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Labile and anion extractable phosphate in calcareous soils and their suitability for characterizing the phosphate availability

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Introduction

Beckett and White (1964) suggested the use of the term "labile" for phosphate ions of soil which equilibrate with the soil solution within periods of few hours. The labile phosphate pool is therefore the sum of solution phosphate and that part of solid phase phosphate which is capable of exchanging with the phosphate ions of the solution within short periods.

The labile phosphate (M_i) can be calculated by means of the following equation (Barbier et al., 1954):

$$(1) M_i = m \frac{R}{r} - M_r$$

R = ^{32}P -radioactivity added to a soil suspension (c/min)

M_r = Quantity of the added phosphate with the radioactivity R ($\mu\text{g P/g soil}$)

r = ^{32}P -radioactivity of the filtrate recovered from the soil suspension after a distinct period of equilibration (c/min)

m = Quantity of phosphate in the filtrate with the ^{32}P -radioactivity r ($\mu\text{g P/g soil}$)

It is thus possible to evaluate M_i by suspending unlabelled soil samples in water, shaking them with a ^{32}P -labelled orthophosphate solution for a given period, and analysing the filtrates for ^{32}P and phosphate. The value of M_i increases as the time of shaking is extended, because deeper layers of the solid phase of soil are able to take part in phosphate exchange (Machold, 1962). An insight into phosphate exchange and into phosphate dissolution and precipitation in the soil suspensions may thus be obtained by determining $\frac{R}{r}$ and m after different shaking times. Such results also help to explain the properties of the soil phosphates in the soils investigated. In addition a

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comparison between the M_i values (determined after different shaking times) and phosphate uptake by plants enables conclusions to be drawn as to the availability of the different fractions of soil phosphate. From such data it is also possible to test the reliability of M_i values in comparison to the results of anion resin extraction for characterizing the available soil phosphate in alkaline soils. This last method proved to be the best of all procedures examined in an earlier paper (*Abd El Aal et al.*, 1977).

Material and Methods

Soil samples obtained from different locations in the Arabian Republic of Egypt (ARE) were used. Some characteristics of these soils are given in Table (1). Further details are given by *Abd El Aal* (1975). The samples were grouped into:

a) alluvial soils from the Nile valley (No. 1, 2, 3), newly reclaimed sandy soils from the Nile Delta (No. 4, 5, 6), and highly calcareous soils from the northern west coast of the ARE (No. 7, 8, 9, 10). M_i values were determined using the technique *Machold* (1962). 2.5 g soil (<0.5 mm X particles) were treated with 25 ml redistilled water of pH 6.5, free of CO_2 and containing 0.3 mg sodium azide. The suspension was shaken for 24 hours, then 5 ml H_2O containing carrierfree $\text{H}_3^{32}\text{PO}_4$ (about 25 000 counts/min · ml measured by means of the liquid counter tube) were added. The samples were allowed to equilibrate for different periods of time (1, 6, 24, 48 and 72 hours) shaking 1 hour in every 24 hours. After each period of equilibration, the suspension was centrifuged for 30 minutes at a rate of 4 800 g and filtrated. ^{31}P was estimated in the clear supernatant according to the method of *Murphy and Riley* cited and modified by *John* (1970). ^{32}P was measured in the liquid phase by means of a liquid counter tube combined with a VAM-160 rate meter (VEB MeBelektronik Dresden, GDR).

All results are mean values of three replicates. The M_i values were calculated according to the equation (1) where $M_i = 0$, according to the carrier-free technique. Some of the samples were also treated according to the carrier technique used by *Machold* (1962) where M_i was 22.22. $\mu\text{g P/g soil}$. The values obtained were investigated for correlations with the following parameters: P-uptake by plants, anion resin extractable P and clay content of the soils (*Abd El Aal* 1975).

The P-uptake by plants was determined in a pot onferment using alfalfa plants (*Medicago sativa* L.) Plastic pots were used each containing 1 kg soil. Each pot was supplied with the following quantities of nutrients: 20 mg N as NH_4NO_3 , 20 mg K as K_2SO_4 , 10 mg Mg as $\text{MgSO}_4 \cdot 5\text{H}_2\text{O}$, 1 mg B as H_3BO_3 , 0.5 mg Zn as $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ and 0.2 mg Mo as $(\text{NH}_4)_6(\text{MoO}_4) \cdot 4\text{H}_2\text{O}$. 12 plants were growing in each pot. The soil moisture was always adjusted to 70 % of the field capacity. The plants were harvested 5 times at intervals of 60 days. The shoots were dried, then ashed using the $\text{HClO}_4\text{-H}_2\text{SO}_4\text{-HNO}_3$ acid mixture according to *Jackson* (1958) P was determined on the samples as described above.

The anion resin extractable soil P was assessed according to *Amer et al.* (1955), where 2 g anion resin (Dowex 1 X 10, Cl^- loaded, 0.3 mm \varnothing) were added to 2 g soil (<0.2 mm \varnothing). The mixture was suspended in 100 ml redistilled H_2O , shaken for 1 h and left for 72 h for equilibration. The resin was then separated from the soil by means of a 0.25 mm \varnothing sieve and washed with redistilled water. The phosphate ions adsorbed by the resin were reextracted by

Table 1: Some characteristics of the soils investigated

Einige Kennwerte der untersuchten Böden

Soil sample No.	Location	pH ¹⁾	CaCO ₃ %	Clay ²⁾ %	Textural class	Resin ³⁾ extractable P ($\mu\text{g P/g soil}$)	P uptake ³⁾ $\mu\text{g P/g soil}$
1	Sakyat-Mecky	8.3	3.2	20.3	Clay loam	50.4	118.3
2	Moshtohor	8.1	3.4	52.9	Clay	48.0	100.5
3	Sakha	7.9	1.8	47.2	Clay	47.0	71.4
4	Enshas	8.1	2.3	7.9	Sand	31.0	58.0
5	Abou-Balah	8.2	0.8	7.6	Sand	26.5	31.2
6	North Tahrir	8.4	13.4	4.3	Sand	30.0	36.1
7	Sidi Barrani	8.3	19.1	16.0	Sandy loam	30.5	22.9
8	El-Daba	8.3	38.0	18.8	Sandy clay/loam	31.5	80.1
9	El-Sallum	8.2	44.5	9.5	Loamy sand	19.0	30.0
10	Ras El-Hokma	8.4	76.3	7.6	Loamy sand	26.0	29.8

1) The ratio soil to 0.1 mKCl solution was 1:2.5

2) Determined using the pipette method (< 0.002 mm ϕ)

3) Methodical details see later

shaking with 50 ml of 10 % NaCl solution for 30 min, transmitting the suspension to a funnel, and washing the resin by the NaCl solution until 100 ml were collected. Phosphorus was determined in the solution as described above.

Results and Discussion

As demonstrated in table 2 using carrier-free ^{32}P , the quotient R/r increases as the time of equilibration is extended while m remains constant. This means that labelled phosphate disappears from the solution continuously after the ^{32}P addition and that it is replaced by unlabelled phosphate from the solid phase of the soil. In most cases equilibrium is evidently not reached in the time of the treatment and the resulting M_i values (Table 2) represent only momentary states. It is most interesting that - within the limits of experimental error - the same results for M_i are obtained if a carrier is used (Table 3), although the values of R/r and m are then quite different. The measured M_i values (Table 2) therefore represent reliable data. In agreement with the theory they are independent of the quantity of phosphate (carrier) added. On the

Table 2: Labile phosphate (Mi) as determined by the ^{32}P carrierfree method (data of Mi and m as $\mu\text{g P/g}$ soil, R as ^{32}P counts/min added to the soil suspension), r as ^{32}P counts/min (registered in the soil solution at any given time). The results are means of 3 replicates (standard deviation $s\% \leq 15$)

Gehalte an labilem Phosphat (Mi), ermittelt ohne Trägerzusatz (Angaben von Mi und m in $\mu\text{g P/g}$ Boden, R und r in Imp./min). Die Ergebnisse sind Mittelwerte von 3 Wiederholungen (Streuung $s\% \leq 15$)

Soil sample No.		\bar{x}	Period of equilibration (hours)				
			1	6	24	48	72
a) 1	R/r	6.04	7.44	10.00	12.55	14.29	
	m ¹⁾	3.31	3.31	3.31	3.31	3.31	
	Mi	19.99	24.63	33.10	41.54	47.30	
	2	R/r	11.28	13.33	15.89	21.54	21.82
		m	2.45	2.45	2.45	2.45	2.45
		Mi	27.64	32.66	38.94	52.77	53.46
3	R/r	18.52	26.12	29.63	32.16	35.09	
	m	0.70	0.70	0.70	0.70	0.70	
	Mi	12.96	18.28	20.74	22.51	24.56	
b) 4	R/r	3.11	3.62	4.69	5.20	5.63	
	m	3.82	3.82	3.82	3.82	3.82	
	Mi	11.88	13.83	17.92	19.86	21.51	
5	R/r	2.50	3.07	4.21	4.65	6.84	
	m	3.15	3.15	3.15	3.15	3.15	
	Mi	7.87	9.67	13.26	14.65	21.55	
6	R/r	4.18	7.15	7.76	8.48	11.81	
	m	0.40	0.40	0.40	0.40	0.40	
	Mi	1.90	2.86	3.10	3.39	4.70	
c) 7	R/r	47.41	77.73	95.44	106.39	110.00	
	m	0.10	0.10	0.10	0.10	0.10	
	Mi	4.74	7.77	9.54	10.64	11.00	
8	R/r	22.30	29.09	30.05	31.45	33.90	
	m	0.50	0.50	0.50	0.50	0.50	
	Mi	8.50	11.05	11.40	11.95	12.90	
9	R/r	55.71	60.66	64.58	64.28	65.41	
	m	0.11	0.11	0.11	0.11	0.11	
	Mi	6.07	6.61	7.04	7.01	7.13	
10	R/r	51.92	52.67	70.16	76.35	74.46	
	m	0.13	0.13	0.13	0.13	0.13	
	Mi	6.59	6.70	8.91	9.70	9.45	

1) m values were nearly independent of the period of equilibration. Using variance analysis and F-test the calculated F-values (F cal.) were always smaller than F-value 3.48 of table (e.g. soil No. 3 F cal. = 1.84, soil No. 5 F cal. = 2.75, soil No. 10 F cal. = 2.84). Therefore, the m values present mean values resulting from all determinations (5 periods, 3 replicates).

Table 3: The labile phosphate in some alkaline soils as determined by the carrier-free and the carrier technique. Mi and m as $\mu\text{g P/g}$ soil; R and r as counts/min (see Table 2)

Das labile Phosphat einiger alkalischer Böden, ermittelt mit und ohne Zusatz von Phosphat-Träger zur Bodensuspension

Sample No.	Technique	R/r	Period of equilibration (hours)							
			24 m	48 Mi	72 R/r	72 m	72 Mi			
2	Carrier-free	15.89	2.45	38.94	21.54	2.45	52.77	21.82	2.45	53.46
	Carrier ¹⁾	9.84	5.85	35.36	11.92	5.75	46.32	12.23	6.01	51.28
4	Carrier-free	4.69	3.82	17.92	5.20	3.82	19.86	5.63	3.82	21.55
	Carrier	2.63	16.00	19.86	2.86	15.00	20.68	2.80	15.90	22.30
10	Carrier-free	70.16	0.13	8.91	76.35	0.13	9.70	74.46	0.13	9.45
	Carrier	15.39	1.97	8.08	19.57	1.67	10.44	24.16	1.39	11.49

1) Results are means of 3 replicates. Differences between Mi values determined by means of the carrier-free and the carrier technique are not significant (t-test, $\alpha = 0.01$)

other hand, the phosphate precipitated when a carrier is used (in such cases the m values are always smaller than the added amount of $22.22 \mu\text{g/g}$) evidently takes part in the exchange as a whole. The particles in question consequently have more reactive inner and outer surfaces. Comparing the values of different soil groups (Table 2) alluvial soils (No. 1, 2, 3) have the highest Mi values due to a relatively high equilibrium concentration of phosphate in the solution (m) and considerable exchange rates (R/r 14 after 72 hours). This indicates the presence of phosphates with considerable water solubility and also the occurrence of fairly large amounts of exchangeable phosphate ions in the solid phase of soil. Since there is a rather close correlation between Mi values and clay contents of soils (Table 4) it may be assumed that the particles of the clay fraction play a decisive role as carriers of exchangeable phosphates.

Sandy soils with low CaCO_3 -contents (No. 4, 5) have lower Mi values. The data in table 2 show that this is due to smaller R/r -quotients, where as the m values are the same as those found in the alluvial soils. This shows that water soluble phosphates here represent the main source of the pool of labile phosphate.

The soils with high CaCO_3 -contents (No. 6, 7, 8, 9, 10) show the lowest Mi values (Table 2) caused by very low equilibrium concentrations of phosphate (m) in the solution. This is emphasized by the fact that the added phosphate of carrier-containing solutions is precipitated quickly and almost quantitatively. In this process different types of reaction are most probably taking place.

It thus seems certain that at first there is sorption of phosphate at CaCO_3 -surface (cf. Cole et al., 1953). In this connection the presence of calcite surfaces may play an important role (Griffin and Jurinak 1974). It is possible that in a reaction of the first

Table 4: The linear correlation coefficients r between M_i values determined after different periods of equilibration (carrier-free) and other characteristics of soils.

Die linearen Korrelationskoeffizienten r zwischen den nach verschiedenen Behandlungszeiten (ohne Träger-Zusatz) ermittelten M_i -Werten und anderen Bodeneigenschaften

Soil sample No.	Characteristic according to Table 1	Mi values					Anion resin P
		Period of equilibration (hours)					
		1	6	24	48	72	
1-10	P uptake	0.846 ⁺⁺	0.823 ⁺⁺	0.858 ⁺⁺	0.842 ⁺⁺	0.876 ⁺⁺	0.906 ⁺⁺
1- 5	of plants	-	-	-	-	0.841 ⁺	0.858 ⁺
6-10		-	-	-	-	0.528	0.421
1-10	Anion resin	0.805 ⁺⁺	0.672 ⁺	0.858 ⁺⁺	0.841 ⁺⁺	0.840 ⁺⁺	
1- 5	P	-	-	-	-	0.750	
6-10		-	-	-	-	0.417	
1-10	Clay content	0.757 ⁺⁺	0.843 ⁺⁺	0.740 ⁺⁺	0.729 ⁺⁺	0.682	

+ Significant at 5 % level

++ Significant at 1 % level

order, hydroxylapatite is subsequently formed at such surfaces (*Griffin and Ajurinak, 1974*). It is conceivable, however, that depending on the conditions of concentration in the solution other calcium phosphates are also formed e.g. octocalcium phosphate (cf. *Whitee and Ellis, 1965; Amer and Ramy, 1971*).

It is interesting to compare the M_i values with those obtained from extraction by means of an anion resin. A comparison of Table 2 with Table 1 shows that in soils with low CaCO_3 -contents (No. 1-5) the amount of resin extractable phosphorus and the M_i values correspond well. In calcareous soils however, far more P is measured by means of the anion resin method than as "labile" phosphate. This is illustrated by the following mean values for the soils No. 6-10: 72 hours $M_i = 9.0 \pm 3.2^2$ $\mu\text{g P/g soil}$, anion resin P = 27.4 ± 5.2^2 $\mu\text{g P/g soil}$. Probably in calcareous soils with low equilibrium concentrations of phosphate in the solution, the low phosphate dissociation from resin favours the dissolution of solid phase phosphate. The exchangeable fraction of soil phosphate is too low to compensate this effect. In calcareous soils, therefore, the results of the two methods differ considerably with regard to the determined phosphate quantities.

The correlation coefficients between all M_i values and the phosphate amounts taken up by alfalfa plants (table 4) are above +0.8. (similar to the extraction by means of anion resin) indicating a close correlation between M_i values and phosphate uptake. These results agree with those obtained for other substrates by

Beckett and White (1964), Russell et al. (1954), Olsen and Watanabe (1970), Kunishi and Taylor (1975) and Machold (1962). Our results do not show the closest correlation for a 50-hour shaking time as found by *Machold (1962)*.

A comparison of the absolute P uptake values (Table 1) with the M_i values in Table 2 (e.g. 72 hours) shows that the latter are considerably lower than the former, especially in the case of calcareous soils. As the phosphate extracted by means of the anion resin reaches higher values in these cases than the labile phosphate (compare above) the alfalfa plants apparently react more analogously to the anion resin. When, however, the correlation coefficients between P uptake and M_i as well as those between P uptake and anion resin P are calculated separately for soils No. 6-10, only low, insignificant correlations result in both cases. According to the results of the experiments presented here (few samples) the method of anion resin extraction does not reflect the behaviour of the plant any better than does the determination of M_i values. This should, however, be re-examined on the basis of a larger experiment. Nevertheless it is possible to suppose that the P uptake from calcareous soils is not only controlled by phosphate dissolution and by phosphate diffusion. *Brewster et al. (1976)* point out for soils with very low phosphate solubility that the roots do not react as a simple "sink" for phosphate. Processes of dissolution of phosphates and improvement of diffusion by the root itself seem to play a role under such extreme conditions. *Jenny and Grobenbacher (1963)* demonstrated e.g. that micigel can fill the cavities between cell wall and clay gel and that the diffusion is thereby facilitated (*Jenny 1966*). Evidently they can be simulated neither by means of M_i determination nor with the help of the method of anion resin extraction.

Summary

This paper investigates the "labile" pool of phosphates (M_i) in calcareous soils of the Arabian Republic of Egypt and the reliability of that pool for assessing soil phosphate availability in comparison to the anion resin extraction (Dowex 1 \times 10). The linear correlation coefficients between the P-uptake of alfalfa plants (pot trials, 6 cuts) and the results of soil analysis were used as a measure for the reliability of the method. The following results were obtained:

1. The determination of M_i according to *Barbier's* equation

$$M_i = m \frac{R}{T} - M_r,$$

in which R = radioactivity added to the soil, r = radioactivity in the solution, m = the amount of phosphate in the solution, and M_r = the amount of phosphate added to the soil, yielded values for M_i reproducibly independent of M_r .

2. With periods of equilibration of 1, 6, 24, 48 and 72 hours the linear correlation coefficient r between the M_i values and the P uptake by alfalfa plants was +0.8. The linear correlation coefficient between the results of anion resin extraction and the P uptake was similar.

3. When only the values of the calcareous soils (> 5 % CaCO₃) were used for calculating the correlation coefficient, lower values for r were obtained. The results of anion resin method did not reflect the P uptake of plants any better than the Mi values. Because of the low solubility of soil phosphates in such soils the exudates (mucigel?) of roots may be important for P uptake. Neither the anion resin method nor the Mi determination can simulate this activity.

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„Labiles“ und anionenaustauschbares Phosphat in Kalkböden und ihre Eignung zur Charakterisierung der Phosphatverfügbarkeit

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In der vorliegenden Arbeit wurde an 10kalkhaltigen Böden aus der Arabischen Republik Ägypten geprüft, ob das „labile Phosphat“ (Mi) bei diesen Substraten ein besseres Maß für die pflanzenaufnehmbare P-Menge darstellt als das durch Anionenaustauscher (Dowex 1 × 10) extrahierbare Phosphat. Als Maß dienten die linearen Korrelationskoeffizienten zwischen der P-Aufnahme von Luzerne (Gefäßversuche, 6 Schnitte) und den Ergebnissen der Bodenanalysen. Folgende Resultate sind erzielt worden:

1. Die Bestimmung von Mi nach der Gleichung von Barbier et al. (1954)

$$M_i = m \frac{R}{r} - M_r$$

in der R = zum Boden zugesetzte Radioaktivität, r = Radioaktivität in der Lösung, m = Phosphatmenge in der Lösung und M_r = zum Boden zugesetzte Phosphatmenge bedeuten, lieferte unabhängig von M_r reproduzierbare Werte für Mi.

2. Der lineare Korrelationskoeffizient r zwischen den Mi-Werten und der P-Aufnahme durch die Luzerne erreichte bei 1, 6, 24, 48 und 72 h Behandlungszeit jeweils Werte > + 0,8 und lag damit in der gleichen Größenordnung wie bei der Ionenaustauschermethode.

3. Wurden die Werte der kalkreichen Böden (> 5 % CaCO₃) allein verrechnet, ergaben sich für beide Methoden gleichermaßen viel schlechtere Korrelationen. Die Anionenaustauschermethode spiegelte für diese Böden die P-Verfügbarkeit nicht besser wider als die Ermittlung der Mi-Werte. Wegen der geringen Löslichkeit der Bodenphosphate dieser Böden dürfte das „Aufschließungsvermögen“ der Wurzel (sekretorische Tätigkeit?) wichtig für die P-Aufnahme der Pflanze sein. Sowohl die Anionenaustauschermethode als auch die Mi-Bestimmung können offensichtlich die Vorgänge an der Grenzfläche Boden/Wurzeloberfläche nicht simulieren.

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