

The effects of some humic substances and vermicompost on phosphorus transformation rate and forms in a calcareous soil

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Abstract

The role of some humic substances (HS) on phosphorus (P) bioavailability and fractions was investigated in a greenhouse study using a calcareous soil from Eastern, Iran. Soils were treated with two levels (2 and 4%) of Humic Acid (HA), Fulvic acid (FA), Vermicompost (Verm) and their mixtures including FA+HA and FA+HA+Verm, and incubated for 24, 360, 720 and 1080 h. The Olsen-P was determined in amended and non-amended soils after incubation time. The P release was modeled by Parabolic, Power and Elovich equations to determine the transformation rates. Since the maximum P release was found in vermicompost treated soil, P was sequentially extracted with H₂O, NaHCO₃, NaOH and HCl at 24 and 1080h after incubation. The highest released P was measured in vermicompost treatment and decreased as follows: HA+FA+Verm>HA+FA>HA>FA>CO. The decrease of P release data was best described by Parabolic equation ($r^2=0.83-0.93$, $SE=0.51-2.91$). Higher transformation indices of vermicompost amended soil than other treatments showed more efficiency of vermicompost to release of phosphorus in soil. H₂O-P, NaHCO₃-P and NaOH-P increased after vermicompost application and converted to HCl-P fraction so that at the end of the experiment, calcium phosphates comprised 69 and 43% of inorganic P (Pi) at 2 and 4% vermicompost treatments, respectively. Results of the study showed that humic substances could increase P extractability and availability in soil.

Keywords: Phosphorus fractionation, kinetic models, P release

1. Introduction

Calcareous soils cover 600 million ha of arable lands in the world (Leytem and Mikkelsen, 2005) which their management to increase crop production assumes a great deal of interest in the present time wherein the available land area for cultivation decreases with time. The plant availability of P in these soils is often below the required amount for

optimum crop production due to precipitation of P with calcium ion or sorption of P by carbonate minerals (Bai *et al.*, 2013). Chemical fertilizers have been continuously applied during the last years to raise soil available P (Bai *et al.*, 2013). However, inappropriate P fertilizer management associated with high P fixation capacity leads to P

precipitation that is why P deficiency is reported in many calcareous soils (Jalali and Ranjbar 2010).

Only 10-20% of the P applied with fertilizers is taken up by plants in the year of application because the majority of applied P is rapidly fixed or precipitated into poorly available forms (Alamgir *et al.*, 2012). The P dissolved from fertilizers undergoes various reactions with the inorganic and organic constituents of the soil resulting in products with varying degrees of solubility. The rates of transformation of fertilizer-derived P into the various soil P forms provide invaluable information on the plant availability of soil P at various times after P application (Rivaie *et al.*, 2008). Soil P is found in several chemical forms including soluble and exchangeable, organic matter, Ca-bound, Fe-Al-bound and residual phases (Hedley *et al.*, 1982). These phases are remarkably different in bioavailability, mobility and chemical behavior, thus play different roles in soil fertility (Adhami *et al.*, 2013). Information about these fractions may shed some light in our understanding of the P sinks and sources in soil and are essential for an efficient P management in agricultural fields. Sequential P fractionation can be used to separate the different forms of soil P and is useful to compare the fluxes of various P forms in soil (Reddy *et al.*, 2005; Wang *et al.*, 2008).

Humic substances (HS) are macromolecular complex mixtures comprised of aromatic hydroxy carboxylic acid and play a vital role in the mobility and fate of plant nutrients (Yang *et al.*, 2013). It is well known that humic substances improve chemical, biological and physical properties of calcareous soils which, in turn influence the production capacity (Nardi *et al.*, 2002). Humic acids (HAs) and Fulvic acids (FAs) are two major fractions of HS and influence plant growth via improving the physical properties, fertility and moisture condition of the soil (Cahassapis *et al.*,

2009). The addition of HS to soil can also rectify the problem of P deficiency directly or indirectly through a range of mechanisms including P release from the residues, competition between P and HAs and FAs for mineral adsorption sites, exchange of P sorbed with HS, metal complexation and dissolution reactions (Fuentes *et al.*, 2006; Alamgir *et al.*, 2012).

Many studies have reported P sorption inhibition due to site competition and metal complexation by organic acids (Uburger *et al.*, 2011), animal and green manures (Alamgir *et al.*, 2012; Verma *et al.*, 2005) and bioslids (Pinerio *et al.*, 2009), but little is known about the effect of humic substances on soil P lability, transformation rate and forms over time. Thus, the aims of the present research were to investigate the effects of different levels of HAs and FAs on 1) different P fractions and 2) the pattern and rate of P release over a period of 45 days.

2. Materials and Methods

2.1. Soil

Composite soil sample used in this study was collected from 0-30 cm depths of a calcareous soil in Kerman Province, Eastern Iran. Soil sample was air dried, crushed and passed through a 2 mm sieve for laboratory experiments. Soil pH and EC were determined in 1:5 ratio of soil: water suspension. Organic C (OC) was measured by the wet oxidation method (Walkley and Black, 1934), calcium carbonate equivalent (CCE) by the method of Allison and Moodie (1965) using acid titration and cation exchange capacity (CEC) using NaOAc method (Rhoads, 1986). Available P were determined by the method described by Olsen *et al.* (1954) using 0.5 M NaHCO₃ at pH 8.5 (1g: 20 ml Soil: extract).

2.2. Incubation Study

For the aerobic incubation, one kg soil samples were placed in plastic pots and two levels (2 and 4 %) of Humic acid (HA), Fulvic acid (FA), vermicompost (Verm) and their mixtures including HA+FA and HA+FA+Verm were thoroughly mixed with soil. Blank (no amendment used) was also among the treatments studied as control. Some chemical properties of used

HS and vermicompost are presented in Table 1. All pots were incubated for 24, 360, 720 and 1080 h at field capacity. One soil, 11 treatments, four sampling dates and three replications resulted in a total of 132 experimental units in a complete randomized design. After the specified time, samples were taken and analyzed for Olsen-P (Olsen and Sommers, 1982) with P measured by the method of Murphy and Riley (1962). The decrease of Olsen-P with time was fitted by the kinetic equations listed in Table 2.

Table 1. Selected chemical properties of HA, FA and vermicompost

Humic substances	EC(dS m ⁻¹) (1:10)	pH (1:10)	P (mg Kg ⁻¹)	Zn (mg Kg ⁻¹)	Fe (mg Kg ⁻¹)	Purity
Humic Acid	14.3	9.4	195.5	38.4	7623.0	80%
Fulvic Acid	29.3	5.4	244.8	309.3	933.3	70%
Vermicompost	2.2	7.4	1760	54.0	11000.0	Humic acid:18% Fulvic acid: 15%

Table 2. Equations tested to describe P release kinetic data

Model	Equation	Parameters
Elovich	$q = \alpha + \beta \ln t$	α , initial P-desorption rate β , P-desorption constant
Parabolic	$q = A + Rt^{1/2}$	R, diffusion rate constant
Power	$\ln q = \ln a + 1/n \ln t$	n, an empirical constant

2.3. P Fractionation

The concentration of various P pools was determined by sequential P fractionation at 1 and 45 day after incubation,

based on Hedley *et al.* (1982). One g of soil samples were sequentially extracted (at a 1:30 soil: solution ratio) for 16h with H₂O (Soluble P), 0.5 M NaHCO₃ (Bioavailable P), 0.1

M NaOH (Fe and Al-bound P) and 1 M HCl (Ca-bound P). The amounts of P in all extracts were determined using colorimetric ascorbic acid method (Murphy and Riley, 1962).

2.4. Statistical analysis

The experiment data were subjected to the analysis of variance to test treatment effects on soil Pi fraction and P release. Pearson correlation was used to investigate the relationship between soil Pi fractions with Olsen-P and selected chemical properties of soil.

3. Results and Discussion

3.1. Soil properties

Selected soil physicochemical properties are listed in Table 3. The textural class of the soil was loamy sand. The soil was neutral to slightly alkaline and low in EC and OC, typical for calcareous soils. The low CEC value obtained for the soil might be related to its low clay and OC contents. The low available P suggests apparent P deficiency in the soil.

Table 3. Selected physicochemical properties of the used soil

Texture	Clay	Sand	Silt	pH (extract)	EC (extract) dS.m ⁻¹	OC	CCE	Olsen-P mg kg ⁻¹
-	g kg ⁻¹						g kg ⁻¹	
loamy sand	130.2	580.3	280.6	7.7	1.7	7.00	240.3	7.3

3.2. Released P

Analysis of variance indicated that only main effects of HS addition and time on P release were significant ($P<0.05$). Released P was higher in soils amended with HS and vermicompost than control (Figure 1). The magnitude of the increase in released P due to vermicompost addition was higher than HS treated soils in all time of incubation. This could be due to higher amount of P in vermicompost than in HA and FA. The highest released P (29.4 mg/kg) measured at soil treated with 4% vermicompost. Soil application of HA and FA at two levels of 2 and 4% increased released P compared to the control, significantly. However, no difference was found between HS

treatments. These results are supported by Selim *et al.* (2009) on soils of Egypt.

A significant decreasing trend in released P was found up to 30 day and then leveled off between 30 and 45 days (Figure 2). The decrease in released P in the soil might be attributed to penetration and adsorption of P into the porous solids (Jalali and Ranjbar, 2010) and precipitation of Ca-P minerals during incubation. In their study, Short *et al.* (2007) reported that much of the P applied as mono-calcium phosphate to Australian soils was converted rapidly to insoluble forms after 4 weeks and only 10% of added P remained available (90% fixed) for plant. In the present study, a decrease of 30% in extractable P from amended soils after 45 days indicates the positive effect of HS and vermicompost on P availability.

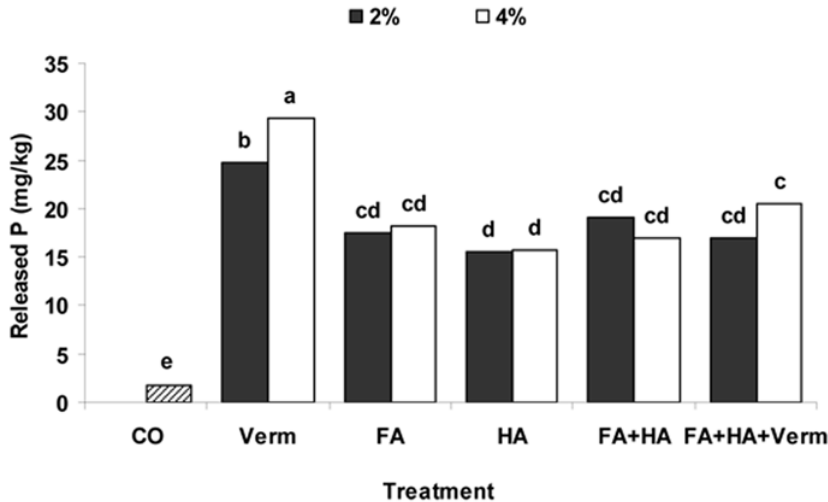


Figure 1. Effect of different humic substances on released P. Values are significantly different ($P < 0.05$) if indicating letter differs

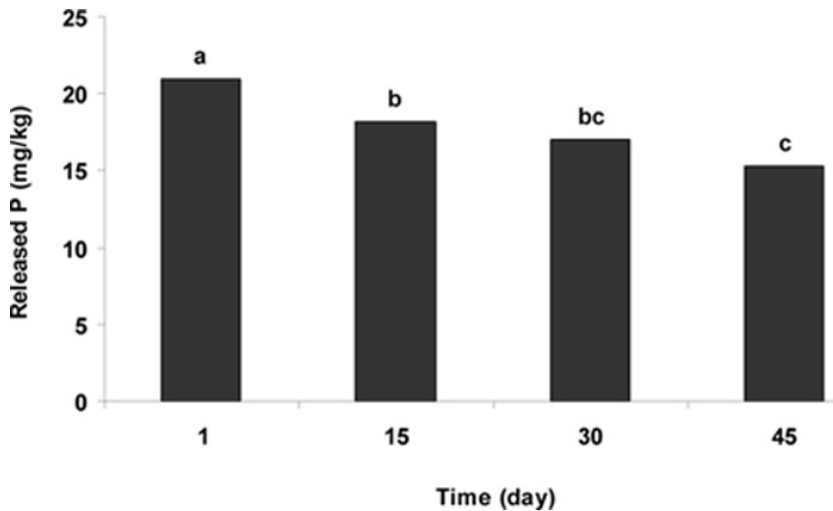


Figure 2. Effect of incubation time on P release. Values are significantly different ($P < 0.05$) if indicating letter differs

3.3. Kinetic models

Different kinetic models were used for explanation of P release from the amended and non-amended soils. Phosphorus release data were best fitted by the semi-empirical and empirical models including parabolic, power and Elovich equations. The coefficients of determination (r^2) and the standard errors of estimate (S.E.) for kinetic equations

tested to describe the P release data (Table 4) and the rate constants derived from these equations are shown in Table 5. Based on the relatively higher values of r^2 and the lower values of S.E., the parabolic diffusion model was considered the most appropriate equation describing the release of P with time in amended soil. Fitting of the data to parabolic equation is shown in Figure 3. The linear fit indicates that the release of P in HS and vermicompost amended soils is a diffusion-

controlled process. Successful description of P release by the parabolic equation model has been reported in the study of Shariatmadari *et al.* (2006) and Jalali and Ranjbar (2010) on some calcareous soils of Iran.

3.4. Phosphorus release rate

The slope of used equation which represents the loss in P extractability with time can be used as an index of P transformation. It has a negative value and was used as the P transformation rate in HS and vermicompost treatments (Table

5). The results showed that P transformation indices increased with application rates in all amended soils. The increase in transformation indices may be due to an increase in the number of adsorption sites. The overall effect of HS and vermicompost on P transformation rates was in the order of Verm>HA>FA+HA+verm>FA+HA>FA. The lowest P transformation rate found in soils amended with 2 and 4% FA shows its efficiency to increase P availability in soil. It seems that FA can decrease the number of adsorption sites and P affinity more than HA and vermicompost.

Table 4. Coefficients of determination (r^2) and standard errors of estimate for kinetic equation used to describe P release in control and amended soils

Treatment	Parabolic		Power		Elovich	
	r^2	SE	r^2	SE	r^2	SE
Vermicompost 2%	0.85**	1.50	0.73**	0.08	0.78**	1.88
Vermicompost 4%	0.93**	2.91	0.81**	0.16	0.85**	4.10
Fulvic Acid 2%	0.79**	0.79	0.60*	0.06	0.62*	1.07
Fulvic Acid 4%	0.76**	0.38	0.58*	0.03	0.59*	0.50
Humic Acid 2%	0.82**	1.16	0.95**	0.04	0.95**	0.59
Humic Acid 4%	0.90**	1.00	0.96**	0.03	0.97**	0.53
(Fulvic Acid+Humic)2%	0.34 ^{ns}	1.50	0.18 ^{ns}	0.09	0.18 ^{ns}	1.68
(Fulvic Acid+Humic)4%	0.93**	6.64	0.99**	0.58	0.99**	10.44
(Fulvic Acid+Humic+vermicompost)2%	0.24 ^{ns}	1.58	0.13 ^{ns}	0.10	0.13 ^{ns}	1.70
(Fulvic Acid+Humic+vermicompost)4%	0.91**	10.5	0.72 **	0.70	0.80**	14.50
Control	0.83**	0.51	0.65*	0.28	0.65*	0.48
Mean	0.75**	2.59	0.66*	0.20	0.68*	3.41

** : significant at $P<0.001$ and * : significant at $P<0.05$

Table 5. Parameters of kinetic equation used to describe P release in control and amended soils

Treatments	Parabolic		Power		Elovich	
	A	R	ln a	1/n	α	β
Vermicompost 1	29.87	-0.25	3.60	-0.07	34.10	-1.67
Vermicompost 2	43.89	-0.69	4.21	-0.15	55.93	-4.70
Fulvic Acid 2%	19.64	-0.05	3.07	-0.02	21.15	-0.28
Fulvic Acid 4%	19.15	-0.10	2.99	-0.04	19.81	-0.62
Humic Acid 2%	19.11	-0.17	3.17	-0.077	22.81	-1.30
Humic Acid 4%	19.74	-0.19	3.23	-0.085	23.71	-1.42
(Fulvic Acid+Humic)2%	20.62	-0.07	3.06	-0.02	21.20	-0.37
(Fulvic Acid+Humic)4%	22.61	-0.27	3.44	-0.11	28.16	-1.99
(Fulvic Acid+Humic+vermicompost)2%	17.54	-0.03	2.88	-0.01	17.83	-0.17
(Fulvic Acid+Humic+vermicompost)4%	28.06	-0.36	3.65	-0.11	34.01	-2.38

3.5. Inorganic P fractions

Since maximum P release was found at vermicompost amended soil, fractionation of Pi was only performed in 2 and 4 % vermicompost treatments. Concentrations of inorganic P fractions are presented in Table 6. In all

treatments, H₂O-Pi constituted the smallest P fraction (2-7 % of total Pi) whereas HCl-Pi (42-89% of total Pi) was the most abundant fraction (Figure 4). This was likely due to calcareous nature of studied soil. Similar results were reported by Pinerio *et al.*, (2009) and Dail *et al.*, (2009) in organic amended soils.

Table 6. Inorganic P fractions in amended and control soils at 1 day and 45 days after incubation

Treatments	H ₂ O-P		NaHCO ₃ -P		NaOH-P		HCl-P	
	24h	1080h	24h	1080h	24h	1080h	24h	1080h
Control	17.8 b	12.7 b	29.0 c	24.4 c	27.6 b	27.9 b	338.5 bc	538.9 a
Verm 2%	36.4 ab	19.8 b	189.4 a	101.1 b	74.2 ab	70.7 ab	459.3 ab	441.1 ab
Verm 4%	37.6 a	36.6 ab	218.1 a	179.0 a	96.5 a	72.5 ab	226.6 c	213.1 c

For each fraction, same letters are not significantly different at 0.05 probability level

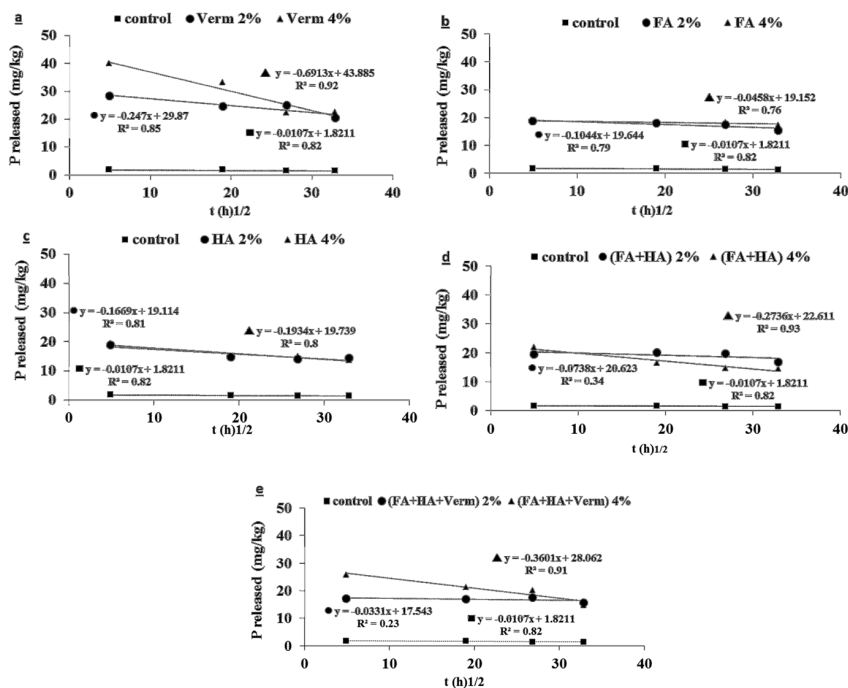


Figure 3. Relationships between observed and predicted P released in HS and vermicompost amended soils as described by parabolic model (a: vermicompost, b: Fulvic acid, c: Humic acid, d: Fulvic acid+Humic acid and e: Humic acid+Fulvic acid+Vermicompost)

The H_2O and $NaHCO_3$ -Pi are considered as labile fractions (Guo *et al.*, 2000). Application of vermicompost at the level of 4% increased both H_2O -Pi and $NaHCO_3$ -Pi compared to control. Verma *et al.* (2005) also reported an increase in $NaHCO_3$ -Pi due to manure application. Although a slight reduction in these fractions was found in amended soils after 45 days, the remained H_2O and $NaHCO_3$ -Pi seems to provide sufficient P for plant growth. Although, $NaHCO_3$ -Pi distribution was approximately unchanged in control after 45d of incubation, it was decreased by 25 and 37% in soil amended with 2 and 4% vermicompost, respectively (Figure 4).

The Pi extracted by NaOH is considered as Pi associated with Fe and Al components (Hedley *et al.*, 1982) and is

often assumed to be a moderately labile fraction (Guo *et al.*, 2000). The NaOH-Pi has been reported to be an important P sink for the crops in low P-input systems (Verma *et al.*, 2005). NaOH-Pi content of soil was higher at 4% vermicompost treatment than control and remained unchanged over time (Table 6). This observation suggested that the NaOH-Pi may maintain adequate plant available-P in studied soil. Supporting this idea is the positive correlation ($r=0.80^{**}$) found between NaOH-Pi and Olsen P. The results are similar to those reported by Dail *et al.* (2009) in the poultry manure amended soil.

The HCl-Pi fraction, which represents Ca-P minerals, is low in availability for plants (Hedley *et al.*, 1982). At the end of the experiment, a significant decrease

was found in soil HCl-Pi fraction after vermicompost application at the level of 4%. HCl-Pi distribution decreased from 89% in control to 69% and 42% in 2 and 4% vermicompost treatments (Figure 4). Calcium phosphates are thermodynamically the most stable P minerals, although they were found to decrease in greenhouse studies (Guo *et al.*, 2000; Uygur and

Karabatak, 2009). The result is similar to the study of Abolfazli *et al.* (2012) who reported that Ca-P mineral reduced following application of cow dung manure. In amended and non-amended soil, calcium phosphate minerals comprised the largest fraction of soil Pi indicating that the added P was transformed into the stable Ca-P minerals at the end of the experiment.

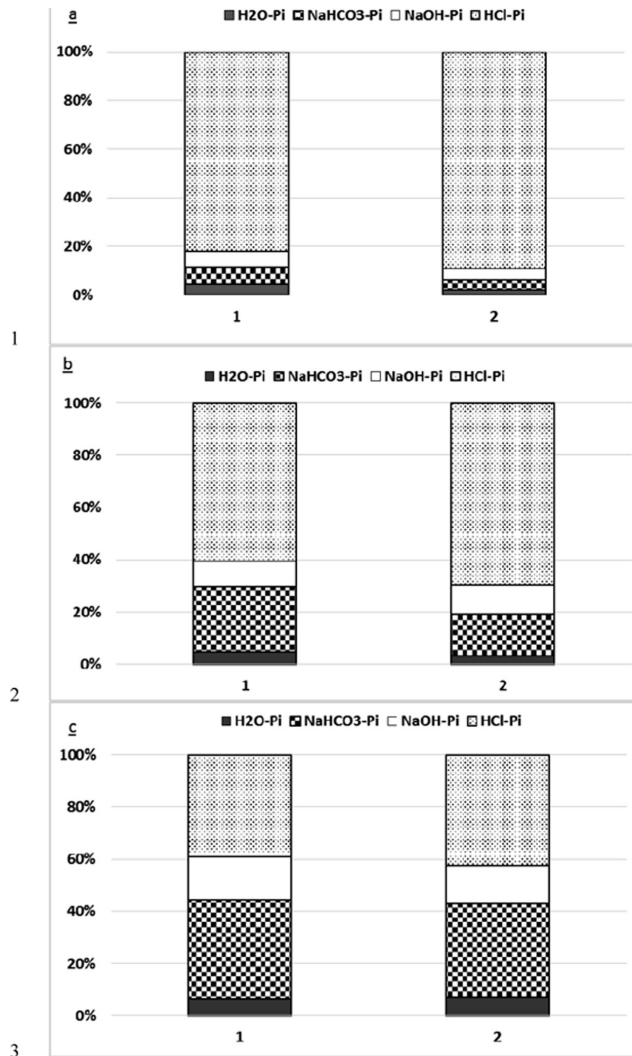


Figure 4. Soil Pi distribution at the beginning (1) and end (2) of experiment at control (a), 2% vermicompost (b) and 4% vermicompost (c) treatments.

3.6. Correlation between Pi fractions with Olsen-P and soil properties

Simple correlation analysis between four Pi fractions with Olsen-P and some soil chemical properties is shown in Table 7. The highest correlation coefficients were found among Olsen-P and NaHCO_3 and NaOH Pi fractions, whereas no significant correlation was found between Olsen-P and HCl-Pi. These results confirmed that NaOH-Pi and NaHCO_3 -Pi in soil

play as a sink of available P. Soil OC was positively correlated with H_2O -Pi, NaHCO_3 -Pi and NaOH-Pi among soil chemical properties, whereas correlation was negative with HCl-Pi. Carbonate calcium is a very strong complexing agent for P in soils. Based on results of the present research, organic matter can increase availability of soil P through dissolution of stable HCl-Pi to more labile forms such as H_2O and NaHCO_3 -Pi. These results are supported by other published reports (Hao et al., 2008; Nafilu, 2009; Dail et al., 2009).

Table 7. Pearson correlation coefficient (r^2) for soil properties and Pi fractions

	H_2O -P	NaHCO_3 -P	NaOH-P	HCl-P	Olsen-P	pH	EC	CCE	OC
H_2O -P	-	0.64**	0.63**	-0.32	0.61**	0.02	0.15	-0.28	0.52*
NaHCO_3 -P		-	0.67**	-0.25	0.90**	0.07	0.40	-0.33	0.76**
NaOH-P			-	-0.28	0.80**	-0.37	-0.37	-0.08	0.61**
HCl-P				-	-0.29	0.13	-0.39	0.11	-0.57**
Olsen-P					-	-0.22	0.37	-0.17	0.75**
pH						-	0.08	-0.51*	-0.15
EC							-	-0.06	0.22
CCE								-	-0.36
OC									-

*, ** significant at $P<0.05$ and $P<0.01$, respectively.

4. Conclusions

This study clearly showed that soil application of HA, FA, vermicompost and their mixtures had different effects on P availability. In all treatments the time-dependent P release processes were best fitted by the parabolic diffusion equation. In spite of higher released P in soil treated with vermicompost, lower transformation rate indices were found in HA and FA amended soils indicating more effectiveness of HS on P availability. In vermicompost amended and non-amended soil, P was associated mainly with HCl-Pi

(42-89%), NaHCO_3 -Pi (4-37%), NaOH-Pi (4-17%), and H_2O -Pi (2-7%). Increased distribution of NaOH-Pi and HCl-Pi with time indicates the transformation of labile fractions (H_2O -Pi and NaHCO_3 -Pi) to these stable fractions. The multivariate analyses confirmed that NaHCO_3 -Pi was the most important fraction contributing to plant available P.

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