2		NMC 1280
62	22PP.001	Pyrophosphate (Tetrasodium)	225.00	7		NMC 1280
63	23PP.001	Pyrophosphate (Tetrasodium)	225.00	9		NMC 1280
64	24PP.001	Pyrophosphate (Tetrasodium)	225.00	12		NMC 1280
		Glycerophosphate	2.25	2		
		Glycerophosphate	2.25	7		
		Glycerophosphate	2.25	9		
		Glycerophosphate	2.25	12		
		Glycerophosphate	22.50	2		
		Glycerophosphate	22.50	7		
		Glycerophosphate	22.50	9		
		Glycerophosphate	22.50	12		
65	33GP.001	Glycerophosphate	225.00	2		NMC 1280
66	34GP.001	Glycerophosphate	225.00	7		NMC 1280
67	35GP.001	Glycerophosphate	225.00	9		NMC 1280
68	36GP.001	Glycerophosphate	225.00	12		NMC 1280
		Potassium dihydrogen phosphate	2.25	2		
		Potassium dihydrogen phosphate	2.25	7		
		Potassium dihydrogen phosphate	2.25	9		
		Potassium dihydrogen phosphate	2.25	12		
		Potassium dihydrogen phosphate	22.50	2		
		Potassium dihydrogen phosphate	22.50	7		
		Potassium dihydrogen phosphate	22.5	9		
		Potassium dihydrogen phosphate	22.5	12		
69	45PDHP.001	Potassium dihydrogen phosphate	225	2		NMC 1280
70	45PDHP.002	Potassium dihydrogen phosphate	225	2		NMC 1280
71	46PDHP.001	Potassium dihydrogen phosphate	225	7		NMC 1280
72	46PDHP.002	Potassium dihydrogen phosphate	225	7		NMC 1280
73	47PDHP.001	Potassium dihydrogen phosphate	225	9		NMC 1280
74	47PDHP.002	Potassium dihydrogen phosphate	225	9		NMC 1280
75	48PDHP.001	Potassium dihydrogen phosphate	225	12		NMC 1280
76	48PDHP.002	Potassium dihydrogen phosphate	225	12		NMC 1280

Index of NMR Runs

Run Number	File Name	Sample	Total P Concentration (mg P/L)	pH Number of Scans	Instrument
		RNA	-3	2	
		RNA	-3	7	
		RNA	-3	9	
		RNA	-3	12	
		RNA	-30	2	
		RNA	-30	7	
		RNA	-30	9	
		RNA	-30	12	
ppt		RNA	-300	2	
77	58RNA.001	RNA	-300	7	NMC 1280
78	59RNA.001	RNA	-300	9	NMC 1280
79	60RNA.001	RNA	-300	12	NMC 1280
		Mixture	3	2	
		Mixture	3	7	
		Mixture	3	9	
		Mixture	3	12	
ppt		Mixture	30	2	
		Mixture	30	7	
		Mixture	30	9	
		Mixture	30	12	
ppt		Mixture	300	2	
80	70MIX.001	Mixture	300	7	NMC 1280
81	71MIX.001	Mixture	300	9	NMC 1280
82	72MIX.001	Mixture	300	12	NMC 1280
83	72MIX.002	Mixture	300	12	NMC 1280
84	73PA.001	Phytic Acid	135	2	NMC 1280
85	74PA.001	Phytic Acid	135	7	NMC 1280
86	75PA.001	Phytic Acid	135	9	NMC 1280
87	76PA.001	Phytic Acid	135	12	NMC 1280
88	77PA.001	Phytic Acid	90	2	NMC 1280
89	77PA.002	Phytic Acid	90	2	NMC 1280
90	78PA.001	Phytic Acid	90	7	NMC 1280
91	79PA.001	Phytic Acid	90	9	NMC 1280
92	79PA.002	Phytic Acid	90	9	NMC 1280
93	80PA.001	Phytic Acid	90	12	NMC 1280
94	81PA.001	Phytic Acid	45	2	NMC 1280
95	82PA.001	Phytic Acid	45	7	NMC 1280
96	82PA.002	Phytic Acid	45	7	NMC 1280
97	83PA.001	Phytic Acid	45	9	NMC 1280
98	84PA.001	Phytic Acid	45	12	NMC 1280
99	84PA.002	Phytic Acid	45	12	NMC 1280

f. DESCRIPTION OF COOPERATION

The UC Riverside group studied extraction procedures of organic-P in soil, and organic-P transformations following incubation and enzymatic hydrolysis reactions. Organic-P species in test solutions and in the studied extracts were analysed by NMR techniques to improve current methodologies and to evaluate the efficiency of the extractants in releasing various organic-P compounds from different soils.

The Israeli Weizmann group developed techniques to determine solid phase P concentrations in soil by MAS NMR spectroscopy. They studied the spectra of predominant organic and inorganic-P species in soil and in gibbsite (single elements and their mixtures) and determined minimal total P concentrations that can be detected by this method.

The principal investigator coordinated the work of the three cooperating groups and prepared a set of soil samples which were enriched with five predominant organic and inorganic-P species at various levels. These samples were incubated for different periods of time, and were analysed later by the Riverside and Israeli groups.

Towards the end of the first year, the PI visited Riverside to review the obtained results and compare data obtained in the three Institutions.

g. EVALUATION OF THE RESEARCH ACHIEVEMENTS

In accordance with the feasibility study objectives, we evaluated in this work the suitability of up-to-date high resolution NMR techniques to determine concentration of various organic and inorganic P compounds in soil and soil extracts. It was established that at P concentrations exceeding 45 mg P/L solution and 1 g P/kg solid phase this technique is a powerful tool to identify and quantify single P species in solution and in solid phases, respectively.

The research identified three main problems that limit application of current NMR techniques in studying organic-P transformations in soil:

(1) It was found that it is practically impossible to distinguish between the NM resonances of orthophosphate and phytic acid when found together in solution. (2) The ^{31}P spectra of all the studied P compounds in the soil-solid phase are very similar, and it is difficult to distinguish among them when found in mixture. (3) None of the studied P extraction methods was sufficiently effective in extracting soil-P for the ^{31}P NMR analysis.

The research results will help to focus future research on organic-P behavior in reactive porous media, especially in developing analytical techniques to determine concentration of organic-P species in solution and solid phases of soils.