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Role of Bacterial Inoculation in Kinetics of Phosphorus Release from Phosphate Rock in Calcareous Soil.

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ABSTRACT

Biological experiment was conducted to study role of *Bacillus ceares* inoculation in phosphorus release from phosphate rock ($Ca_{10}(PO_4)_6(OH)_2$) and to evaluate the phosphorus supplying powder of a calcareous. A loamy soil was collected from College of Agriculture field (Al-Gadria), University of Baghdad. Bacterial isolates were obtained onto phosphorus and used to release phosphorus. The soluble phosphorus was determined at 0, 10, 20, 30, 40 and 50 day of incubation. The results showed that adding the bacterial inoculation significantly increased the amount of the released phosphorus which was 0.473 mg p kg^{-1} soil compared with 0.198 mg p kg^{-1}soil recorded in the control treatment. The amount of the released phosphorus that was 0.833 mg p kg^{-1}soil .There was positive relation between the incubation periods and amount of the released phosphorus in all the treatments and that shows the need of long time of incubation to complete dissolution of phosphate rock. The value of rate of reaction constant (k) was the greatest and reached 0.208 mg p kg^{-1}soil h^{-1} when phosphate rock interacted with the inoculation compared with 0.117 mg p kg^{-1}soil h^{-1} in the control treatment, and the greatest rate of phosphorus release coefficient (kd) was 1.257 mg p kg^{-1}soil h^{-1} in the interaction compared with 0.033 mg p kg^{-1}soil h^{-1} in the control treatment. The result showed a decline in the phosphorus supplying power of phosphate rock in the calcareous soil and increase of rate of phosphorus release coefficient (kd) value to 38.09% when phosphate rock interacted with the bacterial inoculation.

Keywords: Bacterial inoculation, Kinetics of phosphorus release, phosphate rock, calcareous soil.

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INTRODUCTION

Phosphorus is one of the important essential elements of plant nutrition and is the key of life due to the direct important role in many physiological processes of the plant and therefore affects plant growth and productivity [1]. Available phosphorus, in the Iraqi calcareous soils with high pH, suffers of a number of processes that limit the availability such as adsorption and deposition on the surface of carbonate minerals, direct reaction with calcium ions which results in the formation of insoluble phosphorus compounds or reserved on the surface of clay minerals [2-4]. Phosphate rock, [Ca_{10} $(PO_4)_6$ $(OH)_2$], is the main phosphorus source containing 10 – 15% P, but has very low solubility [5] and it possibly to increase its efficiency to supply the phosphorus by using acidity generators, which act increasing the solubility of the phosphate rock, or the benefit of soil microbiology which act increasing its solubility and releasing P by formatting organic and non-organic acids which decreasing soil pH and increasing the solubility [6]. Soil microbiology plays an important role in increasing the availability of phosphorus in the soil from the insoluble phosphorus compounds such as calcium and phosphate rock [7-8]. Darwesh et al. [9] found that bacterial inoculations play an important role in improving soil properties and increasing the availability and absorption of many nutrients like phosphorus; At-Ta'ee and Sa'eed [10] found that the most common of bacterial genera dissolving phosphate compounds were *Bacillus* and *Pseudomonas*. Hasan [11] mentioned that mechanism of dissolving phosphorus compounds in the soil by microorganisms is reflected in the production and secretion of a range of organic acids that reduce soil pH and increase the solubility and most of the bacterial isolates dissolving phosphorus had the ability to secrete these acids dissolving the phosphate.

The study of phosphorus releasing from phosphate rock was by applying velocity laws which all the kinetic equations depended on to calculate the releasing speed coefficient of phosphorus depending on time factor to predict the releasing and its mechanism in the soil [12], kinetic concept was used, which considered as an important measures to study the phosphorus supplying ability [13], for this purpose depending on kinetic equations which built either according chemical basis such as zero, one and two order equation or according physical basis such as diffusion equation or the equations that depending on empirical basis such as exponential and Elovich's equation to describe phosphorus releasing in the soil. Jebur [14], in studying phosphorus releasing from phosphate gypsum in alluvial calcareous soil depending on kinetic equation, indicated that Elovich's equation was the best description for phosphorus releasing; also Manaf [15] pointed to the superiority of Elovich's equation for describing phosphorous releasing from phosphate rock in the soil. As for Sikora et al [16] indicated that, generally, the good kinetic equations describing soil phosphorus releasing were first order, exponential, diffusion and Elovich's equations.

The aim of this paper was to study phosphorus kinetics from soil phosphate rock in calcareous soil treated with bacterial inoculation, know the effect of bacterial inoculation on the released phosphorus quantity and evaluating the phosphorus supplying power according to kinetic chemical standards.

MATERIAL AND METHODS

Soil sample

A loamy texture soil sample, from one of Agriculture College – University of Baghdad fields, was taken and the initial procedures were done to prepare it for the next laboratory procedures. Soil chemical, physical and biological properties (table 1) were estimated according to the methods of Bushoor and As-Sa'egh [17]. A part of the soil was taken and sterilized by autoclave at a temperature of 121º and pressure of 15 pounds / $inch^2$ for 20 minutes. Bacterial isolation, *Bacillus ceares*, was obtained from the National Research centre in Dokki / Egypt and grown in the solid and liquid culture media, N. Ager and N. 7browth, and preserved in the slant solid media until later use.

The biological experiment

The biological experiment was carried out using Petri dishes; 10 g of sterilized soil were put in each dish. 0.0058 g of phosphate rock [Ca_{10} $(PO_4)_6$ $(OH)_2$] containing 10 – 15%P (i.e. 240 Kg $P.ha^{-1}$) was added to each dish. As a nitrogen source, liquid urea (prepared by dissolving 0.01g urea into 500 ml distilled water, added as 5 ml to each dish) was added. Liquid nutrition media, N. broth, was prepared into a set of test tubes sterilized by autoclave and a part of bacterial colony was transferred by the loop from the slant to inoculate

the liquid media in the test tubes under much sterilized conditions. The tubes were incubated in the incubator at 30˚. McFarland's solution was prepared according the method of Baron and Finegold [18] which equal $1*10^8$ bacterial cells.ml^{-1}. Culture media turbidity of the tubes was compared with the standard turbidity of McFarland's solution found in a test tube similar to those culture media. The bacterial inoculation intensity was $1*10^8$ bacterial cells.ml^{-1} for each dish (10 g soil) and distributed by dishes rotation to right and left, then the dishes and their contents were incubated at 30˚ for 50 days. The soil soluble P was estimated in periods of 0, 10, 20, 30, 40, and 50 days. The soil moisture, at 75% of field capacity, was preserved by the difference weight method.

The experiment was conducted according to Complete Randomized Design (CRD) with 3 replicates and 4 treatments:

1- Soil only (a1)
2- Soil + bacterial inoculation (a2)
3- Soil + phosphate rock (a3)
4- Soil + Bacterial inoculation + phosphate rock (a4)

Table 1: some chemical and physical properties of study soil

Property		Value	Unit
pH 1:1		7.61	----------
EC 1:1		3.10	$ds.m^{-1}$
)CEC(		23.20	$cmol^{+}.Kg^{-1}$
Organic Matter		6.54	$g.Kg^{-1}$
Carbonate Minerals		210.20	$g.Kg^{-1}$
Gypsum		0.29	$g.Kg^{-1}$
Cations	Ca	28.50	$Mmol.L^{-1}$
	Mg	16.68	
	Na	10.61	
	K	0.68	
Anions	Carbonate	Nil	
	Bicarbonate	1.82	
	Chlorides	18.91	
	Sulfates	26.80	
Available N		21.10	$mg.Kg^{-1}$soil
Available P		3.12	
Available K		74.31	
Soil Particles	Sand	340.41	$mg.Kg^{-1}$soil
	Silt	390.20	
	Clay	260.39	
Texture		L) (Loamy	----------
Bulk Density		1.31	$Mg.m^{-1}$
Soil Microbiology	Total Bacteria	$10^5 * 1.11$	CFU.g^{-1} dry soil
	Fungi	$10^4 * 0.75$	

The soluble P was estimated by citric acid ($5*10^{-4}$ M) after the ending of each incubation periods according to Olsen and Sommers (1982). Soluble phosphorus kinetics, for each different incubation periods, were studied for all treatments using the below kinetic equations:

1- Zero order

$$(Co - Ct) = Co - Kd^t$$

2- First order

$$\ln (Co - Ct) = \ln Co - Kdt$$

3- Second order

$$1/Ct = 1/Co + Kt$$

4- Exponential function

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$$\ln Ct = \ln Co + Kd \ln t$$

5- Diffusion

$$Ct/Co = Co + Kdt^{0.5}$$

6- Elovich

$$Ct = Co + Kd \ln t$$

Where:
Ct: the released P quantity at time t (extraction time)
Co: P concentration at zero time
Kd: releasing velocity coefficient (releasing velocity constant) $mmol.Kg^{-1}.h^{-1}$

RESULTS AND DISCUSSION

The accumulative quantity of P as a time function

According to table 2, it can be inferred the accumulative quantity of released P (mg $p.Kg^{-1}$ soil) for the different treatments and time periods. Adding the bacterial inoculation led to a significant increment in the amount of released phosphorus noted in a2 treatment, the average of released amount was 0.473 mg P. kg^{-1} soil compared to the treatment without the bacterial inoculation (a 1) of 0.198 mg P. kg^{-1} soil, due to the role of the bacterial inoculation in increasing the solubility of insoluble phosphorus compounds in the soil, thus increasing phosphorus releasing, corresponding with Darwesh et al [9] who indicated that adding bacterial inoculation led to increasing soil phosphorus releasing. Adding phosphate rock led to insignificant released phosphorus (a3) of 0.22 mg $P.Kg^{-1}$ soil compared with control treatment; that might due to solubility lack of soil phosphate rock, corresponding with Menaf [15] who indicated that the solubility lack of soil phosphate rock led to lack of released phosphorus, the highest amount of released phosphorus was in a4 treatment which gave significant increment in released phosphorus of 0.833 mg $P.Kg^{-1}$ soil compared with the control which was 0.198 mg $P.Kg^{-1}$ soil, that was due to the role of bacterial inoculation in increasing the solubility of phosphate rock and the increment of what released from the phosphate rock into soil, that corresponding with Rashid et al [19] and Khan et al [20] who indicated that increasing solubility of soil insoluble compounds due to the formation of organic acids secreted by microorganisms in the soil that produced hydrogen ion (H^+) that had a capability to dissolve soil insoluble phosphate minerals making it available for plants by decreasing soil pH. About incubation periods, results indicated that there was a positive relationship between incubation periods and the accumulative quantity of P of all treatments, but it was insignificant except in a4 treatment in the last period (50 days) of 0.530 mg $P.Kg^{-1}$ soil compared with control treatment of 0.235 mg $P.Kg^{-1}$ soil, that indicated the need of bacterial inoculation a long time to complete dissolving low soluble phosphate rock then increasing released P, that corresponding with Menaf [15] who indicated that there increment in the accumulative quantity of P from phosphate rock with increasing incubation period. The interaction between bacterial inoculation and phosphate rock showed significant increment in the accumulative quantity of P for 4 treatments only and the increment appeared in the 40 days period, gradually increased in the next period of 1.023 and 1.032 mg $P.Kg^{-1}$ soil for the periods 40 and 50 days, respectively.

Table 2: The accumulative quantity of P (mg $P.Kg^{-1}$ soil) for different periods and treatments

No.	Treatments	Periods (days)					Mean
		10 b1	20 b2	30 b3	40 b4	50 b5	
1	a1 Soil only (control)	0.110	0.183	0.224	0.235	0.238	0.198
2	Soil + Inoculation a2	0.290	0.415	0.517	0.570	0.576	0.473
3	a3 Soil + Rock	0.115	0.200	0.250	0.271	0.275	0.222
4	a4 Soil + Rock + Inoc.	0.425	0.746	0.941	1.023	1.032	0.833
	Mean	0.235	0.386	0.483	0.524	0.530	0.431

$LSD_{0.05}$ (a) = 0.261
$LSD_{0.05}$ (b) = 0.292
$LSD_{0.05}$ (a * b) = 0.584

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The Description of Releasing P According Kinetic Criteria

The Kinetic equations depended to describe releasing P based on scientific principles (chemical and physical) to explain releasing mechanism. For the purpose of better knowledge of the equations that describe P releasing process from phosphate rock with or without bacterial inoculation in the soil, statistical analysis was performed for the values of the determination coefficient (R^2) and standard error (SE) for these treatment, the best equation is that achieved the highest value of the R^2 and the lower value of SE. Table 3 showed the average of SE and R^2 values of the kinetic equations used to describe P releasing for all treatments. According to table 3, the different kinetic equations which describe the releasing process, the best equation was the exponential achieving the highest value of R^2 of 0.956 and the lower value of SE of 0.041.

Table 3: R^2 and SE values for kinetic equations used to describe the effect of the bacterial inoculation on P releasing from phosphate rock in the soil

No.	Treat.	0 order		1st order		2nd order		Diffusion		Exponential		Elovich	
		R^2	SE	R^2	SE	R^2	SE	R^2	SE	R^2	SE	R^2	SE
1	a 1	0.816	0.207	0.888	0.040	0.435	2.587	0.838	1.280	0.932	0.025	0.954	0.614
2	a 2	0.898	0.045	0.908	0.036	0.702	0.728	0.953	0.014	0.982	0.811	0.982	0.366
3	a 3	0.773	0.038	0.790	0.067	0.716	2.182	0.918	0.032	0.931	0.034	0.971	0.014
4	a 4	0.850	0.122	0.876	0.068	0.740	0.611	0.931	0.028	0.980	0.024	0.969	0.050
Rate		0.834	0.103	0.865	0.052	0.648	1.527	0.910	0.338	0.956	0.041	0.969	0.261

This equation states that the P released amount positively proportional to the time raised to a certain power, thus the releasing process was defined by the reaction time (Sparks, 1985), that corresponded with Sparks (1986) who indicated that the exponential equation was the successful kinetic equation for describing P releasing in the soil, the kinetic equation, for describing P releasing, graded as following in current study:

Exponential Function > First Order > Zero Order > Elovich's > Diffusion > Second Order

Releasing Velocity Coefficient (Kd)

The releasing velocity can be described as it the released amount of element from the soil particles and components, organic matter and additive fertilizers sources into soil compared to adsorbed amount in the soil [21]. For the permanence of supplying nutrients for the plant, the releasing rate of these nutrients must be as much fits with plant requirements. The best way to describe the releasing of nutrients was using the entrance of kinetics and application of velocity laws depending on the time factor to predict the velocity of reaction and its mechanism for the element in the soil [12]. The velocity laws were applied to calculate Kd and the half-life required for the reaction ($t^{1/2}$) which presented the time required to decrease dissolved P to the half to know what occurred for additive fertilizers to the soil and the rest amount of them with the time [22].

The constant of reaction velocity (k) was calculated from the regression value (b) of the superior equation describing P releasing in soil for different treatments and the values were: 0.117, 0.031, 0.178 and 0.208 mg $P.Kg^{-1}$ $soil.hr^{-1}$ for a1, a2, a3 and a4 treatments, respectively, and the half-life time ($t^{1/2}$) was calculated from k values according the equation $t^{1/2} = 0.693/k$ [21-23], the half-life values of the different equation were 5.92, 7.22, 3.89 and 3.33 hr for a1, a2, a3 and a4 treatments, respectively, and this meant that after 5.92 hr dissolved P concentration of a1 treatment will reach the half and so on for the rest treatments.

Table 4: the coefficient of P releasing velocity from the phosphate rock for different treatments

No.	Treatments	0 order	1st order	2nd order	Diffus.	Exp.	Elovich
1	Soil Only a1	0.744	0.278	5.888	11.720	0.033	0.071
2	a2 Soil + Inoculation	2.171	0.778	2.876	0.026	0.978	0.142
3	a3 soil + Rock	0.620	0.470	8.472	0.003	0.087	0.116
4	a4 Soil + Rock + Inoculation	2.251	0.817	1.892	0.004	1.257	0.457

Generally, the low values of releasing coefficient refers to low P supplying ability by phosphate rock in the soil due to the low solubility of phosphate rock in the calcareous soils with high pH, which increasing P deposition and increasing adsorption on the hard surfaces in the soil, and thus be caught by high connect power on those surfaces. The highest value for the releasing velocity coefficient was at a1 treatment, which achieved increment ratio of 38.09% due to the effect of the additive bacterial inoculation to increase the solubility of phosphate rock in the soil and that corresponded with Henri *et al* [24] who indicated that soil pH decreased due to the biochemical activity of microorganisms producing and secreting the organic acids that their carboxyl group chelating soil cations, specially Ca bonded with phosphate, converting them into soluble form.

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