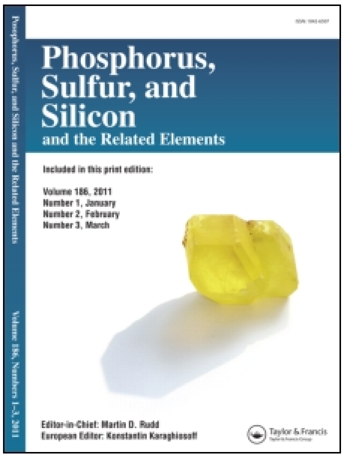

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THE ANALYSIS OF SOIL PHOSPHORUS BY ICP AND ^{31}P NMR SPECTROSCOPY

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The phosphorus content of 14 samples of UK soils has been determined. Aqueous tetra-*n*-butylammonium hydroxide was used to extract organic phosphates and these solutions were analyzed by Fourier transform ^{31}P nuclear magnetic resonance spectroscopy. This permits the composition of this fraction of soil phosphorus to be expressed in terms of the following classes: (i) monoester phosphates (chiefly of inositol); (ii) diester phosphates (such as phospholipids and nucleotides); (iii) polyphosphates; and (iv) non-phosphates. The method is not suited to measuring the relative amounts of the key phosphate esters but it is sensitive enough to show the presence of other phosphorus compounds that are probably of pesticidal origin. The phosphorus content of the extracts was determined with an inductivity-coupled plasma source spectrometer, which was also used to measure the total phosphorus content of the soils after complete solubilization of samples via high temperature ignition followed by $HClO_4$-HF treatment. This method gives higher phosphorus values than that using a mixture of strong mineral acids at 350°C, except for peaty soils.

INTRODUCTION

The phosphorus cycles in nature are clear in outline but not in detail,[1] and nowhere is this more true than in the pedosphere. The composition of soil phosphorus continues to attract a lot of attention. Halstead and McKercher's review[2] summarises the earlier methods of analysis and the compounds characterized. Anderson and co-workers have isolated and identified inositol phosphates,[3] nucleoside diphosphates,[4] glucose phosphates,[5] and other carbohydrate phosphates,[6] that make up a large part of the so-called organic phosphates in soils.[7]

The major organic phosphate is inositol hexaphosphate, IHP. It occurs chiefly as *myo*-inositol but other isomers have also been detected as have the penta- and lower phosphates. The chemistry of these substances has been excellently reviewed by the late D. J. Cosgrove.[8] Up to 460 ppm P (0.046%) inositol phosphates have been measured in some Scottish soils,[9] and up to 75% of the total organic phosphorus may be of this form.

Phospholipids are the second most abundant organic phosphate. They can be extracted using organic solvents such as acetone or mixtures of ethanol and benzene. The major component (*ca.* 30%) of the soil phospholipids is phosphatidyl choline.[10] Up to 23 ppm P of phospholipids may be present in soils.[11]

Inorganic polyphosphates, i.e. long chain condensed phosphates, have been extracted from soils by cold 0.5 M $HClO_4$ solution, detected by gel filtration and shown to be present at *ca.* 5–10 ppm P abundance.[12] The more specific organic

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TABLE I

Soil samples

Soil type	UK location	Land use	Surface texture	Parent material	Drainage (rainfall/mm)	pH
Acid brown earth	A Modbury	Permanent grass	Clay loam	Fragmented bedrock	Moderate (1250)	4.9
	B Newport	Improved grass	Silt clay loam	Weathered bedrock	Good (1100)	4.7
Basic brown earth	A Bedford	Green crops	Sand clay loam	Alluvium	Good (560)	7.4
	B Clitheroe	Permanent grass	loam	Alluvium	Good (1150)	6.0
Gley	A Hambleton	Fallow	Silt clay loam	Weathered bedrock	Poor (850)	6.2
	B Derby	Improved grass	Clay	Alluvium	Poor (700)	6.3
Peaty gley	A Migneint	Unimproved grazing	Organic	Till	Poor (2400)	3.8
	B Maybole	Unimproved grazing	Organic	Fluvioglacial deposits	Poor (1200)	4.4
Podzol	A Penrith	Improved grass	Sand loam	Till	Good (1000)	3.1
	B Pocklington	Conifer forest	Sand	Loess	Good (650)	5.2
Brown podzol	A Ferness	Conifer forest	Sand loam	Fluvioglacial deposits	Good (830)	4.4
	B Grizedale	High forest	Sand slit loam	Till	Good (1900)	4.0
Peaty podzol	A Gruinard Bay	Unimproved grass	Organic	Till from gneiss	Poor (1650)	4.4
	B Ilkley	Unimproved grass	Organic	Hard bedrock	Poor (950)	3.4
Peat	A Saddell Forest	Conifer forest	Organic	Peat	Very poor (1160)	4.0
	B Dornie	Unimproved grass	Organic	Peat	Very poor (1900)	4.1

polyphosphate, ATP, has been extracted from soils by a paraquat based reagent and found at levels as high as 8.7 ppm P.[13] Recently HPLC has been used to identify not only ATP but AMP (and IHP) in soils.[14]

An unexpected discovery was the finding of the compound 2-aminoethyl phosphonate,[15] which is one of the few naturally occurring compounds with a C—P covalent bond. This was identified by its ^{31}P nmr signal,[15] the same technique that was originally used to detect this phosphonate in nature.[16] Choline phosphate was also identified in a soil component by this method.[15]

There still remains certain organic phosphates of high molecular weight that have been extracted from soils but not identified.[17] These are in very low abundance, however.

In the work reported here we have also used FT ^{31}P nmr spectroscopy to analyze extracts from soils. We have chosen a non-metal base, Bu_4NOH, to extract the organic phosphates. As far as we are aware this reagent has not previously been used for this purpose. The soils are listed in Table I.

DISCUSSION

Extraction of phosphorus from soils

(i) *Organic Phosphates.* The extraction of phosphorus compounds from soils is possible with a variety of reagents.[7] The kind of phosphorus which dissolves depends upon the reagent; the accepted solution for extracting organic phosphates is cold NaOH solution of strength 0.1–0.5 M. This does not cause hydrolysis. Acid pre-treatment of the soil with HCl was found to increase the amount of organic phosphate removed by the NaOH but this step is not really necessary.[15] Other pre-treatments involving complexing agents or organic solvent have been tried.[7] Dilute ammonia solution as the basic extractant bears some relationship to the extractant used by us but it too is not ideal in all respects.[18] The basic extractant removing most phosphate is a hot solution of concentrated (3 M) NaOH.[19]

Bearing in mind the complex nature of organic phosphates it is perhaps naive to expect a single extractant to dissolve them all, since they range from very insoluble salts on the one hand to phospholipids on the other. In choosing Bu_4NOH solution as our extractant we hoped to utilize the salting-in effect of the large organic cation,[20] which should favour organic material and discourage the extraction of simple inorganic metal salts which might adversely effect the nmr picture. This reagent, though more expensive, can be used at a dilution of 0.1 M and works well even at room temperature. However it also extracts some of the soluble inorganic phosphate as shown in the ^{31}P nmr spectra of the solutions. The extent of this extraction was estimated by extracting the same soil with Bu_4NOH before and after pyrolysis when all the organic phosphate has been destroyed. The post-ignition extractions brought very little phosphorus into solution as Table II shows. The difference between the ante- and post-ignition extraction, Δ in Table II, is assumed to relate to the amount of organic phosphorus compounds in the soil. Although there are some obvious objections to this simple assumption about Δ it does not lead to inconsistencies with other data in Table II.

TABLE II

Phosphorus analyses of soils (ppm P)[a]

Soil		Bu_4NOH extraction	Ignition + Bu_4NOH	Ignition + $HClO_4$–HF	$HCl–HNO_3$ – H_2SO_4	Weight loss on ignition (%)	Δ^b
Acid brown earth	A	640	140	1160	860^c	8.7	500
	B	390	60	810	860	7.9	330
Basic brown earth	A	350	110	680	480	4.4	240
	B	390	70	460	400	8.1	320
Gley	A	600	70	1180	840	14.3	530
	B	650	50	1510	980^c	16.7	600
Peaty gley	A	780	200	1230	1000^c	45.1	580
	B	500	220	810	710^c	17.6	280
Podzol	A	380	20	590	470	11.6	360
	B	150	80	270	100^c	7.0	70
Brown podzol	A	270	60	540	200^c	10.4	210
	B	610	90	630	700^c	20.4	520
Peaty podzol	A	550	300	980	730^c	42.1	250
	B	500	300	630	590^c	64.4	200

[a] ± 20 ppm P; 1 ppm P = 0.0001% phosphorus by weight.

[b] Difference between extractions with Bu_4NOH before and after ignition.

[c] Data supplied by Dr. A. F. Harrison using method given in A. F. Harrison, *Soil Biol. Biochem.*, **11**, 393 (1979).

The effectiveness of Bu_4NOH was compared with that of other extractants at room temperature at the same concentration; peaty gley (A) was chosen as a representative soil for this purpose. The amounts of phosphorus extracted from it were: Bu_4NOH, 780; NaOH, 783; KOH 788; HCl (1 M) 344; EDTA (0.05 M)[21] 364; and distilled H_2O 32 ppm P (based on original weights of soil). Thus Bu_4NOH is as efficient as the alkali metal hydroxides but no more. The hoped for salting-in effect did not happen suggesting that the counter-cations of the organic phosphates are the single most important fact in solubility.

Leaving a sample of peaty gley (A) in contact with Bu_4NOH (stirred) for 5 days gave a slightly reduced extraction of 750 ppm suggesting either some slight mineralization or break-up of the soil minerals to release metals whose phosphates are less soluble.

Repeated extraction of the same soil with 5 different aliquots of Bu_4NOH solution removed 775, 85, 53, 47 and 38 ppm P representing a total of 998 ppm P and a projected total of 1050 ppm to exhaustive extraction with this reagent. Bu_4NOH followed by HCl (1 M) extraction removed 775 and 63 ppm P respectively, while applied in the reverse order the amounts were 344 and 390 ppm P. The base/acid total of 838 ppm P shows there to be another factor operative in extraction since this is significantly larger than the acid/base total of 734 ppm P.

(ii) *Total Phosphorus.* The total phosphorus content of soils has been determined in a variety of ways but digestion with a mixture of concentrated mineral acids at high temperatures of *ca.* 350° is commonly used such as HNO_3–$HClO_4$–H_2SO_4[22] and $HClO_4$–H_2SO_4.[23] This treatment is then followed by colorimetric determination. These methods were introduced as alternatives to ignition oxidation as this can lead to loss of some of the phosphorus.[24] However complete solubilization is only possible after ignition, and ignition at 500–600°C for 1–2 hours followed by acid extraction may not achieve complete dissolution although it is a recognized method of analyzing soils for their total phosphorus content.[7] Even so this method is not recommended for peats from which loss of phosphorus has been reported on heating at temperatures as low as 400°C. Yet even for peats this method has had its advocates and with temperatures as high as 750°C.[25]

Since we desired complete solution of the soil prior to ICP analysis the pyrolysis was done under the sort of conditions normally used for geological samples: 890° for 30 minutes followed by dissolution in $HClO_4$–HF. The hydrofluoric acid ensures the release of even that phosphate which is incorporated in the silicates and silica of the mineral from which the soil is composed.

Table III shows the results of high ignition on both peats (A) and (B) and a peaty podzol (B) which has over 30% organic content. The results are higher for the lower ignition temperature by *ca.* 40 ppm P, but in the case of peaty podzol (B) the value is still higher than that obtained by the strong mineral acids method.

Table II gives the total phosphorus obtained by both methods, and in almost all cases that from the ignition/$HClO_4$–HF method is the higher. Thus this method is to be preferred except for peats, and even with these it is possible to get good results provided a lower temperature is used, to prevent reduction of phosphate to volatile phosphorus by carbon. The price paid for the lower temperature however is that it becomes very difficult to effect complete solubilization after ignition.

(iii) *^{31}P nmr Spectra of Soil Extracts.* This technique has been used to study New Zealand soils extracted with 0.5 M NaOH. Solutions were concentrated by rotary evaporation prior to their FT ^{31}P nmr spectra being run. The spectra showed the presence of inorganic phosphate, IHP, choline phosphate, diphosphate and polyphosphates.[15] In addition the presence of 2-aminoethyl phosphonate was conclusively demonstrated.

TABLE III

Ignition and analysis of peaty soils (ppm P)

Soil		Organic content (%)[a]	Ignition temperature and P content					
			550°		890°			
			Wt. loss (%)	P (ppm)	Wt. loss (%)	P (ppm)	Bu_4NOH[b]	Acid[c]
Peaty podzol	B	32	62.7	690	64.4	630	500	590
Peat	A	48	97.0	470	94.3	430	510	620
	B	46	86.4	710	87.1	680	690	720

[a] Based on carbon analyses supplied by Dr. A. F. Harrison.

[b] Without ignition.

[c] Data supplied by Dr. A. F. Harrison—see footnote c Table II.

^{31}P nmr spectroscopy would seem the ideal analytical tool for probing phosphorus compounds in soils but in practice there are certain drawbacks to its use. Because of its low sensitivity, highly concentrated solutions are preferred although Fourier transform techniques can enhance signal/noise ratios to enable dilute solutions to be studied, even where the concentration of phosphorus is as little as 10 ppm of phosphate.[26] Overnight operation of a spectrometer will accumulate sufficient scans to distinguish phosphorus compounds in soil solutions.

Since every step in extracting a soil solution seems to incur a penalty[7] it was considered undesirable to use ultrasonics or to concentrate the solution by evaporation.[15] The former was compensated for with a longer extraction time and the latter with a higher number of spectral accumulations to counteract the higher dilution. Moreover because of the known effect of alkali metal cations on phosphate signals the base chosen for extractant was Bu_4NOH, the cation of which has been shown to have no effect in this respect.[27] The pH of the solution can also influence the inorganic phosphate signal.[28]

The chemical shifts of the phosphorus compounds found in soils are as follows:

Inorganic phosphate, +4.00 (high pH) to −0.12 ppm[28]
Inositol hexaphosphate, +3.27 to −1.21 ppm (multiplet)[29]
Phospholipids, −0.2 to −2.0 ppm[30]
Nucleic acids, monoesters *ca.* +1.8 ppm[31]
  diesters *ca.* −1.9 ppm[31]
DNA, −1.7 to −4.5 ppm[32]
Diphosphate or phosphate endgroup −8 to −11 ppm[33]
Polyphosphate or middle group phosphate −20 to −23 ppm[33]
Phosphonates +15 to +40 ppm[16]
  (2-aminoethyl phosphonate, 19.8 ppm[15])

It can be seen that some of these phosphate esters fall in overlapping regions of the spectrum and especially around the $\delta = 0$ ppm region, which unfortunately is where all the organic phosphate esters resonate. All that ^{31}P ppm analysis can distinguish are phosphate monoesters (IHP, mononucleotides) from diesters (phospholipids, polynucleotides, DNA) and even this distinction is somewhat hazy.[15]

The signals obtained from samples of the same soil, podzol (A), extracted by different strengths of Bu_4NOH solution are given in Table IV. This soil was chosen because it gave the lowest phosphorus extraction by Bu_4NOH after ignition which should mean that there is less inorganic phosphate extracted, as indeed is shown in Table II. The profile of the phosphorus of this soil, averaged from all the results of Tables II, III and IV is thus:

Total phosphorus content	590 ppm (0.059%)
Inorganic phosphate	230 ppm
Organic phosphate	360 ppm
Monoesters	88%
Diesters	8%
Polyphosphates	2%
Non-phosphates	2%

TABLE IV

^{31}P nmr analysis of podzol (A)

Extractant	Spectra accumulations	Chemical shifts (δ)	Relative intensity	Identification
Bu_4NOH[a]	85,272	66.85	2	Pesticide residue
		4.80	91	monoester phosphates
		−1.01	5	diester phosphates
		−30.52	2	unknown[b]
Bu_4NOH[c]	89,859	3.92	6	inorganic phosphate
		2.7	79	mainly IHP
		−1.01	13	diester phosphates
		−23.74	2	polyphosphates
Bu_4NOH[d]	32,768	4.01	70	monoester phosphates
		2.66	20	inorganic phosphate
		−1.18	3	diester phosphates
		−9.15	5	diphosphate[e]
		−60.8	2	unknown[b]

[a] 0.5 M.
[b] See text.
[c] 0.2 M.
[d] 0.1 M.
[e] Or end-group phosphate.

Extracting podzol (A) with acetylacetone gave only a single signal after 83885 accumulations and this was in the downfield region of the spectrum at 76.58 ppm, and although this cannot positively be identified it can be compared with the chemical shifts of phosphorus compounds with P—S substituents: eg. $(MeO)_3PS$ $\delta = 73$ ppm; $(R_2N)_3PS$ δ *ca.* 75 ppm. There are known pesticides which have PS and PN bonds,[34] and these are likely to be the explanation of the substance extracted by acetylacetone. This is the first time that a phosphorus pesticide or its residue has been detected in soils by this method.

Table V gives the results on other soils, and here too unexpected signals are found in some spectra. Signals upfield at −58 ppm suggest trivalent phosphorus compounds, e.g. Me_3P $\delta = -62$ ppm, but this oxidation state is unlikely to occur in nature unless some strongly reducing process is taking place. Signals at *ca.* −28 ppm might be due to ultraphosphates, i.e. $O{=}P(OPO_3^{2-})_3$ compounds. Although this grouping should be relatively easily hydrolysed, similar signals have been observed in aqueous solutions of polyphosphates produced by the heating ammonium phosphates and urea.[35]

Amido and amino phosphates might also be present in soils since these can be produced by algae, but it would be impossible to distinguish them by ^{31}P nmr since their chemical shifts would overlap those of the esters.[36]

As a technique for investigating soils ^{31}P has some advantages in revealing unexpected compounds and measuring the polyphosphate content. However it is expensive in machine time and unlikely to replace conventional methods for estimating the amounts of the main constituents, IHP, phospholipids, etc. Nor can the relative intensities reflect the true composition of the organic phosphates present in some of the soils, especially the peats and peaty podzol soil samples. Despite its

TABLE V

^{31}P nmr analysis of representative soils extracted with 0.1 M Bu_4NOH solution

Soil	Spectra accumulations	Chemical shifts (δ)[a]	Relative intensity	Identification
Acid brown earth (B)	80,285	5.05 4.52 4.16	90	Inorganic, IHP, and other monoester phosphates
		−1.33	6	diester phosphates
		−10.66 −19.46 −26.28 −28.80	2	polyphosphates
		−58.0	2	unknown
Basic brown earth (A)	32,768	91.72	1	pesticide residue
		4.76 4.48 3.92	92	inorganic, IHP, and other monoester phosphates
		−11.06	7	diphosphate or end-group
Gley (B)	174,584	3.92 3.31	100	inorganic, IHP, and other monoester phosphates
Peaty gley (B)	53,886	18.1	10	2-aminoethyl phosphonate
		3.96 2.91 2.01	80	inorganic, IHP, and other monoester phosphates
		−0.77	8	diester phosphates
		−57.8	2	unknown
Brown podzol (A)	99,662	4.48	80	inorganic, IHP, and other monoester phosphates
		−0.44	10	diester phosphates
		−11.16 −28.8	10	polyphosphates
Peaty podzol (A)	87,802	−1.13	100	diester phosphates
Peat (B)	151,963	11.86	70	phosphonate?
		2.0	30	inorganic, IHP, and other monoester phosphates

[a] ppm with respect to 85% H_3PO_4. Signals mainly broad, δ quoted for strongest signals.

promising start[15] ^{31}P nmr has a long way to go before it becomes the method of choice for analysing soils for their phosphorus compounds.

EXPERIMENTAL

Instruments. The inductively coupled plasma source spectrometer was a Philips/MBLE System. In this equipment the sample of aqueous solution to be analyzed is pumped by a peristaltic pump into a spray chamber from where a sample is carried as an aerosol by argon gas to the centre of an argon plasma. This consists of a stream of argon atoms inductively heated by a radio frequency coil operating from a generator at 50 MHz. Passage through the r.f. field strips the electrons from the argon atoms to produce a plasma of argon ions with an operating temperature at its centre of 6000–10,000°K. This plasma is ignited by a high frequency Tesla spark. Light from the plasma then enters the spectrometer through a 20 μm slit, and after diffraction and photomultiplication the signal is computed and the data processed (Philips P852, CESPEC III). The instrument is calibrated with phosphate solutions of known concentration.

The nuclear magnetic resonance spectrometer was a Bruker HFX 90 machine operating at 36.4 Mhz. Samples were recorded at 307 ± 3°K with pulse Fourier transform and broad band decoupling. Spectra were recorded overnight or longer (15–36 hours) with signal averaging representing 50,000–200,000 accumulations. Samples were held in 10 mm external diameter tubes with a few drops of D_2O added to provide a deuteron nmr lock signal. EDTA was also added to prevent line broadening by paramagnetic metal ions extracted from the soil. Peak positions were measured in ppm from 85% H_3PO_4 as external standard. Filter band width 10,000 Hz/cm, sweepwidth 6000–10,000 Hz/cm; offset 4500–8500 Hz; Pulse-Fourier transform: 7.5–12.5 μseconds pulsewidth; time constant −2 to −10 seconds; flip angle 30–51°.

Analyses of the Soils. All the soils, air-dried and sieved to < 2 mm, were supplied by Dr. A. F. Harrison of the Institute of Terrestrial Ecology, and their description is given in Table I. The soils were dried at 100°C for 2 hours prior to extraction. The loss in weight caused by this heating was small, 0–5%, and was attributed to surface water.

Extraction with tetra-n-butylammonium hydroxide. To a 0.500 g sample of the finely ground soil was added 1.75 cm^3 40% Bu_4NOH solution (2.57 M) and 45 cm^3 water (equivalent to an extractant of 0.1 M). The mixture was stirred at room temperature for 24 hours, centrifuged, filtered and made up to 50 cm^3. Samples were then used immediately or stored in a deep freeze until required for analysis.

Ignition and extraction with $HClO_4$–HF. A 7.000 g sample of the finely ground soil was heated in a porcelain crucible at 890°C for 30 minutes, allowed to cool, reweighed, and reground. To a 0.500 g portion of the ignited soil in a platinum crucible was added 4 cm^3 $HClO_4$ (72% solution) and 15 cm^3 HF (40% solution). The mixture was evaporated to dryness, a further 2 cm^3 $HClO_4$ added and evaporation to dryness repeated. A further 4 cm^3 of dilute $HClO_4$ was added and the crucible warmed until solution was complete. The cooled solution was made up to 50 cm^3. Some peat soils were ignited at 550°C for 1 hour, since it has been shown that some phosphorus can be lost from soils by heating at high temperatures. These soils, of high organic content, are susceptible to phosphorus loss probably through the reduction reaction: $4PO_4^{3-} + 10C \rightarrow 10CO + P_4\uparrow + 6O^{2-}$. The carbon is available from the pyrolysis of the organic material. This reaction occurs readily at temperatures above 1200°C. That there is some loss can be seen from Table III. However, given the choice of incomplete pyrolysis, which hinders solubilization, or complete pyrolysis at 890°C with some phosphorus loss, the latter was preferred. Loss of phosphorus has also been observed by $HClO_4$ digestion itself.[37]

Extraction with Mineral Acids. 1.000 g of soil was added to 10 cm^3 of a freshly prepared mixture of HNO_3, H_2SO_4 and $HClO_4$ (Allen *et al.*)[22] and the mixture heated to dryness. The process was repeated. The sample was subsequently extracted with distilled water (30 cm^3), filtered, and made up to 50 cm^3.

Analysis for Phosphorus by ICP. Samples of solutions obtained by the above methods were submitted to ICP spectrometric analysis. The results are listed in Tables II and III, each figure being the average of two results rounded to two significant figures; the error is estimated to be *ca.* ±20 ppm P.

Analysis for phosphorus compounds by ^{31}P nmr spectroscopy. Tables IV and V show the results obtained by this technique on soil extracts.

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REFERENCES

1. J. Emsley, "The Handbook of Environmental Chemistry, Vol. 1, Part A," ed. O. Hutzinger, 147–167, Springer-Verlag, Berlin, 1980.
2. R. L. Halstead and R. B. McKercher, "Soil Biochemistry," eds. E. A. Paul and A. D. McLaren, Vol. 4, Chap. 2, Marcel-Dekker, New York, 1975.
3. R. B. McKercher and G. Anderson, *J. Soil Sci.*, **19**, 302 (1968).

4. G. Anderson, *Soil Sci.*, **86**, 169 (1958); *ibid.*, **91**, 156 (1961); *J. Soil Sci.*, **12**, 276 (1961); *ibid.*, **21**, 96 (1970).
5. G. Anderson and R. E. Malcolm, *J. Soil Sci.*, **25**, 282 (1974).
6. M. V. Cheshire and G. Anderson, *Soil Sci.*, **119**, 356 (1975).
7. G. Anderson, "Soil Components; Vol. 1 Organic Components," ed. J. E. Gieseking, Chap. 4, Springer-Verlag, Berlin, 1975.
8. D. J. Cosgrove, "Inositol Phosphates," Chap. 13, Elsevier, Amsterdam, 1980.
9. R. B. McKercher and G. Anderson, *J. Soil Sci.*, **19**, 47 (1968).
10. C. G. Kowalenko and R. B. McKercher, *Can. J. Soil Sci.*, **51**, 19 (1971).
11. R. T. Baker, *J. Soil Sci.*, **26**, 432 (1975).
12. C. P. Ghonsikar and R. H. Miller, *Plant & Soil*, **38**, 651 (1973).
13. D. S. Jenkinson, S. A. Davidson and D. S. Powlson, *Soil Biol. Biochem.*, **11**, 521 (1979).
14. R. G. Gerritse, *J. Sci. Food Agric.*, **29**, 577 (1978).
15. R. H. Newman and K. R. Tate, *Commun. Soil Sci. Plant Anal.*, **11**, 835 (1980); *Soil Biol. Biochem.*, **14**, 191 (1982).
16. T. Glonek, T. O. Henderson, R. L. Hildebrand and T. C. Myers, *Science*, **169**, 192 (1970).
17. R. L. Veinot and R. L. Thomas, *Soil Sci. Soc. Arn. Proc.*, **36**, 71 (1972).
18. J. F. Dormaar, *Can. J. Soil Sci.*, **44**, 265 (1964).
19. R. L. Halstead and G. Anderson, *Can. J. Soil Sci.*, **50**, 111 (1970).
20. J. E. Desnoyers and C. Jolicoeur, "Modern Aspects of Electrochemistry," eds. J. O'M. Bockris and B. E. Conway, Vol. 5, Butterworths, London, 1969.
21. F. E. G. Harrap, *J. Soil Sci.*, **14**, 82 (1963).
22. S. E. Allen, H. M. Grimshaw, J. A. Parkinson and C. Quarmby, "Chemical Analysis of Ecological Materials," ed. S. E. Allen, Blackwells, Oxford, 1974.
23. U. Kafkafi, "Analytical Chemistry of Phosphorus Compounds," ed. M. Halmann, Chap. 19, Krieger, Melbourne, 1972.
24. J. G. Dormar and G. R. Webster, *Can. J. Soil Sci.*, **44**, 1 (1964).
25. A. Mehlich, *Phosphorsaüre*, **23**, 330 (1963).
26. T. W. Gurley and W. M. Ritchley, *Anal. Chem.*, **47**, 1444 (1975); **48**, 1137 (1976).
27. T. Glonek, R. A. Kleps, E. J. Griffith and T. C. Myers, *Phosphorus*, **5**, 157 (1975).
28. M. M. Crutchfield, C. F. Callis, R. R. Irani and G. C. Roth, *Inorg. Chem.*, **1**, 813 (1962).
29. J. Emsley and S. Niazi, *Phosphorus & Sulfur*, **10**, 401 (1981).
30. T. O. Henderson, T. Glonek and T. C. Myers, *Biochemistry*, **13**, 623 (1974).
31. M. Mandel and J. W. Westley, *Nature*, **203**, 301 (1964).
32. S. Hanlon, T. Glonek and A. Chan, *Biochemistry*, **15**, 3869 (1976).
33. T. Glonek, M. Lunde, M. Mudgett and T. C. Myers, *Arch. Biochem. Biophys.*, **142**, 508 (1971).
34. J. Emsley and C. D. Hall, *Chemistry of Phosphorus*, Chaps. 3 and 12, Harper and Row, London (1976).
35. J. Emsley and S. Niazi, *J. Chem. Soc. Dalton Trans.*, 2527 (1982).
36. M. M. Crutchfield, *et al.*, "^{31}P Nuclear Magnetic Resonance," Topics in Phosphorus Chem., Vol. 5, M. Grayson and E. J. Griffith, editors, Interscience, N. Y. 1967, pp. 353–4.
37. P. C. Brookes and D. S. Powlson, *J. Sci. Food Agric.*, **32**, 675 (1981).