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THE APPLICATION OF RADIOISOTOPES IN THE STUDY OF SOIL FERTILITY, by Sigurd LARSEN, M. Sc.,
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The historical background of the use of radioisotopes in plant nutrition studies goes back to 1923 when Dr G. HEVSEY, the founder of the isotopic tracer method, used radio-lead, which occurs in nature, in a study of the ion uptake by plants. When the artificial production of radioisotopes was discovered in 1934 (by Monsieur and Madame JOLIOU-CURIE) the potential application of radioisotopes as tracers was extended to elements which do not occur as radioisotopes in nature. The most important of these isotopes in the 1930's was radiophosphorus (P^{32}) whose nucleus contains one neutron more than the stable phosphorus isotope P^{31} , but, as is well known, it is now possible to obtain radioisotopes of almost all elements. This has been due to the vast development of atomic research and the establishment of reactors in most parts of the world. The production of radioisotopes was followed by a rapid development of instruments designed for estimating radioisotopes. The facilities for the application of radioisotopes in research have, therefore, increased tremendously in recent years, and this has resulted in the publication of a vast number of papers dealing with the matter, especially in the field of soil and fertiliser studies. Space does not permit a review of all those papers, but some principles and applications of the use of radioisotopes in the study of soils and fertilisers will be outlined.

The most marked advance from introducing radioisotopes in chemical and biological experiments is the possibility of distinguishing between the added compound and similar compounds or elements from other sources, which in soil and fertiliser studies means nutrient elements in the plant originating from the added fertiliser and from the soil reserves. If, for instance, a phosphatic fertiliser is "labelled" with radiophosphorus (P^{32}), the content of radiophosphorus in the plant will indicate the utilization of the fertiliser, and further, the ratio between P^{32} and P^{31} in the plant compared with the same ratio in the fertiliser will give the relative content of soil and fertiliser phosphorus in the plant. This second possibility arises from the fact that there is dilution of the added fertiliser phosphorus by 'available' soil phosphorus, assuming that the soil contains some available phosphorus. This fundamental principle - the dilution of added phosphorus - can be used either for the comparison of different fertilisers or for the estimation of the phosphatic fertility of different soils. In the first case a standard soil, or range of soils, covering the main soil types on which the fertilisers will be used, is treated with the labelled experimental fertilisers, while in the second case a standard

labelled fertiliser, e.g. superphosphate, is added to the soils under examination. From a technical point of view this seems straight-forward but there are, however, some snags in it which we shall deal in the following :

The first snag is the effect of radiation from the isotopes, and several research workers have investigated this problem in relation to the growth of plants and animals during the half century this kind of radiation has been known, but the position is still not clear. When experimenting with plants and fertilizers, one does not necessarily need to take all possibilities of radiation damage into consideration. As long as the radiation does not influence the uptake of fertiliser phosphorus in relation to uptake of soil phosphorus it does not matter. Where the total uptake of phosphorus and the production of dry matter are significant for the experiment the next condition is that the radioactivity of the labelled fertiliser should not influence those criteria.

Most unfortunately, the radiation has, under certain circumstances, a serious effect upon the uptake of phosphorus from the fertiliser. The circumstances which produces the most favourable condition for a radiation effect on the uptake of fertiliser phosphorus in relation to soil phosphorus is uneven distribution of the fertiliser in the soil due to either fixation of soluble phosphate in the upper layer of the soil, as shown by Dr R. Scott Russell of Oxford, or the use of insoluble phosphate which can only be mixed mechanically but not chemically in the soil. Both cases of uneven distribution of labelled fertiliser result in uneven radiation of plant roots causing a decreased uptake of fertiliser phosphorus. This was the case in some experiments of the author from which the figures in table I are derived.

TABLE I

Soil (pH)	Phosphate	P ³² in fert. at planting. Microcuries per g P ³¹ .	% fertiliser P in plant P (barley)				Average
			days after planting				
			24	45	70	94	
7.5	monocalcium	0.01	36.0	43.5	48.3	42.0	42.5
		0.10	40.6	46.5	42.1	47.5	43.2
	dicalcium	0.01	40.4	45.7	39.6	41.6	41.7
		0.10	32.7	52.8	41.6	42.9	42.5
	hydroxyapatite	0.01	3.3	5.5	23.0	32.6	(32.6)
		0.10	0.9	1.2	1.3	1.3	(1.3)
5.7	monocalcium	0.01	25.2	49.2	45.3	51.1	42.7
		0.10	36.3	52.2	45.9	48.3	45.7
	dicalcium	0.01	25.2	48.0	-	48.1	40.4
		0.10	22.2	47.4	44.2	49.5	40.8
	hydroxyapatite	0.01	10.7	35.4	32.1	35.0	(34.2)
		0.10	1.2	7.8	-	7.0	(7.4)

It will be seen from Table I that no differences of any significance were observed in the content of fertiliser phosphorus/soil phosphorus for mono- and dicalcium phosphates. The uptake of phosphorus from hydroxyapatite was, however, greatly suppressed by P³² in this phosphate. The difference in the behaviour between mono- and dicalcium phosphates and hydroxyapatite can only be attributed to the fact that the former are dissolved in the soil solution and in this manner distributed uniformly with the plant available

part of the soil phosphorus, while the latter granulates with the slightly moist soil. In all cases the phosphate was mixed throughout the soil before this was placed in the pots.

In some early field experiments conducted by the author, where the aim was to compare rock phosphate with superphosphate, the content of P^{32} and other radioisotopes in the rock phosphate invalidated the result completely. The adopted (and, as we know, wrong) procedure was the following. A sample of approximately 100 g rock phosphate was neutron irradiated in the atomic pile at Harwell. By this means some phosphorus was converted into radiophosphorus (P^{32}) and some calcium into radiocalcium (Ca^{45} from Ca^{44}). This irradiated sample was mixed with some kg. unirradiated rock phosphate and half of the mixture was converted into superphosphate. Field experiments were carried out with the two fertilisers but while up to 40% of the plant phosphorus could be traced back to the superphosphate it was not possible in any cases to trace any phosphorus from rock phosphate in spite of the fact that this phosphate was mixed into the soil (by harrowing) to the same extent as the superphosphate. Even with lupins grown in a very acid soil (pH 5) no phosphorus from rock phosphate could be traced at all.

In the opinion of the author this can only be due to the concentrated radioactivity in some of the fine particles of rock phosphate.

One learns from this that one cannot be too careful in planning experiments with labelled fertilisers. The condition must be that the "label" occurs in the same position in the fertilisers which are to be compared, further, the behaviour of the fertiliser in the soil must be the same (solubility etc.), in other words, it is hard to see how fertilisers can be compared by means of labelling with P^{32} . The same applies, of course, to comparison between different placement of phosphate in soils. The only way out is, as pointed out by Dr Scott Russell, to employ different levels of P^{32} in the fertiliser. Only where this is done and agreement between the levels is obtained can the results be taken seriously. No "safe level" has been discovered up to the present, and it is doubtful if a general rule covering all possibilities can be given.

As mentioned earlier, the phosphatic fertility of soils can be estimated by employing a standard fertiliser. The basic idea is as follows: Assuming that the phosphorus in soils can be divided into an unavailable fraction, consisting of organic and inorganic phosphorus, and an available fraction, the latter can be determined in the following way: After adding a known amount of labelled phosphate to the soil and after growing plants in the soil, the phosphorus in the plant will consist of a mixture of "soil" and "fertiliser" phosphorus. By means of the radioactivity in a sample derived from the fertiliser and a sample derived from the plant the amount of fertiliser phosphorus in the plant phosphorus can be estimated, and the amount of "available" or "labile" phosphorus in the soil can then be calculated as follows:

If X = amount of added fertiliser phosphorus

L = amount of labile soil phosphorus

C_x = specific content of P^{32} in fertiliser phosphorus

C_p = " " " " P^{32} in plant phosphorus

Then/content of soil phosphorus in plant phosphorus is:

$$C_x - C_p$$

The following relationship can be calculated: $\frac{L}{X} = \frac{C_x - C_p}{C_p}$

Hence :
$$L = \left(\frac{C_x - C_p}{C_p} \right) X = \left(\frac{C_x}{C_p} - 1 \right) X$$

This estimation of labile phosphorus in soil by means of labelled fertiliser can, of course, only be reliable under certain conditions. The influence of radiation on the result can be avoided by mixing a soluble fertiliser throughout the soil which can only be done in pot trials. Further, the influence of seed phosphorus must be taken into consideration by correction in the formula, or, better, by continuing the experiment under such conditions that the amount of seed phosphorus becomes negligible in relation to the total uptake of phosphorus by ^{the} plant.

What is then the advantage of this new method for estimating the phosphatic fertility of soil? Space does not permit a complete review of all the problems which can be attacked by this method, but a few examples, mostly taken from the work of the author, will be given. The following graph (fig. 1) illustrates the relationship between the L value and the total amount of inorganic phosphorus in 33 soils both expressed in the same unit (millimol PO_4 per kg of soil). The amount of inorganic phosphorus was determined by adding a known amount of labelled phosphate to a certain weight of soil (10 g) and adding diluted sulphuric acid to ensure a pH below 2; after shaking (2 hours) the ratio between added phosphate and phosphate already in the soil was determined as described above for phosphate from plant samples. The calculation of the content of inorganic phosphorus was carried out by means of the same formula used for estimating L values. The advantage of the method is that the ratio of soil to solution, shaking time, reprecipitation and refixation of phosphate and temperature do not influence the result. The only condition is that the suspension is acid enough to ensure dissolution of all ^{the} inorganic phosphate in the soil.

Figure 1 (see p. 7)

Fig. 1 shows that complete agreement between inorganic and available phosphorus only exists in three cases (out of 33), namely in soils with an L value below 1.5 and containing no free calcium carbonate. In the other 30 soils the amount of labile phosphorus is less than the amount of inorganic phosphorus, this indicating that inorganic phosphorus in these soils is not fully available. Further, it is remarkable that the L values above 1.5 of non-calcareous soils vary independently of the content of inorganic phosphorus, whilst calcareous soils show no significant variation in value. The difference between non-calcareous and calcareous soils is possibly due to inclusion of phosphorus in chalk granules in the latter.

Another example will illustrate how the new method can produce additional information in traditional experiments. The problem concerned was the influence of soil steaming on the phosphatic fertility of soils. The steam treatment was carried out in iron containers and lasted three and a half hours. The soils were placed in pots taking 20 kg soil which was fertilised with adequate amounts of nutrients other than phosphorus. Some results are given in table II (see page 5).

The phosphorus absorbed by the plant increased more rapidly than the production of dry matter, which indicates that the productive efficiency per unit of phosphorus decreased with increased absorption. Since the levels of other nutrients were estimated to be optimal, the increase in dry matter production must be accounted to the increased absorption.

The increased absorption of phosphorus can be due to either an increase of the amount of available phosphorus in the soil or an increase in the availability of the soil phosphorus.

TABLE II

Soil	Treatment	Dry matter g/pot	P in dry matter g/pot	Dry matter phosphorus	L value
L	1 no P no steam	73.6	0.077	955	-
	2 no P steam	100.7	0.139	724	-
	3 + P no steam	105.3	0.202	523	0.57
	4 steam + P	123.2	0.232	532	0.59
	5 + P steam	135.7	0.216	627	1.14
M	1 -	82.7	0.147	560	-
	2 -	106.8	0.213	501	-
	3 -	96.5	0.201	483	1.72
	4 -	130.4	0.354	368	1.39
	5 -	121.1	0.287	422	2.61
H	1 -	79.3	0.183	423	-
	2 -	148.2	0.453	328	-
	3 -	93.2	0.238	391	2.30
	4 -	144.3	0.454	318	2.26
	5 -	151.6	0.480	316	4.11

L, M, and H = Low, Medium, and High in available phosphorus respectively.

P = 0.5 millimols per kg soil as monocalcium phosphate.

Test crop : Barley grown to maturity

The unchanged L value (in soils L and H) in treatments 3 and 4 indicates that an increased availability offers the probable explanation. As can be seen from Table II, steaming after adding the phosphate has caused a marked increase in the L value which can be due to either a liberation of fixed soil phosphorus or a fixation of the added standard fertiliser. If the first possibility were true, an increased uptake of phosphorus should have been obtained, at least in soils L and M, but the opposite was the case, indicating that fixation is more likely the true explanation.

It will be clear that if the amount of the standard phosphate is halved the L value consequently will be doubled :

$$L = \left(\frac{C_x}{C_p} - 1 \right) \frac{X}{2}$$

$$2L = \left(\frac{C_x}{C_p} - 1 \right) X$$

This seems to have been the case in those three soils where steaming was carried out after adding the standard fertiliser.

The fixation of phosphate resulting from steaming does not necessarily apply to the phosphate already present in the soil provided this has not recently been added. Soil M which was known to respond to phosphate had been fertilised with superphosphate each year and therefore contained recently applied fertiliser phosphate. In this case there was a drop in the L value (from 1.72 to 1.39) due to steaming before applying the standard phosphate. Soil L was a reclaimed sea-bed soil which had never received any fertiliser, while soil H was known to be rich in available phosphorus, and had not received any fertiliser phosphate for several years. Steaming did not significantly alter the L values in those soils. The explanation of the decrease in the case of soil M may be that steaming had immobilized some available phosphorus from the residues of earlier applications of superphosphate.

Needless to say, the suggested explanation of these observations requires further experimentation, but it is clear that the L value offers a better approach to the answer, whatever it may be, than any other value obtainable.

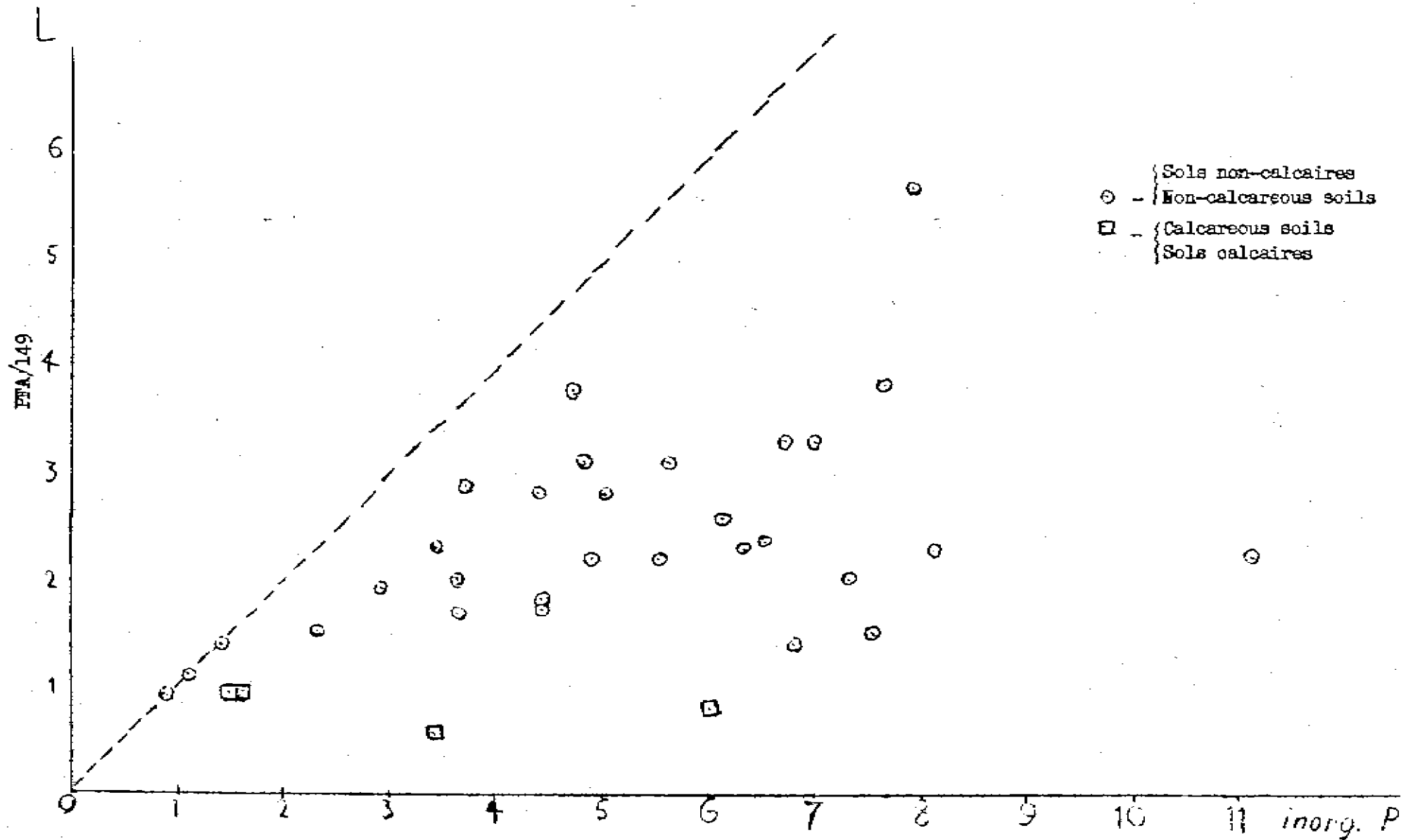
As previously mentioned, it seems rather difficult, if not impossible, to label different phosphatic fertilisers and compare their agricultural value by estimating radiophosphorus in the crop, but the practical difficulties can be overcome by labelling the soil phosphorus instead of the fertiliser phosphorus. This can be done by adding a soluble labelled phosphate to the soil and then comparing the utilization of the labelled soil phosphorus with the unlabelled fertiliser phosphorus. This becomes of particular interest where different placements or application techniques are going to be compared. If the aim is to compare the availability of phosphorus in different fertilisers, this can be done simply by mixing the soil(s) with the fertiliser and measuring the increased L value and comparing this with the content of phosphorus in the fertiliser, as illustrated in fig. 2.

fig. 2 (see p. 8)

This method is far simpler than labelling the commercial fertiliser.

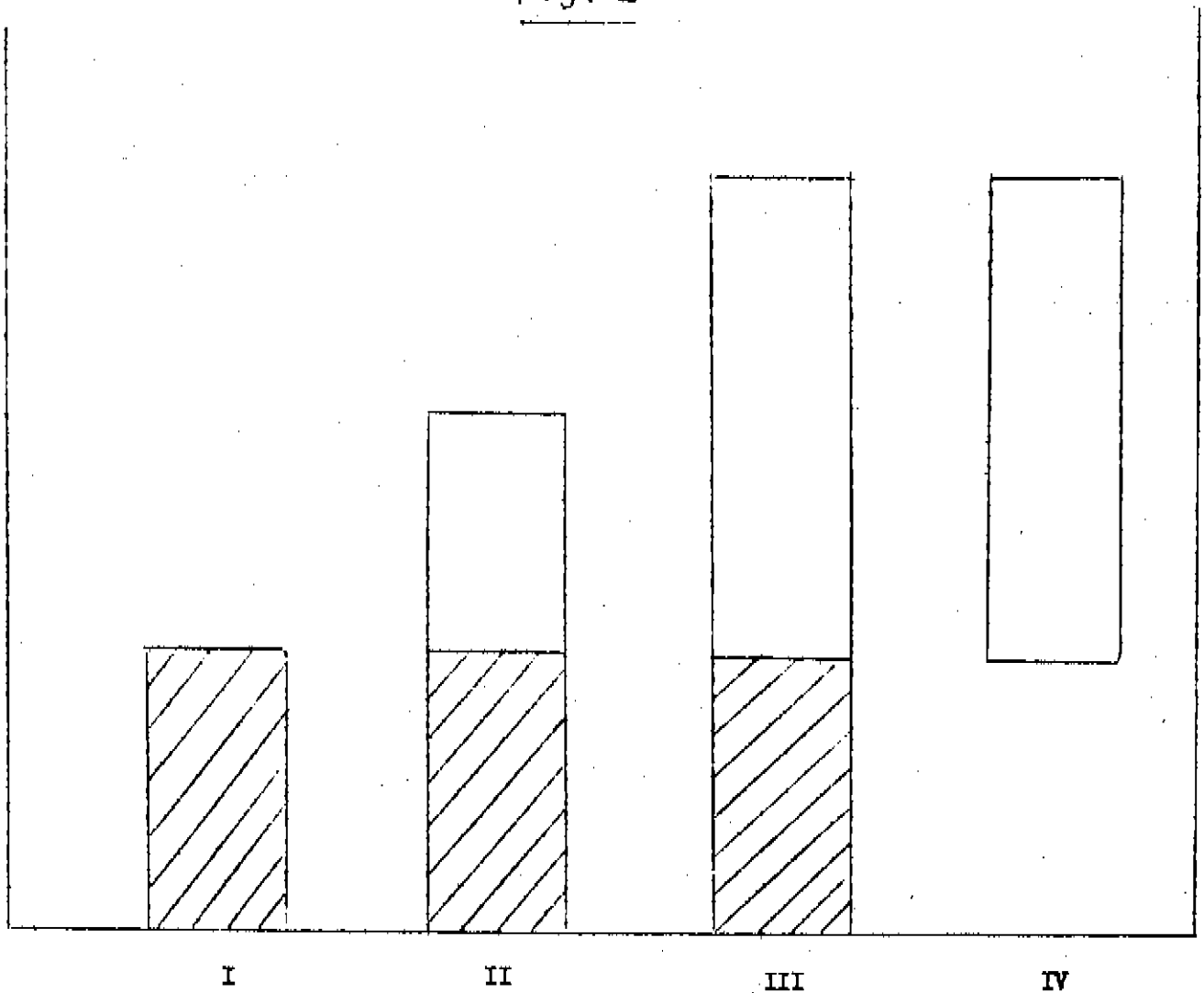
It is not the intention to claim that this paper can give an exhaustive presentation of possible soil and fertiliser studies in the Atomic Age, but if the paper can open a fertile discussion it will have served its purpose.

Fig. 1



L value of soil
Valeur L du sol

Fig. 2



- I No fertiliser added (sans engrais)
 II Fertiliser A half available (engrais A à-demi assimilable)
 III Fertiliser B full available (engrais B entièrement assimilable)
 IV Amount of fertiliser phosphate added (quantité d'engrais phosphaté ajoutée)