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Pollen Studies on *Vicia faba* L. I, Germination Medium and Incubation Duration and Temperature

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Key words: *Vicia faba;* **pollen; germination medium; incubation**

RINGKASAN

Keadaan optima untuk kajian debunga V. faba *di makmal telah disiasatkan dengan menggunakan kombinasi-kombinasi faktorial kepekatan agar dan sukros dengan berbagai tempoh dan suhu pengeraman. Percambahan dan pertumbuhan tiub debunga yang maxima telah diperolehi dengan media yang mengandungi 2% agar dan 25% sukros dan dipengeramkan selama dua jam pada 25°C. Pemecahan debunga dan tiubnya adalah minima di bawah keadaan-keadaan ini.*

SUMMARY

The optimum culture conditions for V. faba *pollen* in vitro *were determined using factorial combinations of agar and sucrose concentrations with various incubation durations and temperatures. Maximum pollen germination and the growth was obtained using a culture medium consisting of 2% agar and 25% sucrose and incubating for 2 hours at 25°C. The bursting of pollen grains and tubes were minimum under these conditions.*

INTRODUCTION

Plant fertility studies are incomplete without an assessment of pollen viability. Standard tests of viability generally involve the germination of a sample of pollen *in vitro*. Little information is available on pollen studies in *Vicia faba.* The hanging drop technique was used by Rowlands (1958), and an agar-sucrose medium made up of 0.75% agar and 25% sucrose was adopted by Drayner (1959) in their studies on *V. faba* pollen.

The hanging drop technique has several limitations among which the more serious is the difficulty in making germination counts owing to the concentration of pollen grains at the apex of the drop due to surface tension. Agar culture media are more widely used. They are easy to handle and pollen growth substances can be readily incorporated. Besides, they also supply moisture at a constant humidity and aerobic conditions on the surface of the medium are good (Stanley and Linskens, 1974).

In the assessment of pollen viability in *V. faba* various incubation durations were reported. Rowlands (1958) assessed pollen viability after 19 hours while Drayner (1959) made germination counts after one hour.

The discrepancies in the techniques reported and the lack of direct information on the optimum composition of the culture medium and the incubation duration and temperature necessitated this study as *a priori* to further investigations on *V. faba* pollen.

MATERIALS AND METHODS

The study was carried out in several stages. Initially the optimum concentration of agar and sucrose was determined using factorial combinations of 2 and 3g agar with 15, 25 and 30g sucrose in each 100g of media. The agar-sucrose combinations were set up in perspex culture blocks (Lim, 1978) and incubated at 20°C for 1, 2 and 24 hour durations. There were six replications.

From the results of the initial study the optimum agar concentration of 2% was adopted for subsequent studies in which the effect of sucrose concentration and incubation temperatures was investigated. There were three levels of sucrose (15, 25 and 35%) and six incubation temperatures ranging from 5° to 30°C in 5° increments. A multiple temperature incubator incorporating a "cold" and a "hot" end was used.

In the studies, pollen growth was arrested after the necessary incubation by treating the pollen with acetocarmine stain (Johansen, 1940). Pollen grains were considered as germinated if the pollen tube produced exceeded the diameter of the grain. The length of pollen tubes was measured with an occular micrometer.

RESULTS

Experiment 1. Germination medium and incubation duration

Pollen germination was not influenced by the concentration of agar in the culture medium. Agar at 2 or 3% gave equally good germination, 142.4 and 141.9 $\pm$ S.E. 0.48 respectively (Table 1). The concentration of sucrose and the incubation duration, however, significantly influenced pollen germination. Highest germination was obtained with media containing 25 and 35% sucrose. The maximum germination was attained after 2 hours of incubation and prolonged incubation up to 24 hours did not result in further germination. However, germination was incomplete when the pollen grains were incubated for one hour. There was no significant interaction effect among the various levels of agar and sucrose, and the duration of incubation on the germination of the pollen.

Bursting of pollen grains and tubes were found in varying degrees due to the various combination of agar, sucrose and incubation duration. Significant interactions were found between the concentrations of agar and sucrose, agar and incubation duration, and incubation duration and sucrose. Media containing low concentrations of agar and sucrose had a higher incidence of ruptured pollen grains and tubes than media with higher concentrations of soilds (Table 2). Minimum bursting occurred in media containing 35% sucrose. The effect of incubation duration appeared to be additive to the effect of media, the longer the duration the more pollen grains or tubes ruptured. Highest damage

TABLE 2

The effect of agar and sucrose concentration and incubation duration on the number of pollen grains and tubes that burst

a. *Agar × Sucrose*

Agar (%)	Sucrose (%) 15	25	35	S E.
2	112.3	42.1	28.2	±4.33
3	85.8	51.9	35.6	

b. *Agar × Incubation duration*

Agar (%)	Incubation duration (hr)			S.E.
2	51.9	56.6	74.1	±4.33
3	28.4	64.6	80.1	

c. *Sucrose × Incubation duration*

Sucrose (%)	Incubation duration (hr) 1	2	24	S E.
15	86.6	104.4	106.0	
25	20.6	47.9	72.4	±5.30
35	13.2	29.4	53.0	

* Mean of 150 pollen grain samples.

TABLE 1

The effect of agar and sucrose concentration and incubation duration on pollen germination.

Factor	Level	No. pollen germinated	(%)	S.E. of pollen germinated
Agar	2%	142.4	(94.9)	±0.48
	3%	141.9	(94.6)	
	15%	140.4	(93.6)	
Sucrose	25%	143.1	(95.4)	±0.59
	35%	142.9	(95.3)	
	1 hr.	136.9	(91.3)	
Incubation	2 hr.	144.6	(96.4)	±0.59
	24 hr.	144.8	(96.5)	

occurred under prolonged incubation (24 hours) at all levels of agar and sucrose.

The pollen tube length was measured for treatments incubated for two hours. Significant differences in the length of pollen tubes were found for the various levels of agar and sucrose as well as their combinations. At the lowest sucrose concentration (15%), an increase in the quantity of agar inhibited tube growth (Table 3). At higher sucrose levels, however, the inhibitory effect of high agar concentration on tube growth was not significant. A medium containing low levels of agar and sucrose gave rapid pollen tube elongation.

TABLE 3

Pollen tube length after 2 hours incubation ($\times 10\mu$)

Agar (%)	Sucrose (%)			Mean
	15	25	35	
2	67.0	61.2	42.1	56.8
3	33.8	52.6	40.3	42.2
Mean	50.4	56.9	41.2	

S.E. of treatment means (Agar $\times$ Sucrose) = ± 5.62.

Experiment 2. Sucrose concentration and incubation temperature

The germination of pollen grains was significantly influenced by the sucrose concentration as well as the incubation temperature. An incubation temperature of 5°C with a high sucrose concentration was unfavourable for pollen germination (Table 4). Temperatures above 10°C gave high germination at all sucrose concentrations.

TABLE 4

The effect of sucrose concentration and incubation duration on pollen germination

Temperature °C	Sucrose (%)		
	15	25	35
5	43.3‡	4.8	2.3
10	95.3	92.0	86.5
15	95.7	96.5	94.2
20	94.5	96.5	93.8
25	94.0	95.3	96.8
30	94.5	96.0	95.5

S.E. of treatment means = ± 1.63
‡ Mean germination of 100 pollen grain samples from 6 replications.

Although germination occurred over a wide range of temperature the optimum for pollen tube growth was between 20° and 25°C. Outside these temperatures many tubes were found to have ruptured (Table 5a). The small number of tubes bursting at the low temperature of 5°C was due to low germination and at that temperature all pollen grains that germinate burst (Table 5b). The minimum rupturing of pollen tubes occurred in media containing 25% sucrose incubated at 20° to 25°C.

TABLE 5

The effect of sucrose concentration and incubation temperature on the bursting of pollen grains and tubes

a. *Mean number of burst grains*

Temperature °C	Sucrose (%)		
	15	25	35
5	35.2	4.3	2.3
10	79.8	54.8	14.3
15	77.2	70.7	55.2
20	37.0	9.0	32.0
25	45.2	8.3	11.5
30	92.7	74.7	83.8

S.E. of treatment means = ± 2.30

b. *Percentage of germinated pollen burst*

Temperature °C	Sucrose (%)		
	15	25	35
5	82.5	91.2	100.0
10	83.8	59.5	16.5
15	80.7	73.2	58.6
20	39.4	9.4	34.2
25	48.1	8.7	11.9
30	98.0	77.7	87.8

Pollen tube length on culture media with 25% sucrose showed significant differences due to the incubation temperature. Maximum growth of pollen tubes was obtained at 25°C (Table 6). No measurement was made on pollen incubated at 5°C as only a few grains germinated and these were in the "beak" stage. At 30°C growth was retarded due to excessive bursting of the tubes.

TABLE 6

The effect of temperature on pollen tube length (×10).†

Repľication	Temperature °C				
	10	15	20	25	30
1	10.4	21.0	42.5	47.5	13.8
2	9.0	21.0	42.3	49.4	11.9
3	10.3	16.7	37.4	51.6	12.4
4	10.2	16.5	36.8	50.7	14.4
5	11.2	16.2	36.1	41.0	11.8
6	8.9	20.5	37.2	50.6	17.5
Mean	10.0	18.6	38.7	48.5	13.6
	S.E. = ±1.91				

† Mean of 10 pollen tubes.

DISCUSSION

The germination and growth of pollen *in vitro* is influenced by the composition of the culture media, the duration and temperature of incubation. The culture media must provide adequate moisture for germination and a substrate for continued growth. In the study, growth of *V. faba* pollen was affected by the viscosity and the prevailing osmotic tensions of the media. These factors were modified by varying the agar and sucrose concentrations. Too much agar resulted in very firm media which affected tube elongation although germination was unaffected. Sucrose provided nutriment for pollen growth but it also influenced the osmotic tensions of the media. Because of this relationship, media with low concentrations of sucrose caused much rupturing of pollen grains and tubes. Media with high concentrations of sucrose produced the opposite effect. Pollen tube growth was slowed down and less rupturing of tubes occurred. The regulation of pollen growth by agar and sucrose concentrations can, therefore, be generalized to be due to the effect of 1) firmness of the media and 2) osmotic relationships. A balance between these constituents results in optimum growth.

The influence of incubation duration on pollen growth was largely an addictive effect. A minimum duration of incubation is necessary to enable all viable pollen to germinate. One hour was inadequate while incubation beyond two hours was unnecessary. The additive nature of incubation duration was also seen in the bursting of pollen grains or tubes. The bursting was accentuated by prolonged incubations although the composition of the media was also responsible. An excessively long incubation duration would, therefore, be deterimental to pollen growth and could affect measurements.

Pollen germination and tube elongation are biological processes and as such are inhibited by very low or very high temperatures. *V. faba* pollen was able to germinate between 10° and 30°C and assessments of viability can be made within this range. However, the optimum temperature for pollen tube growth falls within a narrower range. The optimum temperatures were between 20° to 25°C. Outside this range tube growth was slower and much bursting occurred.

No single factor operates independently; and the study has shown that *V. faba* pollen grows best in a medium made up to 2% agar and 25% sucrose by weight incubated at 25°C. A duration of two hours of incubation was necessary for maximum growth *in vitro*.

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REFERENCES

DRAYNER, J. M. (1959): Self and cross-fertility in field beans (*Vicia faba* Linn.). *J. agric. Sci. Camb.* **53**: 387–403.

JOHANSEN, D. A. (1940): Plant microtechnique. New York. McGraw-Hill.

LIM, E. S. (1978): A technique for pollen studies. *Pertanika.* **1**: 59–61.

ROWLANDS, D. G. (1958): The nature of the breeding system in the field bean (*V. faba* L.) and its relationship to breeding for yield. *Heredity.* **12**: 113–126.

STANLEY, R. G. and LINSKENS, H. F. (1974): Pollen: biology, biochemistry, and management. New York. Springer-Verlag.

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Phosphate Adsorption by Some Malaysian Soils

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Key words: phosphate adsorption; Langmuir isotherm; slope; intercept; binding energy; phosphate fractionation

RINGKASAN

Tanah-tanah tropika mempunyai daya penjerapan fosforus yang tinggi. Untuk menilai keupayaan tanah-tanah Malaysia untuk menjerap unsur fosforus, empat jenis tanah yang mewakili order Ultisol dan Oxisol telah digunakan. Satu gram tanah telah diseimbangkan dengan larutan 0.1N KCl yang mengandungi beberapa paras fosforus bukan organik selama 24 jam. Banyaknya fosforus yang hilang dari dalam larutan telah dikirakan sebagai fosforus yang terjerap. Data-data penjerapan ini telah dinilaikan dengan menggunakan persamaan isoterm Langmuir. Disini telah didapati persamaan ini padan digunakan apabila kepekatan P dalam larutan seimbang adalah kurang dari 12 mg l^{-1}. Kadar maxima unsur fosforus yang dapat dijerap oleh tanah-tanah ini adalah 1.08, 0.75, 1.24 dan 1.08 mg P g^{-1} tanah untuk tanah-tanah siri Bungor, Durian, Melaka dan Munchong.

Fosforus yang terjerap telah dipecahkan dan didapati ianya mengandungi lebeh banyak Al-P daripada Fe-P. Ini mengesahkan pengetahuan yang Al memainkan peranan yang lebeh penting daripada Fe dalam peroses pengikatan unsur fosforus oleh tanah.

SUMMARY

Tropical soils absorb a large amount of phosphorus that are being added as fertilizers. To evaluate this for Malaysian soils, four representative soils from the order Ultisols and Oxisols were used. One gram of the soils was equilibrated with 0.1N KCl containing various amounts of inorganic P for 24 hours. The phosphorus disappearing from the solution was taken as the amount adsorbed. The sorption data were evaluated using the Langmuir isotherm equation. It was found that this only fits at equilibrium concentration less than 12 mg P l^{-1}. The maximum amount of phosphorus that can be adsorbed by these soils was calculated to be 1.08, 0.75, 1.24 and 1.08 mg P g^{-1} soil for the Bungor, Durian, Melaka and Munchong soil series respectively.

The adsorbed P was fractionated and found to contain more Al-P than Fe-P, confirming the knowledge that Al plays a more important role than Fe in phosphate fixation.

INTRODUCTION

The mechanism of phosphate adsorption on soils has been studied intensively by several authors and it is agreed that in acid soils, hydrated iron and aluminium oxides play a primary role in this process (Hsu, 1964; Bromfield, 1965 and 1967). It has been firmly established that the adsorption process on synthetic iron oxides is a ligand exchange reaction where pairs of Fe-OH react with phosphate to give a binuclear bridging Fe-O-P (O_2)-O-Fe complex in which two of the O atoms of the phosphate ion are coordinated, each to a different Fe^{3+} ion (Parfit *et al,* 1975 and 1976).

Studies with gibbsite, $Al(OH)_3$ and kaolinite have shown that phosphate is strongly adsorbed at the edge of $Al(OH)H_2O$ groups (Muljadi *et al,* 1966; Kyle *et al,* 1975; Parfit *et al,* 1976; Parfit, 1977). The resulting complex is probably also a binuclear bridging form Al-O-P(O_2)-O-Al (Kyle *et al,* 1975). At higher concentrations, further phosphate adsorption takes place on gibbsite and kaolinite even though the edge sites are fully occupied.

The Langmuir equation has been used to characterise the adsorption of phosphate by soils (Olsen and Watanabe, 1957; Shapiro and Fried, 1959; Gunary, 1970) and soil minerals (Cole

Key to author's name: Zaharah, R.

et al, 1953; Hsu and Rennie, 1962). Limitations in its use have also been studied and reported (Harter and Baker, 1977; Veith and Spasito, 1977). But despite these limitations, the Langmuir isotherm can still be used to give a relative measure of the energy by which phosphate is bounded to the solids and a relative adsorption maximum.

The purpose of this study was to investigate quantitatively the capacity of Malaysian soils to retain phosphate and to relate this to various soil factors, since it is well known that Oxisols and Ultisols contain appreciable amounts of sesquioxides, Al and Fe and others that are active in phosphate fixation.

MATERIALS AND METHODS

Samples from four surface soils (0 – 20cm) were collected from Universiti Pertanian Malaysia campus at Serdang, Selangor. Two of these soils are Oxisols – the Melaka and Munchong series. Both these soils are classified as Tropeptic Haplorthox. The other two soils are Ultisols. These are the Bungor series (Typic Paleudult) and the Durian series (Orthoxic Tropudult). The soils were air dried and ground to pass through a 0.5 mm sieve. Each of the samples was analysed for clay content by the pipette method, organic matter by dichromate oxidation method (Walkley and Black, 1934), pH in 1:2 soil water ratio using a pH meter, amorphous Fe, Al and Si by Tiron extraction at pH 10.5 (Bierman and Baert, 1977), Fe by the O-phenanthroline method (Toth *et al,* 1948), Al by the eriochromcynine R method (Vanderdeelen *et al,* 1973) and Si by the ammonium molybdate method (Weaver *et al,* 1968). Phosphate fractionation was done according to the method of Chang and Jackson (1957). The minerology of the clay samples were done by X-ray.

For the phosphate adsorption studies, duplicate 1 g samples were shaken with 50 ml of 0.1N KCl containing various amounts of inorganic phosphates as KH_2PO_4(0, 5, 10, 20, 30 and 40 μg P ml^{-1}) in 180 ml screw cap polyethylene centrifuge tubes on an orbital shaker for 24 hours at room temperature. The tubes were centrifuged at 3,000 rpm for 15 minutes and aliquots of the clear supernatant liquid used for colorimetric determination of inorganic phosphate (Jackson, 1958) using Bosch and Lomb Spectronic 20 spectrophotometer at a wave length of 640 nm. The quantity of P adsorbed was taken to be that lost from the solution during the shaking. The residues were washed twice with NH_4Cl and successively extracted with 25 ml 0.5N NH_4F pH 8.5, 0.1N NaOH and 0.5 N H_2SO_4 in accordance with the fractionation method of Chang and Jackson (1957). The phosphorus in the extracts was determined as above (Jackson, 1958).

RESULTS AND DISCUSSION

Soil Characteristics

Table 1 shows some properties of the four soils used. The pH of all these soils are in the range of 4.5. Organic matter content are moderate except for the Bungor series which contains quite a high organic carbon content. Total amorphous materials of Fe, Al and Si are about 2%, in which Fe and Al amorphous materials dominate. The clay minerals presents are kaolinite, gibbsite and goethite.

Table 2 shows the amounts of phosphate being adsorbed by these four soils at the different rates of P applied. As the rate of P applied increases, the amount of P adsorbed also increases, but the percentage of P adsorbed decreases as the rate of P applied increases.

Evaluation of Sorption Data

The Langmuir adsorption equation was used to study the quantitative measurement of phosphorus being adsorbed by these soils. The equation used can be written as:

$$C_A = K_1K_2C_E/(1 + K_1C_E) \qquad (1)$$

where;

C_A = amount of P sorbed/unit weight of soil

K_1 = a constant related to the binding energy

K_2 = adsorption maximum

C_E = Equilibrium P concentration

The above equation can be rearranged to give:

$$C_E/C_A = (1/K_1K_2) + C_E/K_2 \qquad (2)$$

A plot of C_E/C_A versus C_E gives a straight line from which K_2 (slope $^{-1}$) and K_1 (slope/intercept) can be obtained. Regression analysis was used to compute the straight lines obtained with this Langmuir equation.

The adsorption isotherm drawn for these four soils is shown in Figure 1. These soils showed almost similar patterns of adsorption. The amount of P adsorbed increased as the amounts of P in the equilibrium solution increased and tended to level off up to an equilibrium P

TABLE 1

General characteristics of the soils used in the experiment.

Characteristics	Ultisol		Oxisol	
	Bungor	Durian	Melaka	Munchong
% Clay	58.4	46.4	57.0	67.5
% Silt	13.3	16.2	15.8	7.6
% Sand	28.3	37.4	27.2	24.9
pH (H2O)	4.41	4.54	4.52	4.55
pH (KCl)	3.91	3.62	3.83	4.07
CEC m.e. $100g^{-1}$	8.90	9.95	7.10	5.50
% Organic carbon	3.36	1.78	2.81	2.47
% Amorphous Fe	0.38	1.22	1.41	0.98
% Amorphous Al	0.40	0.46	0.48	1.13
% Amorphous Si	0.03	0.06	0.05	0.16
Clay types	Kaolinite, gibbsite and goethite			

TABLE 2

Amounts of phosphate adsorbed by the soils at the different levels of phosphate added.

Treatments	Ultisol				Oxisol			
(μg P g^{-1} soil)	Bungor		Durian		Melaka		Munchong	
	μg P adsorbed	%	μg P adsorbed	%	μg P adsorbed	%	μg P adsorbed	%
250	249	99.6	230	92.0	228	91.2	244	97.6
500	468	93.6	398	79.6	419	83.8	403	80.6
1000	737	73.7	603	60.3	755	75.5	782	78.2
1500	894	59.6	690	46.0	843	56.2	979	65.3
2000	1087	54.4	702	35.1	911	45.6	1038	51.9

concentration of 12 mg Pl^{-1}. The Bungor series showed an increase in the amounts of P adsorbed after this point. This same observation was explained by Gunary (1970) to be due to the adsorption of P after the monolayer adsorption has been achieved on the adsorption sites. This may also be due to the organic matter present, which may provide a major site for P adsorption (Harter 1969).

From the graph it is seen that the Munchong series, which is an Oxisol, showed the highest level of adsorption at levels above 500 μg of P added which may be due to it being a highly weathered soil (Oxisol), containing high amounts of clay and amorphous materials. The Durian series showed the lowest adsorption capacity at all levels of P added. This may be due to it having a lesser clay and organic matter content as compared to the Bungor series.

The adsorption data plotted according to the conventional Langmuir equation (equation 2) are shown in Figure 2. All the four soils showed a very high correlation coefficient. Table 3 shows the characteristics of the Langmuir type adsorption of P by these four soils. All of them have very high K_2 values indicating that the maximum capacity of these four soils to adsorb P ranges from 0.746 to 1.235 mg Pg^{-1} soil. This corresponds to a rate of 2,238 to 3,705 kg P ha^{-1}.

Fractionation of adsorbed phosphate

Fractionation of the adsorbed phosphate is shown in Table 4. Most of the adsorbed phosphate is present as Al-P followed by Fe-P. Very little of Ca-P was detected in all the samples. This finding is in accordance with those found by Udo and Uzu (1972), and those found by Dunbar and Baker (1965) who found that most of the applied P in soils were extracted with NH_4F and NaOH as Al and Fe phosphates respectively. This shows that Al plays a more important role as phosphate fixers in these soils when compared to Fe and Ca.

Juo and Boyd (1968) have indicated that amorphous and colloidal forms of Al-P and Fe-P are regarded as being readily available to plants because of the relatively slow rate of recrystallization in acidic aqueous medium. Al-P may remain highly amorphous and hence more available to plants than Fe-P which, because of its faster rate of crystallization in acid aqeous medium,

TABLE 3

Characteristics of the Langmuir type of phosphate adsorption by the soils.

Characteristics	Ultisol		Oxisol	
	Bungor	Durian	Melaka	Munchong
Slope	0.927	1.34	0.81	0.93
Intercept	1.051	2.12	2.30	0.90
Maximum adsorption (mg g^{-1})	1.079	0.746	1.235	1.075
Energy of adsorption (l mg^{-1})	0.882	0.632	0.352	1.033

TABLE 4

Fractionation of adsorbed phosphate into Al-P, Fe-P and Ca-P.

Soil	P added	P adsorbed	Al-P	Fe-P	Ca-P
	μg P g^{-1} soil				
Bungor					
	250	249	111	52	5
	500	468	211	77	7
	1000	737	393	114	5
	1500	894	492	115	6
	2000	1087	544	143	7
Durian					
	250	230	97	78	6
	500	398	185	100	6
	1000	603	341	144	11
	1500	690	451	166	7
	2000	702	510	171	8
Melaka					
	250	228	92	70	3
	500	419	147	95	3
	1000	755	260	126	2
	1500	843	336	138	3
	2000	911	366	127	3
Munchong					
	250	244	97	62	1
	500	463	192	96	2
	1000	782	344	132	4
	1500	979	395	150	4
	2000	1038	406	160	5

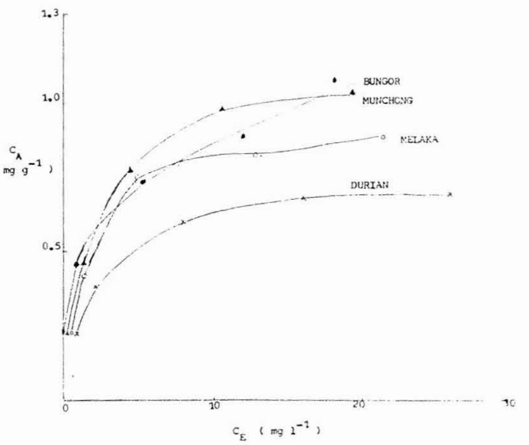

Fig. 1 : Isotherms for the sorption of added phosphate by the soils.

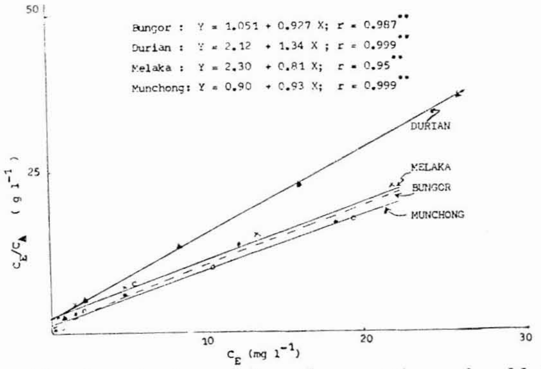

Fig. 2 : Isotherms for the sorption of added phosphate by the soils obtained by using the conventional Langmuir equation.

may exist in a higher state of crystallization in soils, and therefore less available to plants.

The finding of this study shows that the four soils have a very high capacity to adsorb added phosphate but a higher percentage of this sorbed P is in the Al-P form, thus suggesting that the added P is somewhat available to the plants.

Since these soils undergo many cycles of wetting and drying due to the climatic conditions, one would then expect the adsorbed P to revert to the less available Fe-P.

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REFERENCES

Biermans, V. and Baert, L. (1977): Selective extraction of the amorphous Al, Fe and Si oxides using an alkaline Tiron solution. *Clay Miner.* **12:** 127–135.

Bromfield, S. M. (1965): Studies of the relative importance of iron and aluminium on the sorption of phosphate by some Australian soils. *Aust. J. Soil Res.* **3:** 31–44.

Bromfield, S. M. (1967): Phosphate sorbing sites in acid soils. I. An examination of the use of ammonium floride as a selective extractant for aluminium bound phosphate in phosphated soils. *Aust. J. Soil Res.* **5:** 93–102.

Chang, S. C. and Jackson, M. L. (1957): Fractionation of soil phosphates. *Soil Sc.* **84:** 133–144.

Cole, C. V., Olsen, S. R. and Scott, C. O. (1953): The nature of phosphate sorption by calcium carbonate. *Soil Sc. Soc. Amer. Proc.* **17:** 352–356.

Dunbar, A. D. and Baker, D. E. (1965): Use of isotopic dilution in a study of inorganic phosphorus fractions from different soils. *Soil Sc. Soc. Amer. Proc.* **29:** 259–262.

Gunary, D. (1970): A new adsorption isotherm for phosphate in soil. *J. Soil Sci.* **21:** 72–77.

Harter, R. D. (1969): Phosphorus adsorption sites in soils. *Soil Sci. Soc. Amer. Proc.* **33:** 630–632.

Harter, R. D. and Baker, D. E. (1977): Applicationsand misapplications of the Langmuir equation to soil adsorption phenomena. *Soil Sci. Soc. Amer. J.* **41:** 1977–1080.

Hsu, P. H. and Rennie, D. A. (1962): Reactions of phosphate in aluminium systems. I. Adsorption of phosphate by X-ray amorphous aluminium hydroxide. *Can J. Soil Sci.* **42:** 177–209.

Jackson, M. L. (1958): Soil Chemical Analysis. New Jersey. Prentice Hall Inc., Engelwood Cliffs.

Juo, A. S. R. and Boyd, E. G. (1967): Particle size distribution of aluminium, iron and calcium phosphates in soil profiles. *Soil Sci.* **106:** 374–380.

Kyle, J. H., Posner, A. M. and Quirk, J. P. (1966): Kinetics of isotopic exchange of phosphate adsorbed on gibbsite. *J. Soil Sci.* **26:** 32–43.

Larsen, S. (1967): Soil phosphorus. *Adv. Agron.* **19:** 131–210.

Muljadi, D., Posner, A. M. and Quirk, J. P. (1966): The mechanism of phosphate adsorption by kaolinite, gibbsite and pseudoboehmite. I. The isotherms and the effect of pH on adsorption. *J. Soil Sc.* **17:** 213–229.

Munns, D. N., and Fox, R. L. (1976): The slow reaction which continues after phosphate sorption: kinetics and equilibrium in some tropical soils. *Soil Sc. Soc. Amer. J.* **40:** 46–51.

Olsen, S. R. and Watanabe, F. S. (1957): A method to determine a phosphorus adsorption maximum of soils as measured by the Langmuir isotherm. *Soil Sc. Soc. Amer. Proc.* **21:** 144–149.

PARFIT, R. L. (1977): Phosphate adsorption on an Oxisol. *Soil Sc. Soc. Amer. J.* **41**: 1064–1067.

PARFIT, R. L., ATKINSON, R. J. and SMART, R. ST. C. (1975): The mechanism of phosphate fixation on iron oxides. *Soil Sci. Soc. Amer. J.* **39**: 837–841.

PARFIT, R. L., and ATKINSON, R. J. (1976): Adsorption of phosphate on goethite. *Nature* **264**: 740–741.

RAJAN, S. S. S. and FOX, R. L. (1975): Phosphate adsorption by soils. II. Reactions in tropical acid soils. *Soil Sc. Soc. Amer. J.* **39**: 846–851.

TOTH, S. J., PRINCE, A. L., WALLACE, A. and MIKKELSEN, D. S. (1948): Rapid quantitative determination of eight mineral elements in plant tissue by a systematic procedure involving use of a flame photometer. *Soil Sc.* **66**: 459–466.

UDO, E. J. and UZU, F. O. (1972): Characteristics of phosphorus adsorption by some Nigerian soils. *Soil Sc. Soc. Amer. Proc.* **36**: 879–883.

VANDERDEELEN, J., PINO, N. I. and BAERT, L. (1973): Kinetics of phosphate adsorption in a soil derived from volcanic ash. *Turrialba* **23**: 291–296.

VEITH, J. A. and SPASITO, G. (1977): On the use of the Langmuir equation in the interpretation of adsorption phenomena. *Soil Sc. Soc. Amer. J.* **41**: 467–702.

WEAVER, R. M., SYERS, J. K. and JACKSON, M. L. (1968): Determination of silica in citrate-bicarbonate-dithionite extracts of soils. *Soil Sci. Soc. Amer. Proc.* **32**: 497–501.

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