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An isotope (P^{32}) method for determining readily-available phosphate in Lake District Woodland soils

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Introduction

In the current studies of the phosphorus status of some Lake District woodlands (Merlewood Project 307), an essential requirement is a method to assay the amount of readily-available phosphorus in soils. The currently and rather extensively used extraction procedures, using 2.5% acetic acid, 0.002N sulphuric acid (Truogs) and 0.5 M sodium bicarbonate at pH 8.5 (Olsen) solutions were considered to be unsatisfactory for this study for the following reasons:

1) it is customary to air-dry soils before carrying out the extractions; this is known to reduce the amount of labile and extractable phosphorus in soils and to reduce its availability to plants (1, 2, 3).

2) The degree of partition of the phosphate between the soil phase and the extractant solution varies from soil to soil (4). Neither the degree of partition nor its variation from soil to soil are normally taken into account.

3) The extractant may hydrolyse "unavailable" soil phosphorus and the degree of attack may vary from soil to soil (4, 5).

Measurement of the quantity of available phosphorus in soils is perhaps best carried out by isotope dilution methods (6). Such methods, particularly if carried out on fresh soil samples, may overcome the above criticisms. A number of methods for estimation of isotopically-exchangeable phosphate in soils have been proposed (e.g. 7 - 14) and some have been shown to give estimates which correlate well with the amount of phosphorus available to plants (e.g. 15-18).

Unfortunately one cannot assume that any method providing good estimates for one soil series will give equally reliable data with another series. It was necessary therefore to screen a number of methods for their applicability to Lake District woodland soils. The tested methods were compared by calculation of the correlation coefficients between the values obtained and the amount of phosphorus extracted from the soils by nettle (<u>Urtica dioica</u> L.) plants during a short growing period.

This brief report gives details of a) the best method, b) results obtained with it, c) a comparison of these results with those of the 2.5% acetic acid extraction technique and d) general background experimentation and discussion.

Materials and Methods

SOILS

A total of 16 soils, all broadly classifiable as brown earths, were used in this study. At least one soil sample was taken during March from each of the ten woodlands being studied in Project 307. The samples, all from the 0-5cm horizon (litter layer was sparse on sample areas but where it occurred it was removed before taking soil sample) were selected to give a wide range of pH and organic matter conditions. Details of the soils are given in Table 1.

GROWTH OF THE NETTLE PLANTS

All the soils were sieved (5mm) in the fresh condition and an amount equivalent to 200 gms oven dry-wt. (105°C) was weighed into 12.5cm diameter plastic plant pots. Six replicates were prepared for each soil. Rhizome material of the nettle, all from the same clone, was dug up in March from Merlewood grounds before any noticeable growth had been formed. Sections of healthy rhizome 1cm in length each with at least one bud and a small knot of roots were randomized (random number tables) among the pots and planted one per pot to a depth of 0.5cm. The pots were randomized (random number tables) on a bench in a glass-house, without temperature control. The soils were maintained at a moisture content (range 68-96% WHC) approximately that on collection. After a growth period of nine weeks, from the end of March to early June, the total plant was harvested and the root system cleaned of soil particles using a small paintbrush.

ISOTOPE-DILUTION METHOD

Sub-samples (2gm) of fresh sieved soil were shaken for 30 minutes at 20°C in 20ml distilled water. After this 80 mls of water containing 20μg phosphate (KH_2PO_4) and 1 microcurie of carrier-free $P^{32}O_4$ was added. The suspension was shaken for 30 minutes at 20°C. The specific activity of the centrifuged solution was then measured. The isotopically-exchangeable phosphorus was calculated from the formula:-

$$\text{Pexch.} = \left[A \left({}^{Sa}/_{Sb} \right) - 1 \right]$$

where A = amount of phosphate carrier (20μg)

Sa = specific activity of the phosphate added

Sb = specific activity of the solution after shaking

The phosphate in solution was measured by the method of Murphy and Riley modified for soils by John (19, 20). The P^{32} was counted on planchettes using an end-window Geiger-Miller-tube assembly.

EXTRACTABLE PHOSPHATE METHOD

The extractable phosphate values were obtained by a standard Merlewood Chemical Service analytical procedure. The analysis, as is customary, was carried out on air-dried 2mm sieved soils. The procedure involved shaking soil in 2.5% aqueous acetic acid (soil solution ratio 1:25 w/v) for 60 minutes at room temperature. The phosphate in solution was assayed by the stannous chloride/ HCl reduction method.

RESULTS

The total uptake of phosphorus by the nettle plants from the soils is given in Table 2, together with the amount of isotopically-exchangeable phosphorus in micrograms. The coefficients of correlation between the means of total uptake of P by the plants and the isotopically-exchangeable P on the basis of soil weight and soil volume respectively were + 0.798 and + 0.85.

The amounts of phosphorus extractable in the 2.5% acetic acid on a weight and volume basis together with the extractable P values expressed as a % of the isotopically-exchangeable P are given in Table 3. Coefficients of correlation between the uptake of phosphorus by the nettles and the extractable phosphorus on soil weight and volume bases were -0.38 and -0.13 respectively. The extractable phosphorus values are generally considerably less than the isotopically-exchangeable figures.

DISCUSSION

The nettle Urtica dioica L. was selected for these experiments for the following reasons:-

1. The plant species is phosphorus-sensitive and has been recommended for use as an indicator of soil phosphorus availability (21).
2. The plant occurs on a wide range of soil types and tolerates a wide spectrum of soil pH conditions (22).
3. It is a woodland plant and it produces easily obtained and cultured clonal (rhizome) material.

Regarding growth conditions of the nettle plants, a number of points were taken into consideration.
To reduce to a minimum the physiological disturbance to the plants, the rhizome sections were planted before spring growth had begun. The soils were not air-dried before the potting operation and were maintained at approximately the original moisture content; air-drying of soils reduces phosphorus availability to plants (3). The plants were grown in a restricted mass of soil to ensure that the plants produced roots throughout the soil. The growth period was limited to 9 weeks to reduce the time for possible changes in P-availability to occur, though long enough to demonstrate differences in plant response to the soils.

The method for measuring the isotopically-exchangeable phosphorus in soils was the "carrier-method" described by Amer (9), modified as follows:

a) fresh soil was used instead of air-dried

b) the phosphate in solution was measured by a much more sensitive and reproducible method (20).

The comparatively short period for exchange (30 mins) was given so as to reduce the effects of recrystallization and self-diffusion (9) and minimize the interference of micro-organism and soil enzyme activity. Furthermore the aim was to find a method to estimate the readily-available phosphorus; the most rapidly exchanging phosphate is likely to be the most readily available to plants.

The results indicate that only a small proportion of the total phosphate compliment of the soil (from 0.2-6.4%) is readily-available. This proportion appears to be related to the soil pH, the smaller proportions being associated with the lower pH's.

With most of the soils, the extractable phosphate values were considerably lower than the isotopically-exchangeable figures (4.3-34.4%). These lower figures can be mainly attributed to a low degree of partition of the phosphorus from the soil solid phase to the extraction solution. In general the magnitude of the difference in values of the two methods is related directly to the phosphate fixing-capacity of the soils. Air-drying of the soils has probably also reduced the quantity of phosphate extracted by acetic acid. In the case of soil number 3 however, the extractable value was 400% greater than the exchangeable figure. This suggests that the acetic acid had attacked "unavailable phosphorus" in the soil. The same might apply to soil number 8.

These findings indicate that the extraction method using 2.5% acetic acid is of limited value in assaying the readily-available phosphorus in woodland soils. The low negative correlations between the plant uptake of phosphorus and the 2.5% Ha^{c} extractable phosphorus reinforces this view.

From a practical standpoint, the isotope method is easy to carry out and thus amenable to routine work. It does however consume more time or manpower, than the extraction methods. The significance of the results is however far greater and this therefore compensates for the extra effort.

A computer program (FORTRAN) has been written to calculate the quantities of isotopically-exchangeable phosphorus by soil weight and/or volume, from the basic data.

STATISTICAL APPENDIX

1. Correlation between the total phosphorus content of the nettle plants and nettle dry weight was +0.88 significant at the $>$ 0.1% level, d.f.77

2. There was no significant difference in the variation in phosphorus uptake by nettles between soils.

   F ratio 1.4 df 1 and 14

3. Values for P uptake by nettles from any soil generally showed a normal distribution about the mean as shown by the W tests for small samples (23).

4. The correlation coefficients between mean phosphorus uptake by nettles and the isotopically-exchangeable phosphorus in the soils were significant at the 1% level.

5. The correlation coefficients between mean phosphorus uptake by nettles and the 2.5% HA^{c} extractable phosphorus were not significant at the 5% level.

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a) total phosphate contents of the nettles
b) total phosphate contents of the soils
c) Loss-on-Ignition of the soils
d) 2.5% acetic acid extractable phosphate in the soils.

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Table 1

	Woodland of origin	Grid reference (SD)	Tree species associated with area sampled	Solid Geology*	Soil pH	% Loss on Ignition (550°C)
1	Wintering Park	367868	Birch, Oak, Holly	Slate	4.05	21.7
2	Bogle Crag	339929	Oak	Slate	3.95	25.4
3	Town End	362982	Oak, Hazel, Beech	Coniston grit	4.05	33.5
4	Monk Coniston	325977	W. Hemlock	Coniston grit	4.50	19.1
5	Low Wood	337051	Fir, Sycamore, Beech	Borrowdale volcanic	4.40	15.9
6	Intake	342049	Beech, Pine	Borrowdale volcanic	5.65	14.4
7	Birks Brow (a)	41092	Sycamore	Slate	4.25	13.2
8	Birks Brow (b)		Sycamore, Elder	Slate	3.95	20.5
9	High Birks	420907	Oak, Birch, Ash	Slate	3.85	27.5
10	Honeybee (a)	481903	Hazel, Sycamore, Ash, Yew	Carb. limestone	7.65	27.6
11	Honeybee (b)		Yew, Oak, Ash	Carb. limestone	7.85	24.6
12	Meathop (a)	435795	Elm, Hazel, Oak, Birch, Ash	Carb. limestone	5.95	16.8
13	Meathop (b)		Ash, Hazel, Birch, Yew	Carb. limestone	6.05	11.4
14	Meathop (c)		Ash, Birch, Oak, Yew	Carb. limestone	5.15	13.3
15	Meathop (d)		Hazel, Ash, Oak	Carb. limestone	5.80	15.0
16	Meathop (e)		Oak, Hazel, Elder	Carb. limestone	5.2	12.3

* Lake District soils are mostly derived from drift.

Table 2

Soil number	Mean P uptake by nettles	Micrograms isotopically exchangeable phosphate per gm. (oven dry wt)	Micrograms Total Phosphate per gm (oven dry wt)	Micrograms Isotopically exchangeable Phosphate per cc	Micrograms total Phosphate per cc	% Total Phosphate readily exchangeable
1	168	16.2 ± 2.0*	500	7.2	222	3.24
2	75	8.0 ± 1.0	890	1.9	206	0.92
3	75	1.8 ± 1.0	790	0.8	393	0.20
4	123	14.9 ± 1.5	730	7.7	374	2.06
5	261	6.2 ± 1.0	920	3.2	479	0.67
6	296	15.7 ± 0.5	660	8.9	375	2.37
7	269	27.3 ± 5.5	620	15.1	290	5.21
8	30	2.6 ± 0.5	900	1.3	455	0.29
9	266	6.1 ± 1.0	720	4.3	506	0.85
10	108	12.2 ± 1.0	980	8.6	690	1.25
11	418	44.3 ± 1.5	900	18.7	382	4.89
12	483	35.0 ± 2.0	550	27.4	429	6.39
13	337	30.6 ± 2.0	550	16.2	296	5.47
14	316	16.7 ± 3.5	430	13.2	339	3.89
15	265	17.0 ± 1.5	460	10.6	287	3.69
16	138	8.8 ± 0.5	370	7.2	302	2.38

Table 3

Soil	Micrograms Ext. P per gm.	Micrograms Ext. P per ccm	Ext. P as % Labile P
1	1.4	0.62	8.65
2	2.6	0.62	32.5
3	7.2	3.2	400.0
4	2.7	1.4	18.1
5	1.8	0.93	29.0
6	1.7	0.96	10.8
7	2.5	1.38	9.15
8	2.6	1.30	100.0
9	2.1	1.48	34.4
10	2.4	1.69	19.6
11	1.9	0.80	4.3
12.	1.8	1.41	5.15
13	2.4	1.27	7.85
14	1.9	1.50	11.37
15	2.1	1.32	12.23
16	1.0	0.82	11.38