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# Phosphorus fractions in calcareous soils amended with P fertilizer and cattle manure

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## ARTICLE INFO

### Article history:

Received 24 June 2008

Received in revised form 17 January 2009

Accepted 12 February 2009

Available online xxx

### Keywords:

Phosphorus fractions

Calcareous soils

Manure

Olsen P

## ABSTRACT

Studies on phosphorus (P) reactions over time and the role of organic matter in calcareous soils are important for developing P fertilizers and manure management practices. This study was conducted to determine the effects of time, soil properties and manure on Olsen-P and its chemical forms in calcareous soils. Eight soil samples were treated with two levels of inorganic P (0 and 300 mg kg<sup>-1</sup>) and two levels of manure (0 and 1%, w/w). Olsen-P was determined at 2, 5, 10, 30, 60, 90 and 150 days of incubation. Phosphorus fractionated to NaCl+NaOH-P, citrate bicarbonate (CB-P), citrate bicarbonate dithionate (CBD-P), acetic acid-sodium acetate (OAc) and HCl (HCl-P) after 5, 30 and 150 days. Olsen-P, in P-treated soils, showed a positive correlation with the NH<sub>4</sub>OAc extractable Al and active CaCO<sub>3</sub>. Increases in recovery as CBD-P revealed that Fe oxides play an important role in P sorption during 0–30 days. The relative distribution of P fractions and Olsen-P followed the order of HCl-P > OAc-P > Organic-P > CBD-P > Olsen-P > NaCl+NaOH-P. The CB-P fraction was not measurable. Application of manure along with P increased the recovery of applied P and recovery as CBD-P compared with the P treatment. In general, manure application had an appreciable and different impact on the chemical fractions of P in calcareous soils as P from manure gradually turned into available forms over the time.

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## 1. Introduction

Application of phosphorus fertilizers in agricultural calcareous soils has introduced some problems mainly due to P fixation, low recovery and accumulation in soil. Information on the chemical forms of phosphorus is fundamental to understanding phosphorus dynamics and its interactions in calcareous soils that is necessary for management of P. Decrease in availability of P is suggested to be a complex function of several factors such as: soil chemical composition, amount and reactivity of silicate clays, CaCO<sub>3</sub>, Fe oxides, P addition rates and time (Afif et al., 1993). Some of the studies show that availability of P fertilizer in calcareous soils negatively correlate with CaCO<sub>3</sub> content of soil (Borggard et al., 1990; Afif et al., 1993). In contrast, some studies have shown that rapid P sorption has been related to iron oxides and not to the total or active CaCO<sub>3</sub> content in soil (Ryan et al., 1985; Solis and Torrent, 1989). Samadi and Gilkes (1999) found a negative relationship between recovery of P as Olsen-P and clay-related properties (Fe and Al extractable with dithionite, Al extractable with oxalate and clay contents) and positive relationship with carbonate-related properties (active calcium carbonate equivalent) 5–160 d after P application. Organic sources of P are known to increase P availability more than inorganic P fertilizers and enhance efficient use of applied P fertilizer (Mohanty et al., 2006). The synergistic effect of manure application along with P fertilizer on increasing soil test P concentration has been

reported by Toor and Bahl (1997), Reddy et al. (1999), Mkhabela and Warman (2005) and Garg and Bahl (2008). Delgado et al. (2002) reported that organic amendments consisting of a mixture of humic and fulvic acids increased recovery of applied P by bicarbonate (Olsen-P). This was due to decreases in the precipitation rate of poorly soluble Ca phosphate by organic amendments.

Iran is located in an arid region and most of its soils are calcareous. However, data on the chemistry of P in these soils are scarce. Therefore, this study addresses the effects of soil properties on P availability and its chemical forms over time, and the ways manure may affect efficiency of applied P fertilizer in calcareous soils. It also compares the influence of KH<sub>2</sub>PO<sub>4</sub> and manure application on availability of P (Olsen-P) and its fractions over time for a number of calcareous soils which are widely ranged in CaCO<sub>3</sub> content (3.8–57%).

## 2. Materials and methods

Soils used in this study were from sites located in Mashhad, northeastern Iran (36.27°N 59.60°E) within an arid region at average annual rainfall of 269.5 mm. Eight soil samples were collected from different sites to provide a range in properties affecting phosphorus behavior. Selected soil samples were particularly different in terms of their CaCO<sub>3</sub> and clay content. Samples were taken from surface (0–20 cm) horizons of arid, calcareous agricultural soils classified as Aridisols or Vertisols. Because of the different and variable history of manure and fertilizer addition rates and cropping practices in studied soils, a record of the actual P applications was not precisely available. However, the soils generally were under the wheat

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**Table 1**  
Some selected properties of soils

Soil number	Subgroup	O.C g kg <sup>-1</sup>	Al-O	Al-CBD	Fe-O	Fe-CBD	pH	Ece dSm <sup>-1</sup>	CEC Cmol(+) kg <sup>-1</sup>	ACCE (%)	CCE (%)	Clay g kg <sup>-1</sup>
1	Typic haplocalcid	10.4	0.38	0.28	0.24	3.72	8.0	0.95	8.3	13.0	57.3	168
2	Typic calcitorrent	3.6	0.29	0.30	0.24	4.45	7.8	2.10	11.7	12.3	46.3	257
3	Tyoic haplotorrent	13.4	0.45	0.33	0.59	3.75	8.0	1.46	15.2	11.8	29.5	389
4	Typic haplocambid	2.5	0.08	0.20	0.09	1.92	7.9	1.46	3.0	4.3	33.8	80
5	Typic haplocambid	4.2	0.20	0.22	0.12	3.56	8.2	0.57	5.2	4.1	18.1	130
6	Typic haplosalid	5.6	0.29	0.36	0.45	6.16	7.3	19.57	8.3	4.5	11.5	254
7	Typic haplocambid	9.2	0.26	0.34	0.40	6.41	8.1	2.04	5.2	2.8	3.8	140
8	Typic haplocambid	29.6	0.35	0.25	0.46	6.02	7.4	1.40	12.6	6.3	13.0	245

ACCE: Active Calcium Carbonate Equivalent.

CCE: Calcium Carbonate Equivalent.

CBD: Citrate–Bicarbonate–Dithionite.

O: Oxalate.

monoculture cropping system. A composite sampling technique was used for taking soil samples at each site. Then, the samples were air-dried and ground to pass a 2 mm sieve prior to analysis. Selected soil properties were determined according to standard methods (Sparks, 1996). Clay content was determined by the hydrometer method, soil organic C was analyzed using Walkley and Black method, pH was measured in saturated paste using a glass electrode, Ece was determined in a soil saturated extract, cation exchange capacity was measured by 1 M NaOAc buffered at pH 8.2 and active CaCO<sub>3</sub> was determined using potassium permanganate solution. Available P was determined by the Olsen method (Olsen et al., 1954). Citrate–dithionite–bicarbonate (CBD), extractable Fe and Al (Fe–CBD and Al–CBD) and oxalate extractable Fe and Al (Fe–O and Al–O) were determined according to the procedures suggested by Jackson et al. (1986), McKeague and Day (1966) and Loeppert and Inskeep (1996), respectively. Total CaCO<sub>3</sub> equivalent (CCE) was measured by acid neutralization (Richards, 1969). Phosphorus fractionation was carried out according to the Olsen and Sommer scheme (1982). The process involves four sequential chemical extractions as: 1) 0.1 M NaOH and 1 M NaCl (NaCl–NaOH–P) for nonoccluded Al and Fe bound P and P adsorbed on mineral surfaces; 2) 1 M NaCl and citrate–bicarbonate (CB–P) for phosphate hydrolyzed from Fe/Al phosphates that were reabsorbed by calcite in the previous step and highly soluble precipitated phosphates (Harrell and Wang, 2006); 3) Citrate–bicarbonate–dithionite (CBD–P) to remove P occluded within Fe oxides and P adsorbed on high energy sites; 4) 1 M HCl (HCl–P) for P precipitated as poorly soluble Ca phosphates mainly lithogenic apatite. Except for the step before HCl extraction, soil samples were treated with 1 M CH<sub>3</sub>COOH–CH<sub>3</sub>COONa buffer pH=4 (OAc–P) to remove the more soluble and pedogenic hydroxyapatite (Ruiz et al., 1997; Delgado and Torrent, 2000; Delgado et al., 2002). All extracts were analyzed for P by the method of Murphy and Riley (1962). Total P content was determined by the ignition method (Saunders and Williams, 1955) and inorganic P measured by 1 M H<sub>2</sub>SO<sub>4</sub> extraction (Walker and Adams, 1958). Organic P was calculated as the difference between total and inorganic P.

### 2.1. Experimental design

100 g of each soil were treated with two levels of inorganic P (0 and 300 mg kg<sup>-1</sup> soil) as KH<sub>2</sub>PO<sub>4</sub> and two levels of manure (0 and 1%, w/w) in the form of cattle manure that was passed through a 0.5 mm sieve with three replications. Under field conditions, P concentration near a granule, powder or droplet of liquid fertilizer is very high therefore this situation probably can be simulated by an incubation experiment at high P application rates between 10<sup>2</sup> to 10<sup>3</sup> mg P kg<sup>-1</sup> soils (Afif et al., 1993). The treated soils were transferred to pots (15 cm in diameter), incubated at 25 °C for up to 150 days and maintained at field capacity by weight calibration. A 5 g sub sample of soils was obtained from each pot

after 2, 5, 10, 30, 60, 90 and 150 days. Soil samples were air-dried and analyzed for Olsen–P and pH. Soil pH was determined in 1:2.5 soil/water suspensions after 0.5 h with a combination pH electrode. Phosphorus was fractionated from soil collected at 5, 30 and 150 days.

Phosphorus percentage recovery for Olsen–P and each P fraction at each sampling time was calculated as:

$$P_{\text{corrected}} = P_{\text{amended}} - P_{\text{control}}$$

$$\text{Recovery \%} = 100 \times (P_{\text{corrected}} / P_{\text{added}})$$

Where:

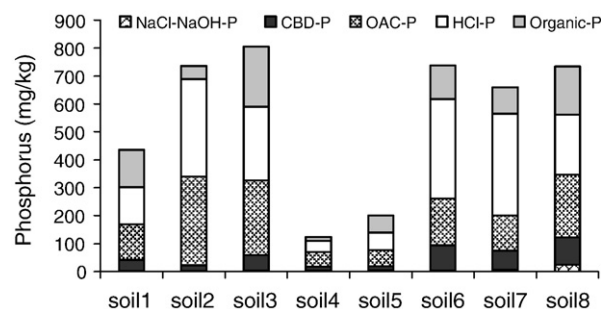
$P_{\text{amended}}$  Olsen–P or P fraction in each P treatment  
 $P_{\text{control}}$  for P and manure treatments = P in control soils  
 $P_{\text{control}}$  for P+manure treatment = P in manure treatment  
 $P_{\text{added}}$  for P and P+manure treatments = 300 mg P kg<sup>-1</sup> soil  
 $P_{\text{added}}$  for manure treatment = 120 mg P kg<sup>-1</sup> soil

Total P, organic C, pH, and Ece in manure were determined according to the procedures referred by Peters (2002). With application of 1% of cattle manure with 22.7% organic C, Ece 7.2 (1:5), pH 7.4 (1:5) and 1.2% P in manure amended soils, 120 mg P kg<sup>-1</sup> was added in studied soils.

### 3. Results and discussion

Some chemical and physical properties of the studied soils are given in Table 1. Clay and CaCO<sub>3</sub> content ranged from 80 to 389 g kg<sup>-1</sup> soil and from 3.8 to 57.3 g kg<sup>-1</sup> soil, respectively. Electrical conductivity was less than 2.5 dSm<sup>-1</sup> except for soil 6 (Ece = 19.57 dSm<sup>-1</sup>).

The P contents in different fractions of the soils are given in Fig. 1. The sums of OAc–P and HCl–P in all soils were greater than 50% of the total P, suggesting that most of the soil P was in carbonate phase. In



**Fig. 1.** Distribution of P fractions in the studied soils: organic–P, hydrochloric acid (HCl), acetic acid–sodium acetate (OAc) citrate bicarbonate dithionite (CBD) and sodium chloride plus sodium hydroxide (NaCl–NaOH) extractable P.

**Table 2**

Simple correlation coefficient between Olsen-P, total and organic P and some selected soil properties in control soils

	Clay	CCE	O.C	CEC	ACC	Fe-CBD	Fe-O	Al-CBD	Al-O
Olsen-P	0.115	-0.240	0.911**	0.353	-0.070	0.394	0.241	-0.263	0.269
Total-P	0.807**	-0.279	0.563	0.795*	0.278	0.743*	0.890**	0.744*	0.756*
Organic-P	0.766*	-0.162	0.709*	0.764*	0.369	0.377	0.857**	0.243	0.907**

\*Significant at  $P \leq 0.05$ .

\*\*Significant at  $P \leq 0.01$ .

soil 3, these two fractions represented more than 90% of total P. Carreira et al. (2006) and Harrell and Wang (2006) showed that HCl-P was the largest extractable P fraction in arid and carbonate-rich soils. Also, Yu et al. (2006) reported that HCl-P accounted for 45–60% of total soil P in neutral and alkaline soils.

The relative distribution of each fraction and Olsen-P in studied soils followed the order of HCl-P > OAc-P > Organic-P > CBD-P > Olsen-P > NaCl-NaOH-P. In general, the CB fraction was not measurable. Delgado and Torrent (2000) showed that HCl-P (Ca phosphates) was about 80% of the sum of fractions in the Olsen and Sommer scheme. They had not separated OAc fraction in their studies.

Olsen-P in control soils had a significant positive correlation with organic C, but was not correlated with other soil properties (Table 2). Sharpley (1985) showed that available P was closely correlated with organic P and phosphatase enzyme activity in unfertilized soils with maximum P sorption in fertilized soils. Organic-P was significantly and positively correlated with organic C, ammonium oxalate extractable Al and Fe and clay content (Table 2). Total P showed a significant and positive correlation with citrate-dithionite-bicarbonate and oxalate Al and Fe extractable and clay content. No relationships were evident between CaCO<sub>3</sub> content and total P (Table 2) in all soils. These findings are consistent with Turner et al. (2003) who reported that organic P was positively correlated with organic C, clay content and oxalate extractable Al, Fe and Mn.

Table 3 presents correlation coefficients between selected soil properties and Olsen-P at the end of each incubation time in P treated soils. Negative significant relationships were observed between Olsen-P and ACCE, Al-O and clay after early stages of the experiment (when Olsen-P was high) whereas, these coefficients were positive at the end of the experiment (when Olsen-P was low). It seems that ACCE, Al-O and clay have an important role in P sorption and desorption in these soils. No relationship was found between CCE and Olsen-P. The importance of active CaCO<sub>3</sub> in arid environments is further verified by Carreira et al. (2006) and Shaheen et al. (2007).

Correlation coefficients between Olsen-P and P fractions in control soils showed that Olsen-P had a positive significant relationship ( $P \leq 0.01$ ) with NaCl-NaOH fraction. At the end of the experiment (150 days) in P treated as well as in control soils, NaCl-NaOH-P was related to the amount of extracted P by the Olsen method (Table 4). It was also observed that Olsen-P and NaCl-NaOH-P were closely related

**Table 4**

Correlation coefficients between P fractions and Olsen-P at each incubation interval in P treated soils

Day	NaCl-NaOH	CBD	OAC	HCL	Organic
5	0.255	-0.235	-0.798**	-0.222	0.665
30	0.319	-0.843**	-0.007	-0.038	0.495
150	0.873**	-0.835**	0.474	-0.114	0.116

HCl: Hydrochloric acid extractable P.

OAc: Acetic acid-sodium acetate extractable P.

CBD: Citrate bicarbonate dithionate extractable P.

NaCl-NaOH: Sodium chloride plus sodium hydroxide extractable P.

\*Significant at  $P \leq 0.05$ .

\*\*Significant at  $P \leq 0.01$ .

to ACCE and Al-O (Table 5). The results indicate that the NaCl-NaOH fraction and Olsen method could be used as an index of weakly adsorbed P on surfaces of calcite and amorphous Al oxides in these soils. Shaheen et al. (2007) showed that NaOH+CB-P was accounted for 3.8 to 6.3% of the total P in all soils followed by NaHCO<sub>3</sub>-P, which accounted for 1.6 to 4.3% of total P. They reported that NaOH-P was positively correlated with clay content, OM content, CEC and active CaCO<sub>3</sub> ratio. Subramaniam and Singh (1997) showed that only Ca-P and Al-P were the major contributors for the plant available P fraction in their soils. In some calcareous soils Shariatmadari et al. (2006) reported that the rate of P release from solid to solution phase had positive significant relationships with clay, active CaCO<sub>3</sub> and citrate-bicarbonate-dithionite extractable Al content of the soils. In all treatments, the P recovery as Olsen-P (%) decreased with time (Fig. 2).

In P treated soils, sorption by soil particles was rapid at the beginning but slowed down with time, so that P recovery as Olsen-P decreased up to 75% after 5 d of incubation. In this period (first time interval), P recovery as Olsen-P in manure treatment was lower than from KH<sub>2</sub>PO<sub>4</sub>. The reason is that soon after addition of manure and fertilizer, P from manure would not be immediately available, while KH<sub>2</sub>PO<sub>4</sub> is completely soluble in water. Change of P recovery as Olsen-P with time in manure treated soils was more consistent. At the end of the experiment, P recovery as Olsen-P was about 17 and 34% of total applied P in fertilized and manured soils, respectively (Fig. 2). Laboski and Lamb (2003) showed that P from manure was more available than fertilizer P for 1 to 9 months of incubation. In the control treatments, Olsen-P increased over incubation time, likely as a result of P mineralization which occurred when dried soils were rewetted (data not shown). Laboski and Lamb (2003) reported similar results in their experiment.

Application of manure and fertilizer P combined increased P recovery as Olsen-P compared with separate application of fertilizer P (Fig. 2). This result shows that application of manure increased the efficiency of fertilizer P, that this effect was greater in the period between 5 to 60 days and caused P to remain in available form for a longer period. Toor and Bahl (1997) showed that the combined addition of poultry manure and fertilizer P had a synergistic effect and increased Olsen extractable P compared with fertilizer P alone. Garg

**Table 3**

Correlation coefficients between selected soil properties and Olsen-P at the end of each incubation interval

Time (day)	Clay	CEC	OC	CCE	ACCE	Fe-CBD	Fe-O	Al-CBD	Al-O
2	-0.451	-0.489	0.064	-0.478	-0.754*	-0.250	-0.203	-0.537	-0.643
5	0.035	0.032	0.410	-0.235	-0.244	-0.055	0.305	-0.117	-0.090
10	0.578	0.669	0.744	-0.024	0.277	0.176	0.594	0.064	0.444
30	0.669	0.670	0.370	0.316	0.545	-0.368	0.409	-0.064	0.451
60	0.798*	0.796*	0.500	0.258	0.630	-0.165	0.612	0.162	0.703
90	0.648	0.647	0.223	0.555	0.782*	-0.404	0.323	0.031	0.642
150	0.737	0.732	0.323	0.482	0.793*	-0.261	0.489	0.192	0.748*

\*Significant at  $P \leq 0.05$ .

**Table 5**

Correlation coefficients between P fractions and some selected soil properties after 150 days incubation of P treated soils

	Clay	CCE	O.C	CEC	ACC	Fe-CBD	Fe-O	Al-CBD	Al-O
NaCl-NaOH	0.481	0.546	0.419	0.584	0.786*	-0.133	0.365	0.123	0.782*
CBD	-0.369	-0.745*	-0.374	-0.551	-0.822**	0.340	-0.099	0.187	-0.512
OAC	-0.066	0.623	0.398	0.382	0.624	-0.127	-0.250	-0.432	0.304
HCl	0.144	0.002	0.495	0.356	0.087	0.406	0.021	-0.157	0.109
Organic	-0.205	-0.426	0.426	-0.140	-0.337	0.056	0.142	-0.154	0.036

\*Significant at  $P \leq 0.05$ .

\*\*Significant at  $P \leq 0.01$ .



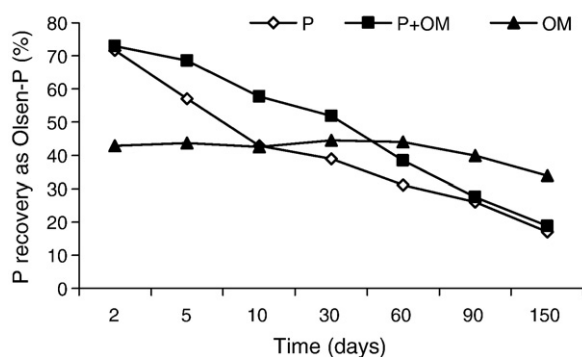


Fig. 2. Changes in phosphorus recovery as Olsen-P (%) over time after amendment with phosphorus (P), phosphorus+manure (P+OM) and manure (OM) treated soils.

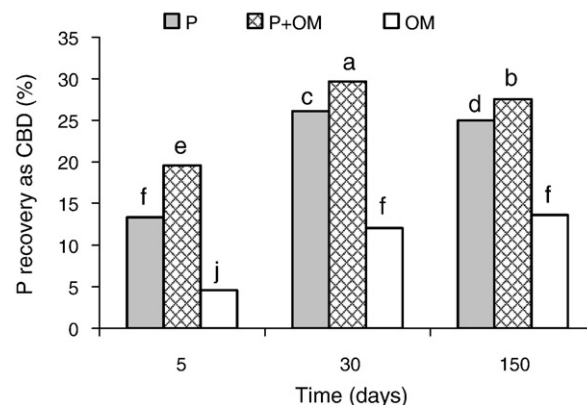


Fig. 4. Changes in phosphorus recovery as CBD over time in phosphorus (P), phosphorus+manure (P+OM) and manure (OM) treated soils.

and Bahl (2008) reported that organic manure addition along with inorganic P, irrespective of the source, increased Olsen extractable P throughout the incubation period. Ohno et al. (2005) showed that application of animal manures may increase the bioavailability of soil P by increasing the concentration of soil dissolved organic C (DOC). No significant difference was found between pH in treated soils during all incubation period.

In P treated soils, addition of P into soils led to a significant increase ( $P \leq 0.05$ ) in all fractions of P with the exception of HCl-P. Recovery percent as NaCl-NaOH-P in manured soils was significantly greater than P and P+manure treatments (Fig. 3). This result shows that P from manure gradually turns into available forms over the time. Manure application along with P tends to decrease recovery as NaCl-NaOH-P at 5 and 150 days as compared to P treatment alone (Fig. 3). No significant difference was found between these treatments at 30 days. It is possible that formation of organic complexes with P affects its extraction by NaCl-NaOH. This effect could be due to the formation of organo-metal (mainly Fe) compounds. Increase in CBD recovered P may explain this observation (Fig. 4). It may be possible that organo-metal complexes mainly, Al, Fe and Mn, remove P from solution and reduce the amount of P loss via runoff and leaching by formation of a cationic bridge between the organic C and P (Leytem and Westermann, 2003).

Increase in recovery as CBD-P in all of treatments until 30 days revealed that Fe oxides play important roles in P sorption in these soils during 0–30 days and had the highest amount in P+manure treatment (Fig. 4). Several researchers have shown that Fe is a major sink for P adsorption especially in soils with a high content of organic C (Borggard et al., 1990; Subramaniam and Singh, 1997). Decrease in OAc recovered P after 30 d of incubation (Fig. 5) and increases in P recovery as Olsen-P in the period between 5 to 60 days (Fig. 2) in P+manure treatments may show that adsorption of P by these organo-metal

compounds has slowed down precipitation of P as Ca phosphates. It suggests that the adsorption of organic components interferes with nucleation of Ca-phosphate on the mineral surfaces to delay the precipitation reaction (Castro and Torrent, 1998; Cooperband and Good, 2002). In addition, complexation of Ca with organic matter reduces formation of Ca-phosphate minerals (Inskeep and Silvertooth, 1998). Formation of complex bonds of Fe-P to organic C and specific bonds to clay particles was reported by Subramaniam and Singh (1996). Furthermore, Leytem and Westermann (2003) found that organo-metal complexes were involved in the inhibition of Ca-P precipitation. In all treatments, a significant increase ( $P \leq 0.05$ ) was observed in OAc-P fraction with time (Fig. 5). Recovery as OAc-P in manure-treated soils was significantly lower than P and P+manure treatments for all incubation times. Manure application with P decreased the recovery as OAc-P fraction significantly at 30 d.

#### 4. Conclusions

In all treatments, the P recovery as Olsen-P (%) decreased with time. However, P recovery as Olsen-P in manure treated soils did not alter dramatically with time. Application of manure plus P fertilizer increased P recovery as Olsen-P compared to a separate application of P. In P treated soils, ACCE, Al-O and clay had an important and complex role in P behavior as there were negative relationships between Olsen-P and ACCE, Al-O and clay in early stages whereas positive relationships were observed at 150 d. In P treated soils, Olsen-P and NaCl-NaOH-P were closely related to ACCE and Al-O. Therefore, it may be concluded that the NaCl-NaOH fractions and Olsen method could be used as an index of weakly adsorbed P on surfaces of calcite and amorphous Al oxides in these soils. Addition of P into soils led to a significant increase in all

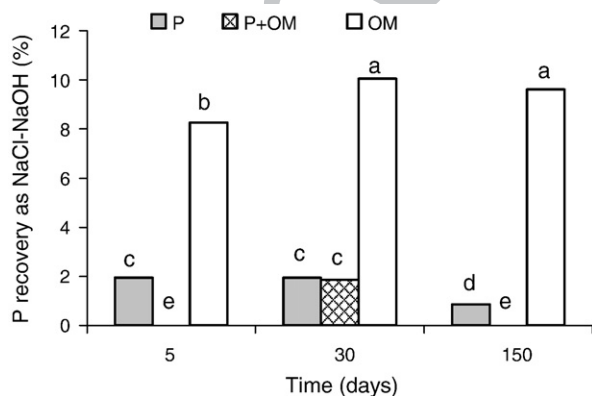


Fig. 3. Changes in phosphorus recovery as NaCl-NaOH over time in phosphorus (P), phosphorus+manure (P+OM) and manure (OM) treated soils.

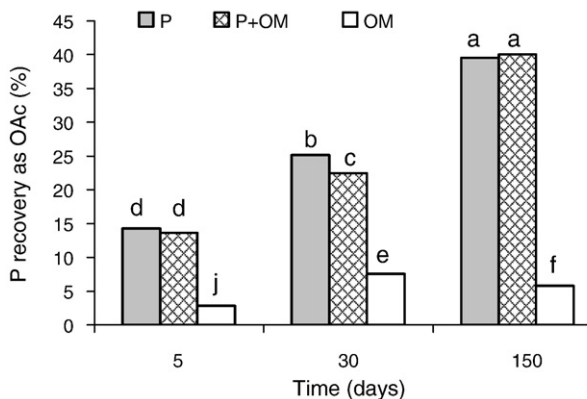


Fig. 5. Changes in phosphorus recovery as OAc over time in phosphorus (P), phosphorus+manure (P+OM) and manure (OM) treated soils.

285 treatments with the exception of HCl-P. Increased P recovery as CBD in  
 286 all of treatments revealed that Fe oxides influenced P sorption in these  
 287 soils during 0–30 d. Recovery as OAc-P in manure treated soils was  
 288 significantly lower than P and P+manure treatments for all incubation  
 289 times.

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