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INFLUENCE OF INCUBATION PERIOD, TEMPERATURE AND DIFFERENT PHOSPHATE LEVELS ON PHOSPHATE ADSORPTION IN SOIL

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ABSTRACT

Phosphorus (P) is the most important element after nitrogen but P sorption properties are poorly documented in many soils. In this study, P sorption capacity in relation to incubation period and temperature under various P levels were determined for two different soil series (Rustam and Miani). Phosphorus was added at 5, 10, 25, 50, 100, 250, 500 and 1000 μ g g⁻¹ to the soils and incubated for the period of 1, 3, 7, 15, 30 and 60 days at 25 and 35±1°C temperatures. Phosphorus sorption was significantly influenced by the temperature, added P and incubation period. Clay content has major influence on P sorption. Rustum Series adsorbed more P than that of Miani Series. The P sorption was faster at high temperature (35°C). At the end of period in the Rustum Series with added 2.5 and 1000 μ g g⁻¹ P level, P sorption were found 0.7 and 943 μ g g⁻¹, respectively, at 25±1°C temperature, whereas at 35±1°C temperature the respective P sorption for same P levels were 1.8 and 987.0 μ g g⁻¹. In the Miani Series at 25±1°C temperature, P sorption was 0.7 and 941 μ g g⁻¹, while at the temperature of 35±1°C, P sorption were 1.0 and 986 μ g g⁻¹ soil. Increasing P levels, temperature and incubation, resulted in higher P sorption. Hence, high clay in combination with calcium carbonate contents enhanced P adsorption, while high organic matter and high native soil P resulted in lesser P adsorption.

Keywords: Incubation, Clay Content, Phosphorus, Soil Series, Sorption, Temperature

1. INTRODUCTION

Phosphorus is most important element for natural ecosystems throughout the world (Onweremadu, 2007). Its availability is limited by fixation in soil. Soil pH is one of the important factors affecting P retention in soils. P sorption reactions with Fe and Al occur at pH<7, while at pH>7.0 Ca and Mg ions in the presence of carbonates help precipitate added P (Bubba *et al.*, 2001). P has high affinity for mineral surfaces and thus, it is often the least available nutrient to plants. Efficiency of the applied P fertilizer is only about 20% under alkaline calcareous soil conditions. P requirement of crops depends mainly on imported P fertilizer, which is expensive. The bioavailability of P is

essential in understanding the processes involved in its sorption because high quantity (>90%) of applied P in soil can be fixed after its application. Sorption of P is a process in which readily soluble P is changed to less soluble forms by reacting with inorganic or organic compound of the soil so that P becomes immobilized and is reduced for plants uptake (Khan *et al.*, 2007).

Clay content has a major effect on P retention in soil (Devau *et al.*, 2010). Likewise, calcium carbonate in soils affects P fixation. After application of P fertilizer to calcareous soils, a decrease in P concentration level occurred within 24 h. The adsorbed P might be diffused inside the CaCO₃ aggregates or P may diffuse inside the existing cracks

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and crevices of $CaCO_3$ aggregates during the overnight period of incubation (Brady, 1999).

Soluble P compounds when added to soil react rapidly with various compounds like $CaCO_3$ (Bertrand *et al.*, 2003), sand (Leclerc *et al.*, 2001), clay (Johnston *et al.*, 1991; Toor *et al.*, 1997), organic matter (Daly *et al.*, 2001), iron and aluminum oxides (Toor *et al.*, 1997) and are quickly converted to slowly available forms (Castro and Torrent, 1995). In addition to these properties, adsorption also depends on contact time between soil and P with temperature (Indiata *et al.*, 1999). Adsorption from added P was found at initially low levels but, slowly increased with increasing incubation period. This might be due to desorption of greater number of P ions (Agbenin and Tissen, 1995).

Surface adsorption and precipitation are the major P retention processes limiting the availability of applied P. However, the need for caution is stressed in interpreting correlation between P retention capacity (Bertrand *et al.*, 2003) and soil properties because of the inter correlations among soil properties (Torrent, 1995). A simple correlation analysis may not be sufficient in evaluating direct influence of soil properties on P retention capacity (Zhang *et al.*, 2005). Hence, this study was conducted to determine the relationship between P adsorption by soil at varying P levels and the period of incubation, which can help in P fertilizer recommendation and improvement of P fertilizer management.

2. MATERIALS AND METHODS

2.1. Physico-Chemical Properties of the Soil

The study was carried out on two soil series (Rustam and Miani) and soil samples were air-dried, crushed and passed through a 2 mm sieve. The soil samples were analyzed for their physico-chemical properties. Soil texture was analysed by Bouyoucos' hydrometer method, organic matter by Black and Walkley method (Jackson, 1958), lime content by Acid neutralization Purie's method (Kanwar and Chopra, 1967) and electrical conductivity by electrical conductivity meter (1:5 soil water extract). Soil pH was measured (water 1:5 extract) using PHM210 Standard pH meter at 30°C (Jones, 2001) and available phosphorus by AB-DTPA method (Soultanpur and Schwab, 1977).

2.2. Phosphate Adsorption

Phosphate adsorption data were obtained by shaking 10 g soil samples with 100 mL of 0.01 M CaCl₂ in 300 mL glass bottles. The samples were prepared in triplicates. Varying P amounts of 0, 2.5, 5, 10, 25, 50,



100, 250, 500 and 1000 μ g g⁻¹ soil were added as KH₂PO₄ and 5 to 8 drops of toluene (C₆H₅CH₃) were also added to each bottle to stop microbial activity. The samples were incubated for 1, 2, 3, 7, 15, 30 and 60 days at the constant temperature of 25 and 35±1°C on an orbital shaker at the speed of 150 rpm. The suspensions were then filtered through Whatman filter paper No.42. Finally, the P was determined followed by the Murphy and Riley (1962) method. The amount of P adsorbed was calculated following Fox and Kamprath (1970).

The experiment was conducted in *in vitro* condition following completely randomized design with three replications for 60 days of incubation period.

2.3. Statistical Analysis

The data so obtained were statistically analyzed using the SAS Software Program (Version 9.2) and the treatment means were separated using Tukey's test (p<0.05).

3. RESULTS

3.1. Physico-Chemical Properties of the Soil

Analytical data on the physico-chemical properties of the soil are presented in **Table 1**. Results showed both of the soil series (Rustam and Maiani) were clay loam in texture, with slight salinity (pH 7.5 to 7.92). The electrical conductivity was low (<4 dSm⁻¹) and the soils were calcareous in nature (>14% carbonate). The organic matter was low, ranging from 0.77 to 0.98% and the AB-DTPA extractable phosphorus was deficient (0.17 to 0.2 $\mu g g^{-1}$) (**Table 1**).

3.2. Phosphate Sorption in Rustum Series

Figure 1a shows the effect of phosphorus levels and incubation period on phosphate sorption in Rustum soil series at $35\pm1^{\circ}$ C. The effect of phosphorus levels and incubation period on phosphate sorption at $25\pm1\div$ C. P sorption was increased with increasing incubation period (). During the initial 2 days of incubation period, most of the added P was adsorbed (40-84%). Then adsorption of P decreased slowly until 60 days of incubation (**Table 2**). The highest P sorption (83.2%) was observed for the 25 μ g g⁻¹ treatment, but after 60 days of the incubation the highest P sorption (95.8%) was observed when 500 μ g g⁻¹ of P was applied. This was statistically similar from 25 to 100 μ g g⁻¹ of added P. In general the majority of the P sorbed occurred at the higher amounts of the added P.

Figure 1b shows the Rustum soil, the P sorption was faster at the temperature of 35° C compared to that at 25° C.

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Table 1. Physico-chemical properties of soils

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Parameters	Rustam series	Miani series			
Sand (%)	1.0	6.5			
Silt (%)	46.5	46.0			
Clay (%)	52.5	48.5			
Textural Class	Clay loam	Clay loam			
Soil reaction (pH)	7.95 (H ₂ O), 7.5 (CaCl ₂)	7.80 (H ₂ O), 7.61 (CaCl ₂)			
Electrical Conductivity (dS m ⁻¹)	0.28 (H ₂ O), 1.86 (CaCl ₂)	0.28 (H ₂ O), 1.86 (CaCl ₂)			
Lime content (%)	14.2	14.3			
Organic matter (%)	0.98	0.77			
AB-DTPA P ($\mu g g^{-1}$)	0.17	0.2			

AB-DTPA = Ammonium Bicarbonate-DTPA



Fig. 1. Effect of phosphorus levels and incubation period on phosphate sorption in Rustum soil (a) at 25°C temperature (b) 35°C temperature



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PA (μg g ⁻¹)	Days							
	1	2	3	7	15	30	60	
2.5	28.0e	40.0d	40.0e	40.0c	44.0d	44.00d	44.0d	
5	40.0d	56.0c	52.0d	72.0b	72.0c	72.00c	72.0c	
10	60.0c	74.0b	76.0ab	79.0ab	79.0b	79.00b	80.0b	
25	83.2a	84.0a	86.8a	88.8a	88.8a	88.8ab	88.8a	
50	75.0b	80.0a	83.2a	88.0a	88.0a	88.0ab	92.0a	
100	63.7c	70.0b	78.1a	82.3a	86.0ab	86.30b	90.0a	
250	29.6	56.0c	61.6c	76.6b	89.6a	89.60a	89.6a	
500	17.6f	67.6b	72.4b	88.4a	95.0a	95.80a	95.8a	
1000	36.3e	50.0d	54.5d	73.8b	86.3ab	93.80a	94.3a	

Table 2. Effect of phosphorus levels and incubation period on phosphate sorption in Rustum soil at $25\pm1^{\circ}$ C

Means within the same column followed by the same letters are not significantly different at p<0.05

 $PA = Phosphorus Added = \mu g g^{-1}$, $PE = Phosphorus at Equilibrium = \mu g 10 mL^{-1}$, $PS = Phosphate Stored by Soil = \mu g g^{-1}$

 Table 3. Effect of phosphorus levels and incubation period on phosphate sorption in Rustum soil at 35±1°C

 P sorption (%) in Rustum soil 35°C

PA ($\mu g g^{-1}$)	Days							
	1	2	3	7	15	30	60	
2.5	72.0b	72.0b	80.0ab	80.0b	80.0b	80.0b	80.0b	
5	62.0c	62.0c	62.0c	76.0b	76.0b	76.0b	76.0b	
10	72.0b	75.0b	75.0b	80.0b	80.0b	80.0b	80.0b	
25	82.4a	84.4a	86.4a	90.0a	90.0a	90.0a	90.0a	
50	79.4a	86.4a	87.2a	92.2a	92.2a	92.2a	92.2a	
100	77.5a	78.5b	79.6b	85.7a	86.0ab	91.0a	91.0a	
250	75.2ab	81.2ab	90.4a	92.8a	95.6a	96.8a	96.8a	
500	78.2a	88.6a	92.2a	96.2a	97.2a	98.2a	98.2a	
1000	48.5d	55.0d	65.0c	90.3a	97.3a	98.7a	98.7a	
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Means within the same column followed by the same letters are not significantly different at p<0.05 PA = phosphorus added = μg g⁻¹, PE = phosphorus at equilibrium = $\mu g 10 \text{ mL}^{-1}$, PS = phosphate stored by soil = $\mu g g^{-1}$

About more than half of the P was adsorbed (48.5-82.4%) after 1 day of incubation period for all treatments (**Table 3**). The highest P sorption was recorded (82.4%) for the 25 μ g g⁻¹ treatment, which was statistically similar to 500 μ g g⁻¹ treatment. However, after 60 days of incubation the highest P sorption (98.7%) was observed for the 1000 μ g g⁻¹ treatment and this was statistically similar to the 25 to 1000 μ g g⁻¹ treatments. The amount of the P sorption was higher at the temperature of 35°C compared to that at the lower temperature. It shows that P adsorption is significantly affected by the temperature. The rate of P adsorption decreased after 7 days of the incubation, but it increased after 60 days where most of the added P was adsorbed (60-98.7%).

3.3. Phosphate Sorption in Miani Series

Figure 2a shows Effect of phosphorus levels and incubation period on phosphate sorption in Miani soil at $25\pm1^{\circ}$ C. The Miani soil almost gave the same trend of the P sorption at both temperatures. The soil P sorption

increased with the incubation period. The highest P sorption (79.2%) was observed in 25 and 50 μ g g⁻¹ treatments, but after 60 days of incubation the highest P sorption (96.6%) was observed in 500 μ g g⁻¹ treatment and this was statistically similar to that of 10 to 1000 μ g g⁻¹ treatments. During the first 3 days of incubation period, most of the added P was adsorbed (28-85.2%) then the rate of adsorption decreased after 60 days of incubation (**Table 4**). At 60 days of incubation period, P sorbed was 32-96.6%. It shows that most the added P was adsorbed slowly and significantly increased at the higher rates of P addition.

Figure 2b shows like Rustum soil, P sorption in Miani soil was faster at 35°C compared to that at 25°C during the incubation period. Almost more than half of the added P was adsorbed (29.9-82%) after 1 day of the incubation at all levels of P (**Table 5**). At 60 days of the incubation, the highest P sorption (98.6%) was observed in 1000 μ g g⁻¹ treatment, which was statistically similar to that of 25 μ g g⁻¹ treatment. The



rate of P sorption was higher at 25° C compared to that at higher temperature. It shows that temperature affected the P sorption in the soil. After 3days of incubation, the amount of the P adsorbed decreased.

3.4. Phospahte Equilibrium in Rustam Series

Figure 3a shows the effect of phosphorus levels and incubation period on phosphate equilibrium in Rustum soil at $25\pm1^{\circ}$ C. The P levels affected the equilibrium of P

in Rustum soil with its equilibrium decreased with the incubation period. During the first three 3 days of incubation, it was rapidly reduced then the reduction slowed down except for the P level of 1000 μ g g⁻¹, followed by 500 μ g g⁻¹).

Figure 3b shows the effect of phosphorus levels and incubation period on phosphate equilibrium in Rustum soil at $35\pm1^{\circ}$ C. At higher temperature, the P equilibrium showed the same trend like at $25\pm1^{\circ}$ C.



Fig. 2.Effect of phosphorus levels and incubation period on phosphate sorption in Miani soil (a) at 25°C temperature (b) 35°C temperature

Table 4. Effect of phosphorus levels and incubation p	period on phosphate sorption in Miani soil at 25±1°C
P sorption (%) in Miani soil at 25°	C

PA ($\mu g g^{-1}$) 1	Days	Days							
	1	2	3	7	15	30	60		
2.5	20de	28d	28e	32d	32d	32d	32d		
5	56b	62b	64c	66c	66c	66c	66c		
10	62b	75a	77ab	81b	83b	83b	83b		
25	79.2a	84.8a	85.2a	85.2a	85.2ab	85.2ab	85.2ab		
50	75.6a	83a	84a	84.6a	84.6ab	84.6ab	84.6ab		
100	53.1c	66.1b	68.4b	81.3b	84ab	84ab	84ab		
250	24.8d	53.2c	55.6d	86a	90.4a	90.8a	90.8a		
500	15.2e	67.2b	72b	90.6a	94.8a	96a	96.6a		
1000	32d	48c	51d	59c	85ab	93.3a	94.1a		

Means within the same column followed by the same letters are not significantly different at p<0.05

 $PA = Phosphorus Added = \mu g g^{-1}$, $PE = Phosphorus at Equilibrium = \mu g 10 mL^{-1}$, $PS = Phosphate Stored by Soil = \mu g g^{-1}$



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PA (μg g ⁻¹)	Days							
	1	2	3	7	15	30	60	
2.5	40d	40c	48d	48d	48d	48d	48d	
5	56c	56b	64c	66c	66c	66c	66c	
10	74b	80a	80b	80b	82b	82b	83b	
25	82a	85.2a	85.6a	85.6a	85.6a	85.6a	85.6a	
50	77.8a	87.8a	88a	89.8a	89.8a	90a	90a	
100	64c	68.9	73.8b	84.5a	88.3a	90a	90a	
250	29.9	82.6a	85.6a	91.6a	94.4a	95.6a	95.6a	
500	79.8a	87.4a	90.6a	95.4a	96.4a	98a	98a	
1000	46.5d	52.4b	61.3c	91.2a	96.7a	98.5a	98.6a	

Table 5. Effect of phosphorus levels and incubation period on phosphate sorption in Miani soil at $35 \pm 1^{\circ}$ C

P sorption (%) in Miani soil at 35°C

Means within the same column followed by the same letters are not significantly different at p<0.05

PA = Phosphorus Added = $\mu g g^{-1}$, PE = Phosphorus at Equilibrium = $\mu g 10 \text{ mL}^{-1}$, PS = Phosphate Stored by Soil = $\mu g g^{-1}$



Fig. 3. Effect of P levels and incubation period on P equilibrium in Rustun soil (a) at 25°C temperature (b) 35°C temperature







Fig. 4. Effect of incubation on P equilibrium in miani soil: (a) at 25°C temperature (b) at 35°C temperature

During the first 2 days of incubation, all P equilibrium levels were low, except for $1000 \ \mu g \ g^{-1}$ treatment. After 3 to 7 days, almost more than half of the equilibrium levels of P were reduced. Whereas, at the highest P level ($1000 \ \mu g \ g^{-1}$), it was observed that P equilibrium in the soil was not achieved even after 60 days of incubation, but it was higher at 35°C than it was at 25°C.

3.5. Phospahte Equilibrium in Miani Series

The effect of phosphorus levels and incubation period on phosphate equilibrium in Miani soil at $25\pm1^{\circ}$ C. P levels affected the equilibrium of P in Miani soil during the incubation period. The trend of reduction was similar to that of Rustum soil. The P equilibrium decreased with the incubation period. It was rapidly reduced in the first week of incubation then the reduction was slowed down except for the lower P levels (2.5, 10, 25, 50 and 100 μ g g⁻¹) (**Fig. 4a**).

Figure 4b shows the effect of phosphorus levels and incubation period on phosphate equilibrium in Miani soil at $35\pm1^{\circ}$ C. The equilibrium of P was reduced quickly at all levels, except for the $1000 \ \mu g \ g^{-1}$ treatment (°C). In general, after 15 days of incubation, all levels of P equilibrium had reduced to lower levels. For both temperatures, there was similar reduction found in P equilibrium during the incubation period. Furthermore, at the highest P level (1000 $\ \mu g \ g^{-1}$), it was observed that in soil the equilibrium was not achieved even at 60 days of incubation, but it was different from that at the 25°C.



4. DISCUSSION

Rustum and Miani soils were similar in their properties, except there slightly higher clay content in Miani soil. It is known that clayey soils with high pH have more P problems due to the bondage of P with other cations such as Ca and Na. Result of the current study showed that soil with high clay content adsorbed more applied P. Likewise, soil with CaCO₃ adsorbed more applied P. However, reverse trend was true for the available P and organic matter contents. Soils with more available P as well as high organic matter retained less applied P.

Both of the soils showed similar trend of P sorption during the incubation period. However, a slight increase in P sorption was observed in Miani soil at 60 days of incubation. The P sorption increased with the incubation period. Most of the P was sorbed within one week and the remaining portion was slowly adsorbed thereafter (Cyr et al., 2009). The slow reaction between P in the solution and soil particles continued up to the incubation time, one distinct behavior of P in the soil. The rates of P sorption by the both soils were initially fast and then slowed down for a long time without any evidence of ending. This finding is in agreement with that of Huang et al. (2012). It might be due to the initial sorption of greater number of P ions. Comparatively higher amounts of P sorption occurred with the high amounts of P added to the soil and maximum amounts of the P sorbed in all the added P at the end of incubation period. Singh et al. (1990) found similar trend of P sorption.

The release of the P in the soils depends on the soil characteristics, especially soil texture. The increased P release occurred due to less clay content and more native P along with the increasing initial phosphorus level. Soil P can be affected by clay and $CaCO_3$ content in the soil. The more the clay content is the more P is needed to be applied into the soil. Kaloi *et al.* (2011) reported that P adsorbing capacity of soils always depends on clay contents and native soil P.

Result of this study showed that clay and CaCO₃ contents severely affected the sorption of P. The sorption of heavy textured soils can be decreased using organic matter (Kaloi *et al.*, 2010). Similar findings were observed by Moazed *et al.* (2010) and Javid and Rowell (2002) that P sorption was considerably linked to clay and organic matter content of the soil. Furthermore, Rashid and Rowell (1988) reported that total P absorbed was directly connected to total surface area and soil clay contents. At low P concentration, total P adsorbed increased with increase in total surface area. The soils

containing low content of clay with high native P required lower doses of P as soils have less P adsorbing capacity and adsorbed P can easily be released. In addition, soil P adsorption at lower concentration in equilibrium solution appears to be managed by the iron oxides while at high concentration by the Ca-P precipitation (Mehmood *et al.*, 2010).

Temperature is a crucial factor affecting P sorption in soils. Temperature plays significant role in the sorption of P. Both of the soils under study had similar behavior for P sorption. More P was adsorbed at 35°C than it was at 25°C. This result is consistent with the study of Sinegani and Sedri (2011). Furthermore, P significantly increased the soluble and loosely bound P aluminum-bound and iron-bound P. The amount of Al-P and Fe-P was markedly higher at 30°C than that at 4°C (Huang et al., 2011). For the soils under study, it was observed an increase in P sorption with temperature In addition, at high temperature a large proportion of P adsorbed permanently, thereby decreasing the equilibrium P concentration in soil solution Huang et al. (2012). In addition temperature plays a significant role for the glucose concentrations (Soares et al., 2011) and in soil due to the presence of microbial activity may also alter (Branzini et al., 2014).

Phosphate sorption was reported to increase with increasing temperature. On the other hand, rate of P sorption and desorption decreased when the equilibration temperature is lowered. The effect of temperature on the process was noticed during the first day of equilibration. Less P was observed in the equilibrating solution at the low temperature (Mamo et al., 2005). From the results of the study it was found that P sorption for soil series is of the following order: Miani Soil>Rustum soil at 60 days of incubation period and 35°C temperature. Consistent reports were obtained by Dahar (2002) and Katiar (2002). According to their observation, the soil with the more clay content sorbed more applied P. However, the reverse was true for the available P and organic matter content. The soils with more available P as well as higher organic matter retain less applied P. This trend of increasing P sorption by the soil was demonstrated from the minimum of one day to the maximum of 60 days of incubation. These results are supported by the findings of Memon (2004). The soil has itself buffering capacity which due to the temperature could influence and may effect on the P sorption and other chemical compositions (Al-Dabbas et al., 2010).

For both soils, it was further observed that the equilibrium between P added in solution and sorbed P by the soil was obtained earlier at the low P level. However;



the equilibrium of P in 1000 μ g g⁻¹ treatment could not be achieved before 60 days of incubation. The longer the time of incubation, the higher was the phosphate sorption. Similar result was reported by Huang *et al.* (2012).

5. CONCLUSION

The results of this study suggested that increase in applied P during incubation period resulted in higher P sorption in both soils. The temperature of incubation affected P sorption and that it was higher at 35°C than it was at 25°C. It was found that soil with more clay content had higher P sorption capacity and needed higher amount of P, while more native P in the soil resulted in less P sorption. It was observed from the study that with increase in the temperature directly influences the P sorption and in future might be different soil series could be studied for further aspects.

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