Effect of incubation time on transformation rate and chemical forms of phosphorous in calcareous soils along a climotoposequence

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Efecto del tiempo de incubación sobre el grado de transformación y las formas químicas de fósforo en suelos calcáreos a lo largo de una climotoposecuencia
Efeito do tempo de incubação na taxa de transformação e nas formas químicas de fósforo em solos calcários ao longo de uma climotopossequência

ABSTRACT

Soil phosphorous fertility can be significantly affected by the P transformation rate in soils. The present study was designed to evaluate the application of time-dependent P fertilizer in terms of its availability and chemical forms in 23 soils of a climotoposequence. The collected soil samples were incubated with 200 µg g⁻¹ P as KH_2PO_4 for 1, 20, 60, 100, 150, and 200 days at 25 °C and after that, the available and chemical forms of P were determined for each incubation time. A rapid decrease in available P was observed within 20 days after application of P fertilizer. A 200-day application of P led to a decrease in the available P by 15% compared to the one-day application. In addition, the biphasic pattern of transformation rate of available P fitted well to parabolic and power function models. The results of sequential extraction methods showed that in treated and untreated soils, Ca-bound P, residual P, Fe- and Al-bound P were the highest fractions, while the exchangeable form was the lowest. At the end of incubation, on average, only 15% of total P (200 µg g⁻¹) remained as exchangeable-P, while this amount was 40.6% and 28.5% at days 1 and 20, respectively. In addition, among all soil characteristics, mineralogical properties showed the most obvious effects on controlling the chemical forms of P over time.

RESUMEN

La fertilidad del fósforo del suelo se puede ver afectada significativamente por la velocidad de transformación del fósforo en los suelos. El presente estudio se diseñó para evaluar la aplicacion de un fertilizante de P dependiente del tiempo en términos de su disponibilidad y formas químicas en 23 suelos de una climotoposecuencia. Las muestras de suelo recogidas se incubaron con 200 µg g⁻¹ P como KH_2PO_4 durante 1, 20, 60, 100, 150 y 200 días a 25 °C y después se determinaron las formas disponibles y químicas de P para cada periodo de incubación. Se observó una rápida disminución de P disponible dentro de los 20 días de la aplicación del fertilizante. La aplicación de P durante 200 días dio lugar a una disminución del 15% de P disponible en comparación con la aplicación de 1 día. Además, el patrón bifásico de la velocidad de transformación del P disponible se ajustó bien a modelos de funciones

parabólicas y potenciales. Los resultados de los métodos de extracción secuencial mostraron que en suelos tratados y sin tratar, el P unido al Ca, el P residual y el P unido al Fe y Al fueron las fracciones más abundantes mientras que la forma intercambiable fue la más baja. Al final de la incubación, en promedio, solo el 15% del P total (200 µg g⁻¹) permaneció como P intercambiable, mientras que este porcentaje fue del 40.6% en el día 1 y del 28.5% en el día 20. En cuanto a las características de los suelos, las propiedades mineralógicas fueron las que controlaron de forma más evidente las formas químicas de P con el tiempo.

RESUMO

A fertilidade do solo no que se refere ao fósforo pode ser significativamente afetada pela taxa de transformação das formas de fósforo nos solos. O presente estudo foi desenhado para avaliar a aplicação de um fertilizante de P, em função do tempo, em termos da sua disponibilidade e formas químicas em 23 solos de uma climotopossequência. As amostras de solo recolhidas foram incubadas com 200 µg g⁻¹ P como KH_2PO_4 durante 1, 20, 60, 100, 150, e 200 dias a 25 °C após o que se determinaram as formas disponíveis e químicas de P para cada período de incubação. Observou-se uma rápida diminuição do P disponível nos 20 dias de aplicação do fertilizante. A aplicação de P durante 200 dias levou, em comparação com a aplicação de 1 dia, a uma diminuição de 15% do P disponível. Além disso, o padrão bifásico da taxa de transformação do P disponível ajustou-se bem a modelos de funções parabólicas e potenciais. Os resultados da extração sequencial mostraram que em solos tratados e não tratados, o P ligado ao Ca, o P residual e o P ligado ao Fe e Al foram as frações mais elevadas enquanto a forma de troca foi a mais baixa. Em média, no final da incubação, apenas 15% do P total (200 µg g⁻¹) permaneceu como P de troca, enquanto esta fração correspondia a 40,6% e 28,5% nos dias 1 e 20, respetivamente. Além disso, entre todas as características do solo, a composição mineralógica mostrou ser a mais óbvia no controlo das formas químicas do P ao longo do tempo.

1. Introduction

Phosphorous one of the most important and highly consumed nutrients for growing plants and other living organisms (Nesme et al. 2014), playing an important role in cellular metabolism in the form of adenosine triphosphate (ATP). Apart from the important role of this element in structural and biochemical compounds of cells, its increasing concentration in natural environments due to excessive use of P fertilizers can lead to environmental problems such as eutrophication of aquatic ecosystems (Devau et al. 2011) and surface waters toxicity (Kier and Kirkland 2013). The vital processes of plants are not feasible without P presence and it is very difficult to access an acceptable concentration of available P in the soil (Bünemann et al. 2010). Total P content is high in most inorganic soils; however, its greater part is found in soil as non-available compounds for the plant (Jalali and Ahmadi Mohammad Zinli 2011). Application of P to soils is done to ensure the sufficient crop yield in many agricultural systems, but the recovery percentage of added P by the plants is very low (10 to 30%) in growing seasons. Due to the sorption, precipitation or transformation to other organic forms, more than 80% of P added to soils would be changed to inactive P and eventually it would be inaccessible for the plant (Holford 1997). In most soils, very low concentrations of non-organic P exist in the soil solution. Phosphate ions tend to be sorbed on positively charged minerals including iron and aluminum oxides (Hinsinger 2001). According to Karaman et al. (2001), physico-chemical characteristic of soil, the type of parent materials, the degree of

weathering, and climatic conditions, have a great impact on the P status in the soil environment. So, a proper management of P fertilizers is required to improve the crop yield plant performance due to low levels and low mobility of P in the soil. P in the soil exists in various geochemical forms including the soil solution, exchangeable phases, organic matter-, Ca-, Fe- and Al-bound phases, and residual phases (Jalali and Ranjbar 2010). The degree of association of P with various geochemical forms in soils is different and depends on various parameters such as type of clay minerals, soil clay content, soil pH, the content of iron and aluminum oxides, calcium ions, and organic matter (OM), oxidation and reduction potential, the presence of competitive ions for surface adsorption, and plant factors (Pierzynski et al. 2005). Dynamic, bioavailability, and chemical behavior of each geochemical forms of P is different and these forms can convert to each other under different conditions. Sequential extraction methods are applied to give qualitative and quantitative information about chemical forms of P (Jalali and Ranjbar 2010) and heavy metals (Saffari et al. 2016) in soils. This method can give us useful information about the mobility, leachability, and transformation of P between chemical forms in soils (Jalali and Ranjbar 2010). Previous studies showed that the availability of P in soil was directly impacted by residence time (Jalali and Ranjbar 2010; Javid and Rowell 2002). P had the highest bioavailability immediately after the application to soils and with increasing time, the bioavailability of P decreased (Jalali and Ranjbar 2010). On the other hand, in order to predict P fertilizer needs and develop efficient and accurate P recommendations, it is necessary to evaluate the residual available P in the soil. Thus, this research attempts to study the impact of application of time-dependent phosphorous fertilizer on the availability of P and its chemical forms in five soil orders (Aridisols, Inceptisols, Alfisols, Histosols and Mollisols) from a climotoposequence.

2. Material and Methods

2.1. Area study, sampling design, and laboratory analysis

The study was conducted across a toposequence transect, near Kerman city in the Kerman province, Iran (**Figure 1**). The studied climotoposequence covers an area of about 1,200 km^2. The mean annual precipitation of the area varies between 116.1 and 238.70 mm year^{-1} and the air temperature ranges from 15.7 °C in the beginning of the transect (north of the transect, 1,840 m above mean sea level) to 10.4 °C in the end of transect (south of the transect, 3,207 m above mean sea level), respectively (Meteorological Organization of Iran 2016). Soil moisture regime (SMR) and temperature regime (STR) in the north of the transect are aridic and mesic that change to xeric and mesic in the south of the transect (Banaei 1998). Two geomorphic surfaces, including a piedmont plain and lowlands of the Lalehzar Mountains were evaluated to determine their P status (**Figure 2**). Five representative pedons on two geomorphic positions were described.

All soil horizons were described according to Soil Survey Staff (2014) and some chemical and physical properties were determined using standard methods. Soil texture was performed using the hydrometer method (Bouyoucos 1962). pH and EC (electric conductivity) were measured in saturated pastes (in a 1:1 soil and distilled water mixture) and saturated paste extracts, respectively. Percentage of calcium carbonate equivalent (CCE) was determined by acid neutralization (Loeppert and Suárez 1996). Organic matter (OM) content was determined using wet oxidation (Nelson and Sommers 1996). Cation exchange capacity (CEC) was measured by replacing exchangeable cations with sodium acetate (Sumner and Miller 1996). Olsen-P was determined by using a soil to solution ratio 1:20 and 30 min of shaking (Olsen et al. 1982). Phosphorous in the extractant was determined by using the ammonium molybdate-ascorbic acid method described by Murphy and Riley (1962). For mineralogical analysis, some selected soil samples were washed to remove gypsum and soluble salts. Fe-oxides, carbonate, and organic matter were removed by citrate-bicarbonate-
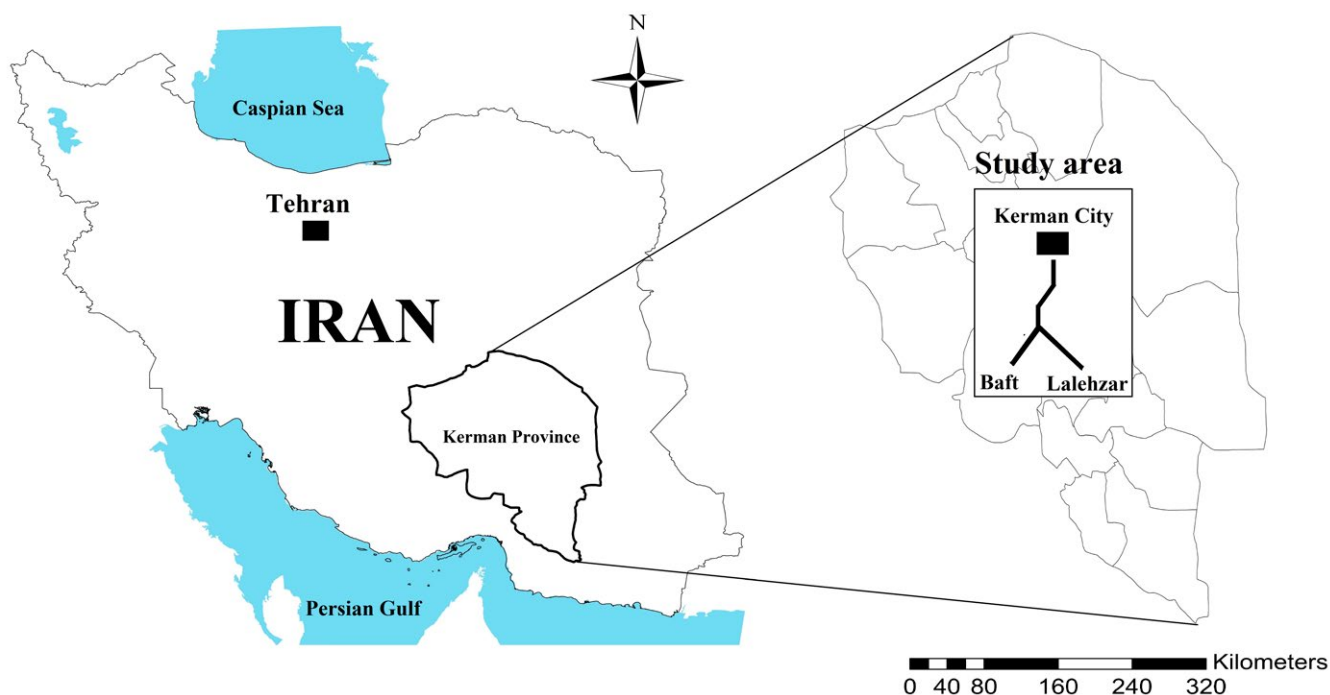
Figure 1. Location of the study area.

dithionite (CBD), sodium acetate (adjusted pH 5), and H_2O_2 (30%), respectively (Mehra and Jackson 1960). The clay fraction of the prepared samples was separated by centrifuge (Kittrick and Hope 1963). The centrifuged clay samples (at 750 rpm for 5.4 min) were treated with Mg (1N $MgCl_2$), Mg/ethylene glycol, K (1N KCl), and K/heated at 550 °C for 2 h. The clay minerals were analyzed (Jackson and Barak 2005), using an X-ray diffractometer (Philips, PW 1130/00), using Ni-filtered CuKα radiation (40 kV, 30 mA). Semi-quantitative estimation of clay minerals performed using the (001) peak intensities of the Mg-saturated and glycerol solvated samples (Johns et al. 1954).

2.2. Incubation study and release kinetic of available P

In order to evaluate the effect of aging on the P transformation rate and chemical fractionation in some calcareous soils, all soil samples were treated with P fertilizer (KH_2PO_4). The soil samples were placed in plastic containers and P was added at the rate of 200 mg kg^{-1} P as KH_2PO_4. After that, the soil samples were incubated for 1, 20, 60, 100, 150, and 200 days

at 25 ± 2 °C. Soil moisture was preserved at field capacity. After each incubation time, sub-samples were air-dried and were analyzed for available P (Olsen et al. 1982) and chemical fractionation of P. Sodium bicarbonate extractant (pH = 8.5) was used for extraction of available P, and concentration of P was determined by the ammonium molybdate-ascorbic acid method (Murphy and Riley 1962). In addition, different kinetic equations (including: zero, 1st, 2nd, 3rd order, power function, and parabolic diffusion), were also used to describe P release in the studied soils (**Table 1**). To choose the best-fitted model, a standard error of estimate (SE) was calculated for each equation. Relatively high values of coefficients of determination (R^2) and low values of SE were used as criteria for the selection of the best-fitted models. The standard error was calculated as follows:

$$SE = \left[\frac{\sum(S - S')^2}{n - 2} \right]^{0.5}$$

where S and S′ are the observed and estimated amounts of P release in soil at time t, respectively, and n is the number of measurements.

2.3. Fractionation of P

Table 1. Equations fitted to describe P release kinetic

Model	Equation	Parameters
Zero order	$q_t = q_0 - k_0 t$	k_0, zero order rate constant (mg P kg^{-1} h^{-1})
First order	$\ln q_t = \ln q_0 - k_1 t$	k_1, first-order rate constant (h^{-1})
Second order	$1 / q_t = 1 / q_0 - k_2 t$	k_2, second-order rate constant [(mg P kg^{-1})$^{-1}$]
Third order	$t\ 1/q_t^2 = 1/q_0^2 - k_3$	k_3, third-order rate constant[(mg P kg^{-1})$^{-2}$ h^{-2}]
Simple Elovich	$q_t = 1 / \beta \ln (\alpha_s \beta_s) + (1 / \beta_s) \ln t$	α_s, initial desorption rate (mg P kg^{-1} h^{-1}), β_s desorption constant [(mg P kg^{-1})$^{-1}$]
Parabolic diffusion	$q_t = q_0 - k_p t^{1/2}$	k_p, diffusion rate constant [(mg P g^{-1})$^{-0.5}$]
Power function	$q_t = at^b$	a, initial desorption rate constant (mg P kg^{-1} h^{-1})^b b, desorption rate coefficient [(mg P kg^{-1})$^{-1}$]

q_0 and q_t are the amount of P extracted from each soil (mg kg^{-1}) at time zero and t, respectively.

Chemical distribution of P in native and treated soils (treated with 200 µg g^{-1} P), in each time, was measured using a modified version of the Hedley et al. (1982) procedure, as outlined by Zhang and MacKenzie (1997). The procedure was designed to separate P into four fractions: 1) soluble and exchangeable P, 2) Fe- and Al-bound P 3) Ca-bound P and 4) residual P. Outline of the method is presented in **Table 2**. The amount of P in each extracted sample was

determined using colorimetric ascorbic acid method (Murphy and Riley 1962).

2.4. Statistical analysis

The regressions and simple correlation were calculated by Microsoft Excel 2010 and SPSS V19.

3. Results

Table 2. Summary of the sequential extraction procedure used in this study

g soil:mL solution	Extracting solution	Shaking time (h)	Chemical form of P	Symbol
0.5:30	0.5M NaHCO$_3$ (pH 8.5)	16	Soluble and exchangeable	Exch-P
0.5:30	0.1M NaOH	16	Fe- and Al-bound P	Fe-Al-P
0.5:30	1M HCl	16	Ca-bound P	Ca-P
0.5:30	5:2 mixture of concentrated HNO$_3$ and HClO$_4$	16	Residual P	Res-P

3.1. Morphology of studied soilss

Figure 2 shows the location of each pedon in different geomorphic surfaces along the sequence. The piedmont plain, as the first geomorphic surface observed in this research, was divided in to 2 parts based on SMR. In the first part of this geomorphic surface (P1) the

SMR was aridic. On the other side, in the second part of this geomorphic surface, the SMR was xeric (for P2 and P3). P1 was classified as Calcic Haplosalid. A black surface as a result of dissolved organic matter was observed in P1, which showed that this soil is a saline-sodic soil (ECe: 86.3 dS m^{-1}, pH: 7.4, SAR: 21.19). Existence of a salic horizon and a petrocalcic horizon in P2 is the reason why this soil was classified as Petrocalcic Calcixerept. In the end
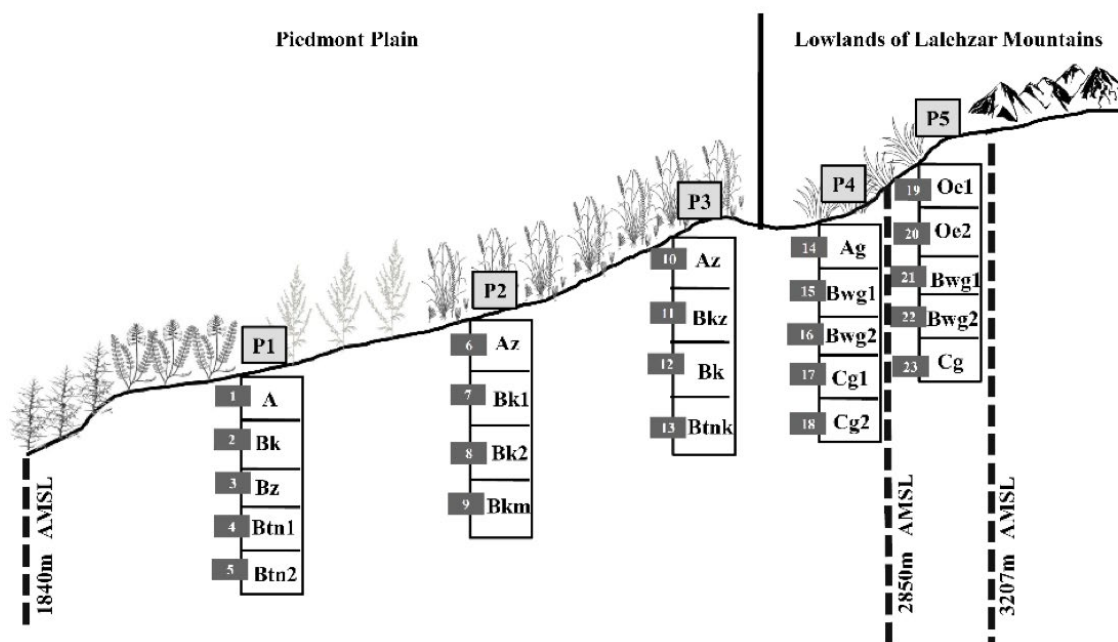
Figure 2. Location of each pedon in the studied geomorphic surfaces of the area.

part of rock pediment geomorphic surface with a xeric moisture regime, P3 (Typic Natrixeralf), formation of argillic horizon is as a result of increase in clay along the depth. In addition, an increase in SAR with depth lead to formation of Btnk horizon, as a result of clay dispersion. P4 and P5 were located in the lowland geomorphic surface of Lalehzar Mountains. In P4, the soil was classified as aTypic Epiaquoll due to existence of a Mollic epipedon with about 32.5% OM and xeric SMR and saturated water for 6 months of a normal year. In P5, the amount of organic matter (46.3%) was higher than P8 (32.5%). This pedon was known as an organic soil with an intermediate decomposition of organic matter (Typic Haplohemists). Stream flow derived from melting snow and rainfall in lowland did not allow the decomposition of organic matter. Results of this part showed that by moving up along the transect, parent materials, topography, and climate changed, that is why soil properties, as well as soil classifications also changed along the transect.

3.2. Physical and chemical properties of studied soils

Selected chemical and physical properties of the studied soils are shown in **Table 3**. The soils were alkaline (except for Mollisols). The pH value in these soils ranged from 6.1 to 7.8. The OM and calcium carbonate equivalent (CCE) values ranged from 0.2 to 46.3% and 7.5 to 32.5%, respectively. Higher OM values were observed in Mollisols (with average 12.71%) and Histosols (with average 22.34%), which their conditions did not provide the decomposition of organic matter. The highest and lowest average of CCE values were found in Inceptisols and Histosols, respectively. The cation exchange capacity (CEC) varied from 6.5 to 120.1 $cmol_{(+)}$ kg^{-1}. The lowest and highest average of CEC values were found in Alfisols and Histosols, respectively. Available P in different soil types was significantly different. Available P (Olsen P) concentration varied from 1.5 to 138.6 mg kg^{-1} with an average of 34.12 mg kg^{-1}. Higher values of available P were observed in Histosols and Mollisols (on average: 123.66 and 19.24 mg kg^{-1}, respectively) than in Aridisols, Inceptiols, and Alfisols (on average: 2.04, 5.97, 9.05 mg P kg^{-1}, respectively). However, the total P concentration in all soil samples varied from 248 to 1812 mg kg^{-1} with an average of 701.739 mg kg^{-1}, but only 20% of the soil samples have an amount of P higher than the critical level (above 18 mg kg^{-1}). Generally, different variations of physical and chemical properties of present soils would be useful to better understand P status and helpful for the best management of P and soil responses to fertilization.

3.3. P releasing over time

Table 3. Selected chemical and physical properties of studied soils

Soil number	Profile number	Taxonomy	Geographic coordinates	Horizon	Depth (cm)	pH	OM	CCE	Clay	Sand	CEC	Olsen-P	Total P
									%		cmol$_{(+)}$ kg^{-1}	mg kg^{-1}	
1	P1			A	0-5	7.4	0.8	25.2	29	16	19.1	3.5	378
2	P1			Bk	5_30	7.3	0.5	25.6	25	37	15.5	1.8	356
3	P1	Aridisols	29º 49'N; 56º 47'E	Bz	30-60	7.5	0.6	19.3	25	75	14	1.8	311
4	P1			Btn1	60-90	7.7	0.2	16	31	35	17.1	1.5	287
5	P1			Btn2	90-120	7.6	0.45	15.1	47	35	25.3	1.6	248
6	P2			Az	0-20	7.4	0.43	21.7	22	62	14.1	8.5	315
7	P2	Inceptisols	29º 45'N; 56º 46'E	Bk1	20-70	7.5	0.35	20.2	16	72	11.4	7.5	288
8	P2			Bk2	70-100	7.8	0.52	25.5	14	72	9.5	5.8	274
9	P2			Bkm	100-132	7.7	0.42	32.5	16	56	10.2	2.1	266
10	P3			A	0-5	7.4	0.85	12.5	19	75	11.3	14.1	413
11	P3	Alfisols	29º 44'N; 56º 46'E	Bkz	5-20.	7.4	0.38	17.3	21	75	14.2	14.5	389
12	P3			Bk	30-65	7.5	0.25	16.8	13	81	6.5	5.2	365
13	P3			Btnk	65-100	7.7	0.2	28.5	23	55	11.7	2.4	378
14	P4			Ag	0-30	6.1	32.5	10.5	44	34	82.36	22.3	873
15	P4			Bwg1	30-60	6.4	11.4	9.8	32	46	43.5	20.5	834
16	P4	Mollisols	29º 28'N; 56º 50'E	Bwg2	60-90	7.5	6.1	9.1	16	66	23.5	21.1	854
17	P4			Cg1	90-120	6.8	6.05	10.2	18	60	25.8	16.8	812
18	P4			Cg2	120-150	7	7.5	8.5	22	56	15.7	15.5	789
19	P5			Oe1	0-30	7.5	46.3	15.1	14	36	120.1	122.4	1812
20	P5			Oe2	30-60	7.1	26.7	9	30	28	86.7	102.5	1687
21	P5	Histosols	29º 27'N; 56º 37'E	Bwg1	60-90	7.1	18.9	12.6	43	5	70.2	138.6	1477
22	P5			Bwg2	90-120	7.2	9.5	7.5	33	32	44.3	120.5	1419
23	P5			Cg	120-150	7	10.3	9.1	26	28	39.1	134.3	1315

OM: organic matter; CCE: calcium carbonate equivalent; CEC: cation exchange capacity; Olsen-P: extracted P by sodium bicarbonate.

Figure 3 shows the amount of P releasing over incubation time in different orders of soils treated with KH_2PO_4. The trend of P release was the same in all soil samples, so that it showed a very high desorption initially and then decreased gradually. A very significant increase occurred in the available P content one day after application of KH_2PO_4. The highest transformation rate of added P was observed at the lowest depth of Inceptisols one day after fertilizer application, so that 87 µg g^{-1} (43.5%) of 200 µg g^{-1} added KH_2PO_4 was transformed to available P form.

On the other hand, the lowest transformation rate of the added P to available form was observed in Histosol topsoil (61.5%) one day after application of KH_2PO_4. On average, one day after application of KH_2PO_4 (200 µg g^{-1}), the available P in Aridisols, Inceptisols, Alfisols, Mollisols, and Histosols were measured as 49.8%, 51.75%, 50.255, 56.8% and 55.8%, respectively. The trend of changes in all treated soils indicated a rapid decrease in the available P, 20 days after application of P compared to the one-day application. The largest and the smallest decrease in the available P, 20 days after P application compared to one-day P application, was observed in the Aridisol and Inceptisol orders, respectively. The results indicated that 10%, 12.8%, 9.6%, 10.9% and 8.2% of available P decreased in the Aridisol, Inceptisol, Alfisol, Mollisol, and Histosol orders, respectively, 20 days after P application compared to the 1-day application. On average, 52.4% of P fertilizer added to the studied soils was available one day after application of P, while this form was observed 42%, 20 days after P application.

A slight decrease in the available P was observed in all treated soil samples within 60 days after application of P fertilizer, which led to a decrease by 1.8%, 2.1%, 1.6%, 1.2%, and 1.5% of the available P in the Aridisols, Inceptisols, Alfisols, Mollisols, and Histosols, respectively, compared to the 20-day application of P. The amount of the available P during the 100-day application compared to the 60-day application decreased by 1.3%, 1.15%, 1.5%, 0.68% and 0.54% in the Aridisols, Inceptisols, Alfisols, Mollisols, and Histosols, respectively. Totally, the results show that only 36.5%, 35.6%, 37.4%, 43.9% and 45.53% of the total P added were available in Aridisols, Inceptisols,

Alfisols, Mollisols, and Histosols, respectively, 100 days after application of the P fertilizer. The trend of changes in the available P over time in both 150 and 200 days was very gradual and low compared to 60 and 100 days, so that the amount of the available P after 150 and 200 days, compared to 100 days, decreased by 1.3%, 1.3%, 1.4%, 1.6%, 1.1% and 1.1%, 1.8%, 2%, 2.4%, 1.6%, 1.4% in Aridisols, Inceptisols, Alfisols, Mollisols, and Histosols, respectively. 200 days after application of P, decrease in the available P by 15% was observed, compared to the one-day application of P in the studied soils. Due to the high amount of the available P as well as high OM content and lower soil CCE content, Mollisols and Histosols showed the lowest P sorption of added P compared to other orders. Due to the changes in the chemical properties of the studied soils, such as CCE, OM, CEC and kind of clay minerals, the amount of available P released in various depths and profiles of different soil orders were not similar. However, soil samples from deeper horizons contained the least amount of available P, which can be attributed to more P fixation at early times. The average P available in the lowest and uppermost horizons of studied soils was obtained 148 and 127 µg g^{-1}, respectively, one day after using the P fertilizer, which decreased to 117 and 97 µg g^{-1}, 200 days after application of the P fertilizer.

3.4. P releasing rate over time

Different equations were used to predict the P release rate in the studied soils. The zero, first, second, and third orders equations are linear and simple. However, the P behavior in soil is very complex, so they did not fit the P release as well as predicted in the studied soils (data not shown). Both parabolic diffusion and power function rate equations were chosen as the best ones for fitting to data in this research. The results obtained from R^2 and SE values in the prediction of P desorption models in the studied soils indicate the superiority of the power function model to the parabolic equation (**Table 4**). **Figure 4** shows the fitting of data obtained from P desorption in treated soils, in each soil order, using the power function equation. Kuo and Mikkelson (1980) reported that an increase in the value of "a" and a decrease in the value of "b" in the power function equation probably
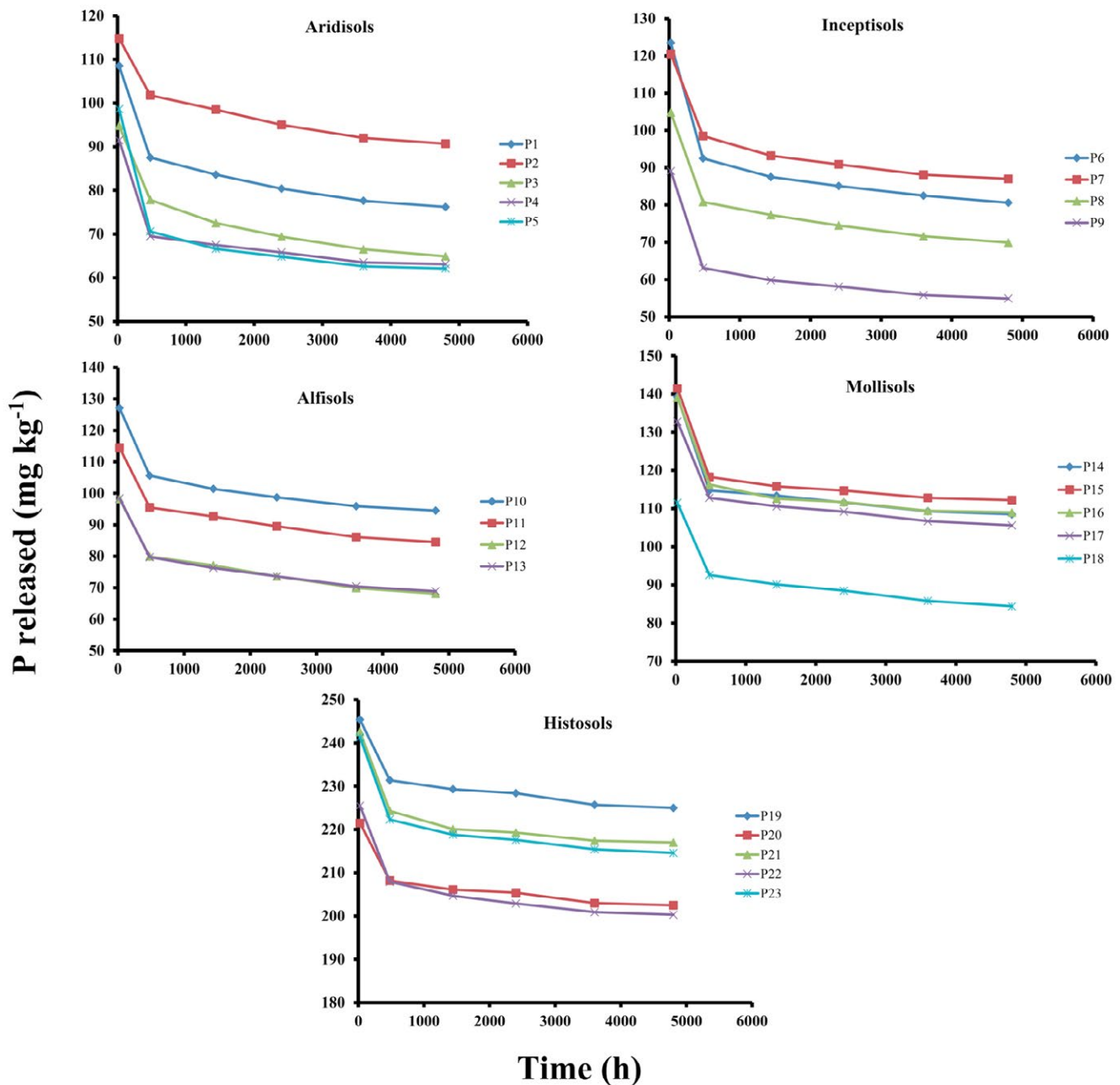
Figure 3. Relationship between Available-P released and time in treated soils.

indicates an increase in the rate of metal release from soils. As it can be seen in **Table 4**, coefficient "a" has the highest value in the Mollisol and Histosol soils. On the other hand, both the Aridisol and Alfisol soils had the lowest the value of this coefficient. Changes in the amount of this coefficient can help significantly to determine the potential for P release.

3.5. Chemical forms of P in untreated soils

Investigating the chemical forms of P in the studied soils before adding P fertilizer shows the superiority of Ca-P form (**Figure 5**). The contribution percentage of the Ca-P, Res-P, Fe-Al-P, and Exch-P forms in all studied soils was obtained as 61.9%, 27.1%, 6.5%, and 4.35%, respectively. Among the different orders studied, Aridisols (71.41%) and Histosols (50.2%) had the highest and the lowest amounts of Ca-P, respectively. On the other hand, Histosols had

Table 4. Values of two equations in examined soils

Soil number	Parabolic diffusion				Power function			
	q_0	K_p	R^2	SE	a	b	R^2	SE
1	112.985	0.059	0.840**	5.341	76.783	0.049	0.987**	0.013
2	116.519	0.046	0.921**	2.776	88.609	0.035	0.993**	0.007
3	97.763	0.056	0.891**	4.071	65.373	0.051	0.998**	0.005
4	97.338	0.049	0.738*	6.138	64.484	0.051	0.948**	0.026
5	106.255	0.064	0.734*	8.030	65.345	0.060	0.949**	0.031
6	131.432	0.075	0.770*	8.579	83.792	0.056	0.963**	0.024
7	125.529	0.061	0.824**	5.868	86.809	0.046	0.986**	0.012
8	110.342	0.062	0.809**	6.274	72.027	0.053	0.975**	0.019
9	96.047	0.060	0.742*	7.320	58.492	0.061	0.950**	0.031
10	131.873	0.059	0.829**	5.575	93.929	0.043	0.985**	0.012
11	118.181	0.054	0.859**	4.584	84.295	0.043	0.987**	0.011
12	101.389	0.055	0.880**	4.229	69.035	0.049	0.988**	0.012
13	102.144	0.053	0.855**	4.595	69.606	0.049	0.988**	0.012
14	145.951	0.053	0.718*	6.884	107.976	0.037	0.938**	0.021
15	147.961	0.050	0.714*	6.657	111.011	0.035	0.942**	0.019
16	145.287	0.053	0.740*	6.531	107.625	0.037	0.955**	0.018
17	137.802	0.047	0.769**	5.408	104.466	0.034	0.962**	0.015
18	115.908	0.048	0.801**	4.946	84.160	0.040	0.972**	0.015
19	248.567	0.036	0.812**	3.632	221.108	0.015	0.978**	0.005
20	224.640	0.034	0.799**	3.531	198.879	0.015	0.976**	0.005
21	247.395	0.046	0.768**	5.240	212.226	0.019	0.972**	0.007
22	229.777	0.045	0.796**	4.777	195.886	0.020	0.980**	0.006
23	246.080	0.047	0.780**	5.240	210.161	0.020	0.974**	0.007

**, * Significant at 0.01 and 0.05, respectively.

the highest amount of Exch-P (9.67%), which was three times more than Exch-P in Mollisols, Alfisols, Inceptisols, and 9 times more than the Aridosol order. Changes in chemical forms of Fe-Al-P and Ca-P did not follow a certain order trough the depth, however both Exch-P and Res-P forms were higher (except Histosols) in the topsoil than subsoil.

3.6. Chemical forms of P in treated soils

Investigating the chemical forms of P in the studied soils, one day after application of P fertilizer, showed many changes in different forms of P (**Figure 5**). The results obtained showed that on average, application of P fertilizer in all studied soils increased Exch-P form from 4.35% to 14.8% and Fe-Al-P from 6.57% to 8.89%. On the other hand, application of P fertilizer in all studied soils decreased Ca-P up to 7% (from 61.9% to 54.9%) and Res-P up to 5.8% (from 27.1% to 21.28%). 40.6%, 14.8%, 39.9%, and 5.34% of total applied P fertilizer entered the Exch-P, Fe-Al-P, Ca-P and Res-P forms, respectively. The highest and lowest change of total phosphorous in the Exch-P form was observed in Histosol and Alfisol soils, respectively. On the other hand, the highest and lowest amounts of P added to Ca-P were observed in Aridosol and Histosol soils, respectively. Because the Exch-P form is considered to be the available form of P for plants as well as it having higher availability for plants and leaching compared to other forms,
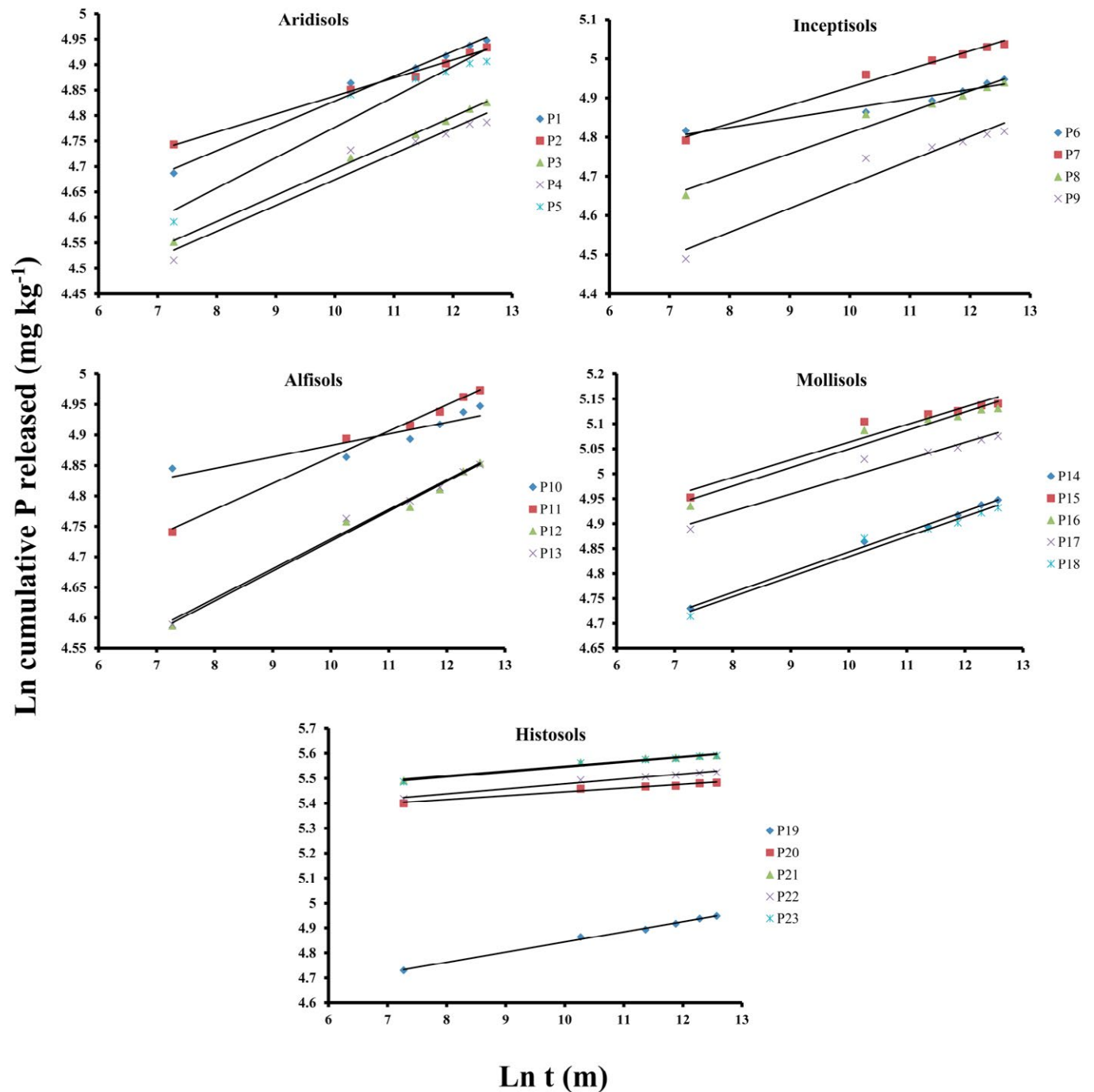
Figure 4. The relationship between observed and predicted P released in treated soils as described by power function equation.

and considering that less than 40% of P fertilizer consumed has transferred to the Exch-P, we can say that more than 60% of the applied fertilizer has changed to non-available form in the studied soils due to their calcareous nature and high pH. However, much of this P is defined as labile and can be released at various stages and introduced as supplier of initial plant needs. Changes occurring in these soils can be very important and critical for fertilizer management.

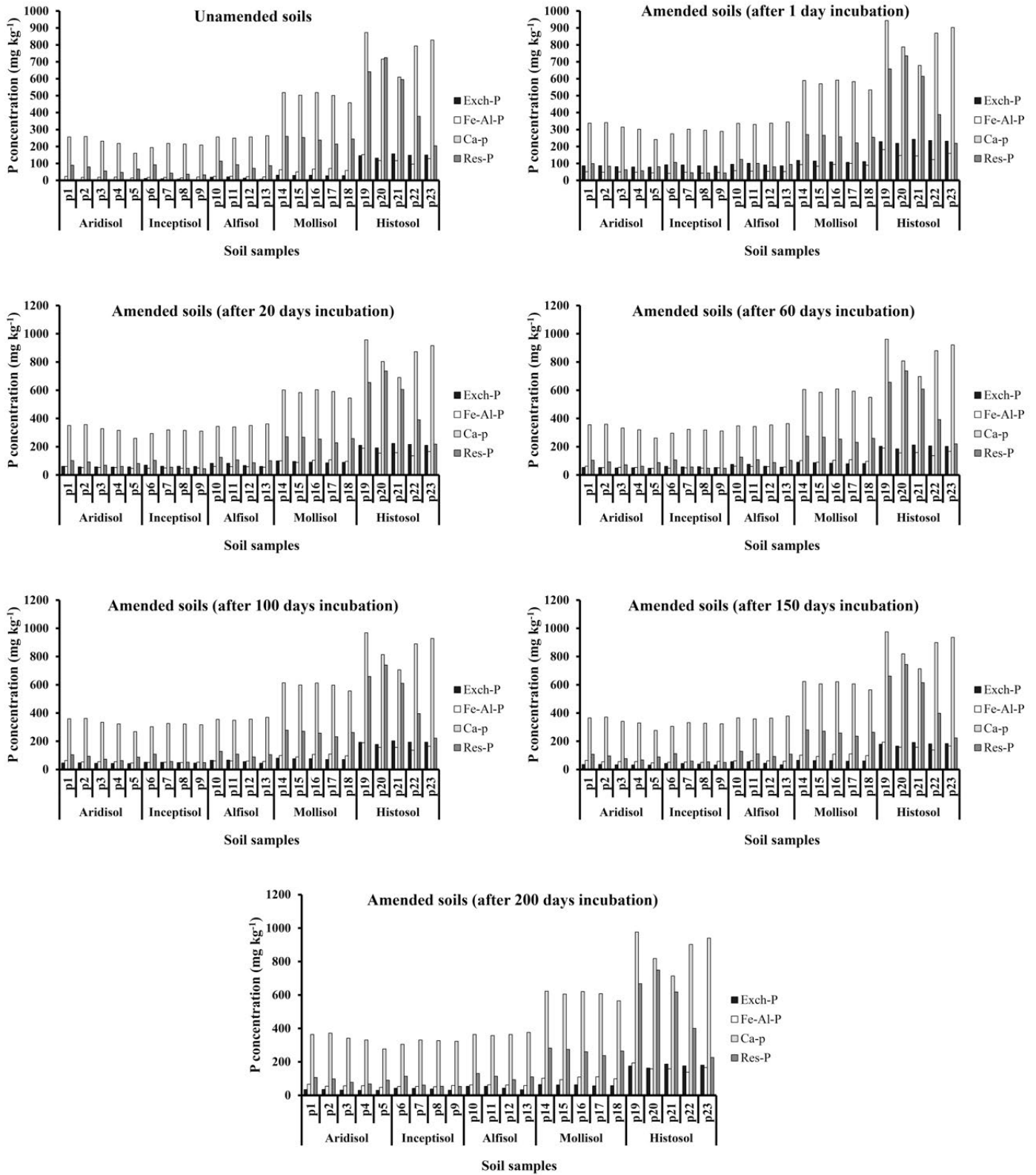3.7. The effect of incubation time on chemical forms of P

Figure 5. Chemical forms of P before (untreated soils) and after incubation.

Investigating the effect of incubation time on P chemical forms can provide useful information about fixation status and availability of P. The effect of different times on P chemical forms has been shown in **Figure 5**. The results obtained show that for a 20-day incubation compared to a one-day incubation, the Exch-P form decreased (from 14.8% to 11.7%), both Fe-Al-P and Ca-P increased (from 8.89% to 9.65% and from 54.9% to 57%, respectively), and Res-P had almost no changes (from 21.2% to 21.5%). Like the effect of incubation time on releasing available P, a severe abrupt change was observed in the amount of available (Exch-P) P after 20-days in all samples. The greatest decrease in Exch-P was observed in the Aridisols (from 16% to 11.3%) and Inceptisols (from 17.8% to 13.5%), and the lowest was observed in Histosols (from 13.4% to 12.2%) and Mollisols (from 10.8% to 8.9%). Moreover, changes made in two forms of Fe-Al-P and Exch-P showed that both the Aridisols and Inceptisols had the largest increase in these forms of P. Changes occurred at days 60, 100, 150 and 200, compared to day 20, were relatively slighter than changes trend at day 20 compared to day 1. A slight decrease in the Exch-P form and a very slight increase in three forms of Ca-P, Fe-Al-P and Res-P were observed in all the soil orders. The greatest decrease of Exch-P and smallest decrease of Res-P form were observed in both Aridisols and Inceptisols 200 days after incubation. On the other hand, the lowest decrease of Exch-P and highest decrease of Res-P were observed in the Mollisols and Histosols. Relative percentage of Exch-P 200 days after incubation showed a decrease by 25.8% (from 40.7 to 14.9%) compared to first day of incubation. On the other hand, 200 days after incubation, three other forms of P, Ca-P, Fe-Al-P and Res-P increased by 15.8%, 5.4% and 4.7%, respectively, compared to one day after application of the total amount of P. Generally, the results of this section of the study showed that the relative percentage of Exch-P form decreased and other parts increased over time. Also, the findings showed that transforming the Exch-P form in both Histosols and Mollisols orders was relatively low compared to other orders over time, while decreasing the Exch-P form and increasing other forms had more changes in the Aridisol and Histosol orders. On average, only 15% of the total P remained in Ex-

P, 200 days after adding the P fertilizer, while this amount was 40.6% and 28.5% at days 1 and 20, respectively.

3.8. Correlation between released P and soil properties

Simple correlations between released P and soil properties show that available P had a significant positive correlation with OM, CEC, initial available P and total P at all desorption times (**Table 5**). On the other hand, the relationship between CEC, EC and sand with released P was significantly negative at all times. The relation between other soil properties and P desorption was not statistically significant (**Table 5**).

3.9. Correlation between chemical forms of P and soil properties

The results obtained from relation between chemical forms of P and properties of the untreated soils (without application of P fertilizer) showed that there was a positive and significant correlation between all forms of P and OM, CEC, and total and available P in soils, and a significant negative correlation between EC, sand, and CCE (**Table 6**). The correlation between chemical forms of P with properties of soils one day after application of P fertilizer was the same as the untreated soils. 200 days after application of P fertilizer made different changes in correlation between chemical forms of P and soil properties, so that no significant correlation was observed except a significant positive correlation between available P (Olsen-P) with Exch-P forms, Fe-Al-P and Ca-P. It seems that chemical forms of P are controlled by other factors over time, like clay minerals.

3.10. Clay minerals and their correlation with P chemical forms

The capacity of soil clay minerals (kaolinite, montmorillonite and illite) has been underestimated for P sorption compared to Fe/Al oxides in many studies. The capacity of P sorption by clay minerals may even exceed the capacity of Fe/Al oxides depending on these minerals' crystallization (for example, the

Table 5. Simple correlation coefficient (r) between extractable P and soil properties

	pH	OM	CCE	Clay	Sand	EC	CEC	Olsen-P	Total-P
OM	-0.477*								
CCE	0.600**	-0.447*							
Clay	-0.449*	0.222	-0.317						
Sand	0.314	-0.472*	0.203	-0.701**					
EC	0.341	-0.442*	0.310	-0.082	0.395				
CEC	-0.450*	0.976**	-0.456*	0.345	-0.588**	-.418*			
P-Olsen	-0.238	0.658**	-0.506*	0.237	-0.591**	-0.410	0.729**		
Total-P	-0.418*	0.826**	-0.633**	0.204	-0.557**	-0.538**	0.860**	0.918**	
†R 1-day	-0.324	0.717**	-0.552**	0.219	-0.576**	-0.416*	0.781**	0.981**	0.944**
†R 20-days	-0.315	0.718**	-0.546**	0.200	-0.570**	-0.432*	0.780**	0.981**	0.949**
†R 60-days	-0.323	0.725**	-0.551**	0.201	-0.573**	-0.438*	0.787**	0.980**	0.953**
†R 100-days	-0.325	0.728**	-0.555**	0.204	-0.575**	-0.442*	0.789**	0.980**	0.955**
†R 150-days	-0.328	0.728**	-0.557**	0.208	-0.578**	-0.444*	0.790**	0.980**	0.955**
†R 200-days	-0.328	0.729**	-0.559**	0.211	-0.581**	-0.446*	0.791**	0.980**	0.956**

*Correlation is significant at the 5% level; **Correlation is significant at the 1% level. †R: Released P in different incubation time. Abbreviations as in **Table 3**. EC: electric conductivity.

Table 6. Simple correlation coefficient (r) between P chemical forms and soil properties in untreated soils and treated soils after 1 and 200-days incubation time

Chemical fractions	pH	OM	Untreated soil					Olsen-P	Total-P
			CCE	Clay	Sand	EC	CEC		
Exch-P	-0.256	0.677**	-0.535**	0.228	-0.578**	-0.426*	0.744**	0.997**	0.937**
Fe-Al-P	-0.389	0.809**	-0.614**	0.153	-0.546**	-0.536**	0.831**	0.925**	0.980**
Ca-P	-0.472*	0.771**	-0.665**	0.148	-0.505*	-0.586**	0.784**	0.889**	0.967**
Res-P	-0.361	0.839**	-0.553**	0.246	-0.547**	-0.453*	0.884**	0.818**	0.943**
				1-day incubation time					
Exch-P	-0.286	0.690**	-0.535**	0.239	-0.588**	-0.434*	0.755**	0.995**	0.943**
Fe-Al-P	-0.404	0.811**	-0.629**	0.153	-0.534**	-0.548**	0.832**	0.918**	0.979**
Ca-P	-0.465*	0.766**	-0.663**	0.142	-0.502*	-0.584**	0.780**	0.889**	0.965**
Res-P	-0.360	0.839**	-0.555**	0.249	-0.550**	-0.450*	0.884**	0.820**	0.944**
				200-days incubation time					
Exch-P	-0.097	0.009	-0.304	0.078	-0.229	-0.152	0.057	0.574**	0.372
Fe-Al-P	-0.164	0.015	-0.279	0.236	-0.366	-0.147	0.071	0.506*	0.311
Ca-P	-0.161	0.021	-0.296	0.169	-0.296	-0.115	0.065	0.453*	0.308
Res-P	-0.169	-0.079	-0.277	0.343	-0.355	-0.108	0.008	0.312	0.207

*Correlation is significant at the 5% level; **Correlation is significant at the 1% level. Abbreviations as in **Table 3**. EC: electric conductivity.

specific surface area) (Gérard 2016). Therefore, the secondary clay minerals were identified in some soil horizons and reported quantitatively (**Table 7**) to investigate the effects of clay minerals on chemical forms of P at different times. Palygorskite, chlorite, kaolinite, illite and smectite minerals were found in the Aridisol order.

The most abundant mineral in this order was smectite and the least abundant were chlorite

and kaolinite. Mineralogical changes in both the Inceptisol and Alfisol orders were similar to the Aridisols, with smectite the most abundant in these orders, and chlorite and kaolinite the least abundant. Palygorskite clay mineral was not found in the Mollisol and Histosol orders due to their high humidity in the slope position, showing that this mineral changed to smectite. Except palygorskite, the minerals present in the three

Table 7. Semi-quantitative analysis of clay minerals in the clay fraction of some soils under study

Pedon	Soil number	Horizon	smectite	illite	chlorite	Interstratified	kaolinite	palygorskite
1	1	A	++++	++	-	*	+	+
1	2	Bk	+++++	++	+	*	+	*
1	3	Bz	++++	+++	+	-	+	*
2	7	Bk1	+++	+++	++	*	+	-
2	8	Bk2	++++	+++	+	-	-	*
2	9	Bkm	+++++	++	-	*	+	*
3	11	Bkz	++++	++	+	-	+	*
4	14	Ag	+++	++	++	*	+	*
4	15	Bwg1	++++	+++	+	*	+	*
5	19	Oe1	+++++	+	+	*	+	*
5	20	Oe2	+++++	+	-	*	+	*

*: not detected, -: < 10%, +: 10-20%, ++: 20-30%, +++: 30-45%, ++++: 45-55%, +++++: > 55%.

previous orders were also observable in both the Mollisol and Histosol orders.

Generally, from the beginning (Aridisols) to the end of the study area (Histosols), with increasing rainfall and moisture, palygorskite would be destroyed due to instability at high humidity, and the smectite and vermiculite minerals would be prevailing. Also, because the Mollisols and Histosols are located in the lowland geomorphological positions, and due to water from melting snow and relatively high rainfall, the conditions are available for formation of smectite in the form of neoformation. The results obtained from the correlation of clay minerals with P chemical forms in all treated and non-treated samples at different times (one day and 200 days after application of P fertilizer) were somewhat similar, so that illite had a significant negative relation with all P forms (**Table 8**).

Moreover, kaolinite had a significant positive relation with Fe-Al-P before treatment, as well as Fe-Al-P and Ca -P one day after application of P fertilizer, and with Res-P form and total P 200 days after adding P fertilizer. The relation between other clay minerals and chemical forms of P were not statically significant.

4. Discussion

According to the results obtained from changes in available P over time, 52.4% (on average) out of total P fertilizer was observed as available one day after application of P fertilizer in all studied soils. The available P decreased severely and reduced to 42% with increasing the

Table 8. Simple correlation coefficient (r) between P chemical forms and clay minerals in untreated soils and treated soils after 1 and 200-days incubation time (n = 11)

Clay minerals	Exch-P	Untreated soil			Total-P
		Fe-Al-P	Ca-P	Res-P	
Kaolinite	0.420	0.671*	0.631	0.517	0.592
Illite	-0.889**	-0.770**	-0.711*	-0.740*	-0.745*
Chlorite	0.170	0.324	0.394	0.234	0.303
Smectite	0.448	0.338	0.264	0.392	0.346
Interstratified	-0.343	-0.421	-0.491	-0.438	-0.455
Palygorskite	-0.315	-0.338	-0.374	-0.342	-0.356
1-day incubation time					
Kaolinite	0.513	0.663*	0.635*	0.520	0.592
Illite	-0.890**	-0.756*	-0.717*	-0.738*	-0.745*
Chlorite	0.187	0.335	0.388	0.239	0.303
Smectite	0.444	0.336	0.273	0.385	0.346
Interstratified	-0.365	-0.411	-0.487	-0.441	-0.454
Palygorskite	-0.332	-0.366	-0.373	-0.339	-0.358
200-days incubation time					
Kaolinite	0.454	0.632	0.612	0.804**	0.703*
Illite	-0.873**	-0.765**	-0.761*	-0.660*	-0.763*
Chlorite	0.115	0.287	0.305	0.417	0.335
Smectite	0.508	0.333	0.336	0.141	0.303
Interstratified	-0.402	-0.485	-0.496	-0.421	-0.481
Palygorskite	-0.255	-0.277	-0.330	-0.259	-0.307

*Correlation is significant at the 5% level; **Correlation is significant at the 1% level.

incubation time (20 days). Passing 200 days from application of P compared to 1 day showed a 15% decrease, on average, in the available P in the studied soils. High concentrations of CCE in all studied soils may be the main reason for significant reduction in available P (less than 50%). P mobility in calcareous soils is controlled by soil properties, which preserves P in the soil strongly and reduces its concentration in soil solution (Delgado et al. 2002). The possible reactions of P in calcareous soils are usually as follows: 1) precipitation in the form of insoluble calcium phosphate such as octacalcium phosphate, hydroxyapatite and apatite carbonate, 2) surface precipitation by free calcium carbonates, and 3) P fixation by calcium-saturated clays. However, in low P concentrations, the exchange process is carried out in the form of sorption silicates and Al-oxyhydroxide on the edge of the minerals.

Reduced phosphate availability by calcium carbonate in calcareous soils is well known (Bertrand et al. 2003). In a study, Jalali (2006) investigated the effect of aging on the available P after fertilizer application in calcareous soils of Iran, and showed that 90 days after application of P fertilizer, only 21% of the applied fertilizer remained available; while in another study by (Jalali and Ranjbar 2010), the same time passing on other regional calcareous soils showed an availability of 32% of the P added to the soil. In a study by Javid and Rowell (2002), it was found that 100 days after adding P fertilizer the availability of the P added to the soil was 20-55%. Reduction of the P available over time is a two-phase process, which is very fast at the beginning, and then it proceeds at a lower speed. The fast process may be attributed to sorption of P on aggregates, and the slow one may be attributed to diffusion of P into Fe and Al

oxides microspores of solid part of the soil (Wang et al. 2000). In other words, P is adsorbed by soil components through a two-phase process (Sims and Pierzynski 2005). In the early stages, high sorption takes place at high speed as non-specific sorption and ligand exchange on the edges of minerals and short links with Fe and Al crystalline hydroxides and calcium carbonates (depending on the type of soil) (Rayment and Lyons 2011). Following these reactions, P moves at a lower speed through diffusion into internal parts of the soil forms, as well as the surface precipitation and P polymerization at minerals surfaces (Correll 1998). Griffin et al. (2003) investigated the effects of time and application of potassium phosphate on extracted P by Mehlich-3 method, and showed that passing 84 days after application of P fertilizer caused a severe decrease in Mehlich-extracted P and an increase in the contents of Exch-P and Fe-Al-P of the soil. After 20 days of using P fertilizer, the available P content had been reduced by 10%, therefore, it is suggested that fertilization should be carried out in several stages shortly after planting. The availability of P in alkaline and calcareous soils is very low due to the high calcium carbonate and clay content (Tisdale et al. 2002). In the present study, since both the Aridisol and Inceptisol orders had the most available P fixation, the recommendation to use less successive P fertilizer in these soils is more necessary than for Histosols and Mollisols with less P fixation. According to the results, only two parabolic and power function equations were able to adequately fit the available P release data in the studied soils. An investigation on the availability of P release models from calcareous soils in Iran, showed that the parabolic equation was the best predictor equation for P release (Jalali and Ranjbar 2010). The researchers stated that the q_0 (slope) coefficient in this equation represents the release of P during time, and may be introduced as an index of transformation. The smaller index indicates less P release in the studied soils. As given in **Table 4**, the index is lowest in Aridisols and Inceptisols, indicating a greater transformation index and less P release in these soils. In treated soils, Ca-P (61/9%), Res-P (27/1%), Fe-Al-P (6/5%) and Exch-P (4.35%) forms have the highest and lowest P fractions, respectively. Among different studied orders, Aridisols (71/41%) and Histosols (52%) had the highest and lowest Ca-P

respectively. On the other hand, Histosols had the most amount of Exch-P (9/67%), which was three times more than the one in the Mollisol, Alfisol, and Inceptisol orders, and nine times more than the one in the Aridisols order. Jalali and Ranjbar (2010) investigated chemical forms of P in 20 calcareous soils and obtained similar results as in this study. They reported the chemical forms of P as follows: Ca-P forms (63/7%) > Res-P (31/1%) > Fe-Al-P (3/3%) > Exch-P (1/9%). In a study on P chemical forms in Alfisols, Entisols, Mollisols and Vertisols soils in California, Linquist et al. (2011) showed that non-organic and organic P content in Mollisols soils, especially chemical form of P extracted with hydrochloric acid (Ca-P), is more than in other orders. Yang et al. (2013) reported that in epipedon A (15 cm from soil surface), the most labile P in soil orders was in Inceptisols, Entisols, Spodosols, Alfisols, Ultisols, and Oxisols orders, respectively. On average, the application of P fertilizer in all studied soils increased the relative percentages of Exch-P and Fe-Al-P, and reduced the relative percentage of Ca-P and Res-P. From total 200 µg g^{-1} of the applied P fertilizer, 40.6% has entered to Exch-P, 14.8% has entered to Fe-Al-P, 39.9% has entered to Ca-P part, and the remaining (5.34%) has entered to Res-P part. Unlike the results of this study, Jalali and Ranjbar (2010) showed that adding P to the soil immediately caused more transformed to Ca-P as well as Fe-Al-P parts. The results of the effect of different time on chemical forms of P in various soils showed that the average percentage of the Exch-P form decreased with time and the Ca-P form had the highest P content like untreated soils. Jalali and Ranjbar (2010) showed that the Exch-P decreased and both Ca-P and Fe-Al-P forms increased with time while Res-P remained unchanged. The results of the relationship between chemical forms of P and chemical, physical and mineralogical properties of soils showed that physical and chemical properties of soils in the early stages of P fertilizer addition were more obviously controlling some chemical forms of P, and the correlation decreases over time (200-days) and shows that the other factors mentioned can affect the chemical forms of P. Therefore, we studied the correlation between chemical forms of P and clay minerals. The results showed that there has been no change in the correlation between clay minerals and chemical forms of P over time, and both kaolinite

and illite minerals got positive and negative meaningful relationships, respectively, with some chemical forms at the beginning and the end of P fertilizer application. The number of hydroxyl groups of 1:1 clays such as kaolinite is more than in 2:1 clays such as montmorillonite. For this reason, the phosphate fixation strength in 1:1 clays is more than in 2:1 clays. In this case, even iron and aluminum ions formed from the breakdown of clay edges are combined with phosphate and make P non-available (Gérard 2016). This can help to justify why kaolinite has a significant correlation with all forms of P except Exch-P. Silicate clays, such as kaolinite, montmorillonite, or illite, have P fixation capability. Hydroxides groups on the surface of clay crystals may be exchanged with hydrogen and phosphate ions and result in P fixation. This may justify the negative correlation of Exch-P form with illite compared to other forms of P.

5. Conclusions

The P reactions added to the studied soils indicated a rapid conversion of available P to relatively less soluble compounds in a short time due to the high adsorption capacities of the studied soils. Investigating the release of available P shows that both the parabolic and power function models have potential to interpret the P release as well. The most extracted chemical forms of P were related to Ca-P in treated and untreated soils. Application of phosphorous fertilizer at first lead to transfer of the highest amount of it to Exch-P form and the least amount to the Res-P form. The relative percentage of the Exch-P form decreased and other parts increased over time. It seems that mineralogical properties can be effective in controlling the P chemical forms of P over longer term compared to the physical and chemical properties.

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