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# Phosphorus release from apatite mineral using some organic amendments and their effect on some clay loam soil properties

Tantawy M. A.\*, Faragallah M. A. A., Awad M. Y., Abd El-Mageed A. M.

Soils and Water Department, Faculty of Agriculture, Al-Azhar University, 71524 Assiut, Egypt

#### Abstract

A pot incubation experiment was conducted to assess the impact of some organic amendments (citric acid, compost and *Bacillus megaterium*) on phosphorus releasing from two apatite levels (2 and 4 g/pot) added to clay loam soil as well as investigating its effect on some soil chemical properties at four incubation periods of total 12 months. The obtained results indicated that the soil electrical conductivity varied from 1.115 to 1.555 dS/m with the application of apatite levels 1 and 2, respectively. However, the highest reduction in the soil pH from 8.05 to 7.57 was found via the citric acid addition compared to the control. Furthermore, it is found that the integrated application of apatite level 2 with compost had the highest capability to increase phosphorus availability while the *B. megaterium* came second. So, the periods mean of level 2 was higher than of level 1 as well as the mean of treatments. Also, the 2<sup>nd</sup> period of the soil incubation showed the highest phosphorus (P) variation. It could be summarized that the apatite+compost followed by the apatite+phosphorus solubilizing bacteria (PSB) had the highest contribution in the P availability.

Keywords: phosphorus, apatite, citric acid, compost, Bacillus megaterium.



### 1. Introduction

Phosphorus (P) is the second essential macro nutrient for crops after nitrogen. It plays an important role in several biochemical processes of plants. The available quantity of phosphorus in most native soils is low, which is present in forms that are quite unavailable to plants (Esther, 2019; Mary et al., 2020). Therefore, organic the amendments represent an adequate approach to increase the P availability in soils (Sardans and Peñuelas, 2015; Sayara et al., 2020; Swoboda et al., 2022). The release of P from primary and secondary P minerals depends upon several factors such as mineral stability and solubility properties, particle size and soil pH (Shen et al., 2011). Rock phosphate (RP) is the important natural most Ρ source supporting the sustainable development of agriculture, but its agriculture application is limited due to its low solubility (Abbasi et al., 2013; Cevik et al., 2010; Cordell et *al.*, 2011; Schroder et al., 2011). However, Elisabeth al. (2020)et underlined the importance of hydroxy apatite (HA) and bone char (BC) as a P source in spite of its slow dissolution compared to vivianite (VI). The combined application of P and citric acid to Typic Quartzipsamment (Entisol) and Typic Hapludox (Oxisol) was reported by Sheila et al. (2017) to increase the dry mass yield and P concentration of corn plants. They also found that the corn growth promotion was observed in Typic Quartzipsamment at levels of 115 mg dm<sup>-</sup> <sup>3</sup> P and 0.7 mg dm-3 citric acid and in Typic Hapludox at levels of 299 mg dm<sup>-3</sup> P and 1.3 mg dm<sup>-3</sup> citric acid. Moreover,

Yang et al. (2019) revealed that the amount of total activating inorganic P (Pi) decreased as the pH of low molecular weight organic acids (LMWOAs) increased. LMWOAs-induced (Pi) activation might be attributed to combine acidity and complex effects. Moreover, the amount of total activating-Pi increased as the concentrations of oxalic and citric acids increase. Microorganisms and biofertilizers, in general, represent an important factor in clean agriculture, as they can provide many important and beneficial nutrients for plant growth through increasing the microorganisms' activities that leads to the decomposition of native and added organic matter (Jurys and Dalia, 2021). Phosphorus solubilizing microorganisms (PSMs) that mediate the bioavailable soil P, play a critical role in P cvcle bv mineralizing organic P. solubilizing inorganic P minerals, and storing large amounts of P in their biomass (Gross et al., 2020; Liang et al., 2020). Although the inoculation with PSMs is a common approach for increasing P availability and agricultural productivity, comprehensive a and complete understanding about the roles of PSM in P geochemical processes (i.e., dissolution-precipitation, sorptiondesorption, and weathering) has not received the merited attentions (Sharma et al., 2013). In addition, several mechanisms are involved to increase P availability including releasing phosphatase enzymes and organic acids, reducing soil pH, and chelation activities increasing with additional P adsorption sites (Motasim-Billah et al., 2019). PSMs can convert soil P or mineral P into soluble and plant available orthophosphate forms (mostly

 $PO_4^{3-}$ ,  $HPO_4^{2-}$  and  $H_2PO_4^{-}$ ) (Jiang *et al.*, 2018; Hao *et al.*, 2020). Therefore, this study aims to evaluate the effect of citric acid, compost and *Bacillus megaterium* as well as the incubation periods on releasing P from the apatite mineral.

#### 2. Materials and methods

An incubation pot experiment was conducted in the Agricultural Experimental Greenhouse of Soils and Water Department, Faculty of Agriculture, Al-Azhar University, Assiut, Egypt during 2019/2020 season to investigate the addition effects of some organic materials (citric acid and compost) and microorganisms (phosphorus solubilizing bacteria) on phosphorus release from the apatite mineral and some chemical properties of

a clay loam soil. The water content of the studied soils was kept at the field capacity to the end of experiment. Also, the pots were taken out and each of them emptied and mixed to obtain samples at the end of each period. The experiment was carried out an incubation pot experiment that was performed for 3, 6, 9 and 12 months. Some physical and chemical characteristics of the experimental soil and organic materials as well as apatite mineral are present in Tables (1) and (2).

#### 2.1 Soil sampling and preparation

Soil samples were taken from Experimental Farm of Al-Azhar University, Assiut, Egypt. The samples were air dried, crushed, sieved with a 2 mm stainless steel sieve and then kept for analysis.

Table (1): Some physical and chemical properties of the experimental soil.

	1
Property	Value
Sand	23.2 (%)
Silt	36.2 (%)
Clay	40.6 (%)
Texture	Clay loam
Calcium carbonate (CaCO <sub>3</sub> )	2.6 (%)
pH (1:2.5)	8.04
Electrical conductivity (EC) (1:2.5)	0.851 (dS/m)
Organic matter (OM)	2.45 (%)
Total nitrogen	0.14 (%)
Total phosphorus	0.06 (%)
Total potassium	0.08 (%)
Available nitrogen	50 (mg/kg)
Available phosphorus	8.72 (mg/kg)
Available potassium	105.51 (mg/kg)

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Chemical property	Apatite	Compost
pH (1:2.5)	7.67	8.05
Electrical conductivity (EC) (1:2.5)	4.09	3.6 (1:10)
Organic matter (OM)		60.50
Total N (mg/kg)		4480
Total P (mg/kg)	21.50%	4740
Total K (mg/kg)		19000

# 2.2 Apatite mineral

The apatite mineral as a natural rock  $(21.5\% P_2O_5)$ , was brought from Mankapad Super Phosphate Factory, Assiut governorate, Egypt. Two levels of fine apatite mineral (2 and 4 g) were used in this experiment the first one equal 2 g while the second equal 4 g.

# 2.3 Organic materials

### 2.3.1 Compost

The compost is a mixture of (70% animal manure + 30% sorghum sticks) was bought from the Egyptian Company for Solid Waste Recycling, El-Minia governorate, Egypt.

# 2.3.2 Citric acid

The citric acid was bought from El-Gomhoria Company, Assiut governorate, Egypt. It was prepared at concentration of 20 mM where 25 ml were used in the treatment.

# 2.4 Experiment design

The experiment was carried out for 12 months started from April 2019 to March 2020. This time was divided into 4 periods, each period was 3 months. The experiment was laid out in a split-plot design (using Statistix 8.1 programme) with three replications the experiment had 10 treatments (main plots) and 4 time periods (sub plots). The treatments included:

1. Control (without addition)

- 2. Recommended dose of chemical NPK /pot
- 3. Apatite only 2 g /plot (level 1)
- 4. Apatite 2 g + Citric 25 ml /pot
- 5. Apatite 2 g + Compost 3 g /pot
- 6. Apatite 2 g + *B. megaterium* 25 ml suspension /pot
- 7. Apatite only 4 g /plot (level 2)
- 8. Apatite 4 g + Citric 25 ml /pot
- 9. Apatite 4 g + Compost 3 g /pot
- 10. Apatite 4 g + B. Megaterium 25 ml suspension /pot

# 2.5 The incubation pot experiment

An incubation pot experiment consist of was conducted pots in the 30 Agricultural Experimental Greenhouse of Soils and Water Department, Faculty of Agriculture, Al-Azhar University, Assiut, Egypt during 2019/2020 season to investigate the addition effects of some organic materials (citric acid and compost) and microorganism В. megaterium on phosphorus release from the apatite mineral and some chemical properties of a clay loam soil. The pots were filled with soil by 3 kg/pot then treatments were added and mixed. The water content of the studied soils was kept at the field capacity to the end of experiment. Also, the pots were taken out and each of them emptied and mixed to obtain samples at the end of each period.

# 2.6 Soil analysis

Particle size distribution was determined using the pipette method (Jackson, 1973). Soil reaction (pH) was measured in (1:2.5)soil-water suspension using Beckman pH meter as reported by McLean (1982). Total soluble salts (EC) were determined in 1:2.5 of soil/water conductivity meter extract using according to Jackson (1973). Total calcium carbonate  $(CaCO_3)$ was estimated using Scheibler Calcimeter (Jackson, 1973). Total N, P and K of the soil as well as total P of apatite were determined according to method described by Page *et* al. (1982).Available nitrogen was extracted with 1% K<sub>2</sub>SO<sub>4</sub> at a ratio of 1:5. Then, 20 ml of the extract were distilled with the addition of 1g Devarda's alloy using a micro Kjeldah's distilling unit into a flask containing 10 ml boric acid-mixed indicator solution until about 50 ml distillate in each flask was collected. After the distillation, available nitrogen  $(NH_4^++NO_3^-)$  content was determined in distillate by titrating with the а standardized 0.01 N sulphuric acid (Jackson, 1973). Available Phosphorus was extracted with 0.5M NaHCO<sub>3</sub> at pH measured 8.5 was and then colorimetrically using stannous chloridephosphomolybdic-sulfuric acid system as describe by Jackson (1973). Available potassium was extracted by ammonium acetate method and measured by flame photometry (Jackson, 1973). Total nitrogen of compost was determined in the compost digest using the Kjeldahl apparatus as described by Jackson (1973). Total phosphorus of compost was determined in the compost digest (0.2g of  $compost + mixture of H_2SO_4 and HClO_4)$ using the Spectrophotometer by the molybdenum blue method as described by Jackson (1973). Total Potassium of compost was measured in the compost digest using the Flame photometer as described by Jackson (1973). The soil organic matter content was determined using the dichromate oxidation method that was described by Jackson (1973).

#### 3. Results and Discussion

#### 3.1 Electrical conductivity (EC)

Electrical conductivity (EC) is a decisive factor particularly in the soils of arid and semi-arid regions since most crops are sensitive to high salts concentrations of these soils (Libutti et al., 2018). Table (3) shows the soil electrical conductivity as affected by the application of apatite individually or combined with the investigated organic amendments. In general, most of the treatments had a positive effect on the soil EC values at all incubation periods. The change in the EC values varied according to the treatments type, its application rate and incubation period. The NPK recommended dose treatment exhibited the highest EC value (1.779 dS/m) overall treatments and it gradually increased with time which could be associated with the increasing of nutrients availability during all periods. The increases in the EC values as a result of the recommended dose addition were 108.07, 119.86, 116.93 and 112.39% for 1<sup>st</sup> 2<sup>nd</sup>, 3<sup>rd</sup> and 4<sup>th</sup> periods, respectively, compared to the control. These results are in an agreement with those obtained by Ali (2020) who found an increase in the EC from 1.13 to 1.34 dS/m as a result of addition of the recommended dose as chemical fertilizer. Also, Luiza *et al.*, (2022) found an increase in the soil EC of 82.1% above the control via the fertilization with N, P and K chemical recommended dose.

Amotito loval	Combination		Moon			
Apatite level		1	2	3	4	Mean
Control		0.855	0.876	0.892	0.912	0.0884
Recommended do	Recommended dose		1.926	1.935	1.937	1.894
2g	Apatite alone	0.882	0.955	1.168	1.195	1.050
	Apatite+Citric acid	0.889	0.962	1.171	1.200	1.056
	Apatite+Compost	0.929	1.289	1.341	1.365	1.231
	Apatite+B. megaterium	0.919	1.173	1.194	1.207	1.123
Mean level		0.905	1.095	1.219	1.242	1.115
	Apatite alone	1.295	1.385	1.629	1.652	1.490
10	Apatite+Citric acid	1.309	1.396	1.634	1.659	1.500
4g	Apatite+Compost	1.348	1.726	1.787	1.815	1.669
	Apatite+B. megaterium	1.320	1.630	1.642	1.651	1.561
Mean level		1.318	1.534	1.673	1.694	1.555
Periods mean		1.111	1.315	1.446	1.468	1.335
LSD of T (0.17), P (0.07) and P×T (0.26)						

Table (3): The effect of apatite individually or combined with the organic amendments on the electrical conductivity (EC) (dS/m) of the clay loam soil.

T= treatments, P= periods,  $P \times T$ = periods × treatments.

Regarding to the apatite treatment effect, the observed results indicated that the application of both apatite levels raised the soil EC values. The electrical conductivity influenced by the lowest apatite level increased by 3.16, 9.02, 30.94 and 31.03% for 1st 2nd, 3rd and 4th periods, respectively, compared to the control, The corresponding EC increases resulting from apatite level 2 were 51.46, 58.11, 82.62 and 81.14%, respectively. In this regard, the highest EC value (1.652 dS/m) was recorded with the addition of the highest apatite level after in the 4<sup>th</sup> incubation period. This could be due to the degradation which leads to the releasing of some soluble salts with time. These are also in the same line with those obtained by Amira et al. (2019). They indicated that the EC values were influenced by increasing the added rate of rock phosphate. On the average basis of treatment values, the lowest EC values were observed with the addition of apatite alone followed by the citric acid combined with apatite treatments that they were increased by 18.78 and 19.46 % for level 1 and 68.55 and 69.68% for level 2, respectively. This illustrates the relation between the RP amount and soil conductivity. In a same regard, the citric acid had a slightly increasing effect on soil EC at all time periods as a result of enhancing nutrient the availability (Shreen *et al.*, 2017). Also. the integration between compost and apatite showed highest significant increases in the EC, especially at 4 g apatite level which could be attributed to the EC value of compost (3.06 dS/m). Bakr (2016) 43 found a slightly increase in the soil EC due to the application of FMC from 5.10 to 6.53 dS/m. In the same line, El-Tayeh et al. (2019) showed that the addition of farmyard manure (FMC) at levels of 10, 30 and 50% resulted in soil EC values of 0.55, 1.64 and 2.73 dS/m, respectively. Moreover, a highly significant positive impact on the soil EC was recorded with applying apatite at 4g/pot combined with B. megaterium at all incubation periods (Table 3). This effect could be due to more P and soluble salt release from the apatite mineral as a result of the bacterial activity (Singh et al., 2022). Furthermore, among all apatite-citric acid combinations a higher increase in the soil EC was obtained at the 2<sup>nd</sup> period compared to that of the 1<sup>st</sup> period, while the augmentation effect was almost steady at the 3<sup>rd</sup> and 4<sup>th</sup> periods (Figure 1). This strongly reflects the negative effects of mineral amount and the incubation period on the soil EC via more release of salts. Yang et al. (2021) mentioned an increase in P release from apatite through the addition of citric acid. Moreover, a gradual increase in the soil EC was observed with time for using the apatite alone either level at 1 or level 2 compared to the control. However, the EC increase was more pronounced in the case of the second level of apatite. The minimum impact on the soil EC was observed with using the citric acid+apatite at level 1 or 2 at all-time periods in comparison with the control or even other treatments. It may be due to the lack of citric efficiency in the decomposition of apatite.



Figure (1): The relative increases in the soil EC (dS/m) induced by the addition of apatite mineral alone or combined with some organic amendments to the clay loam soil.

In addition, the above-mentioned results show that unfavorable effects were obtained with both of compost and B. megaterium combined with apatite at level 2 (4 g/pot) on increasing the soil EC while this impact was less pronounced with apatite at level 1(2g/pot).

### 3.2 Soil reaction (pH)

The effect of apatite, with or without citric, compost and microorganism in comparison to the NPK recommended dose and the control on the soil reaction (pH) of the clay loam soil is present in Table (4). Generally, the results indicated citric that acid had the highest contribution on the soil pH reduction at the 1<sup>st</sup> time period. Meanwhile, the highest soil pH value was obtained with the chemical NPK recommended dose treatment at all time periods which might be due to its high solubility rate; this effect was clearly illustrated in the EC values. Labib et al. (2012) mentioned the same effect on soil reaction by using triple super phosphate application as P source (46%  $P_2O_5$ ). On the other hand, Ali (2020) found that the application of the recommended dose of NPK leads to slight decrease in the soil pH compared to the control. In general, the apatite application levels resulted in a positive effect on the soil pH; however, the effect was more pronounced at the 2<sup>nd</sup> level (4 g/pot). Moreover, a gradual excess was pronounced in the soil pН with increasing the time period via the addition of apatite mineral at both levels as a reflection of its decomposition increase with time. These results are in a harmony with those obtained by Taalab et al. (2019) who found an augmentation impact on the soil pH from 7.5 to 7.9 due to the application of rock phosphate to some soils of El-Nubaria, Egypt. Based on the treatments means, the lowest pH values 7.57 and 8.28 were obtained from applying the citric acid of with apatite at levels 1 and 2, respectively. Also, based on period means, a sharp soil pH decline was recorded at the 1st period and then it tended to rise gradually for the rest of the incubation periods which it could be due to the soil buffering capacity (Na, 2020; Yang et al., 2021). Furthermore, the highest pH values were found at all time periods for applying apatite at levels 1 and 2 combined with the compost which could be attributed to the alkalinity nature of compost (8.05). These results agree with those of Navarro et al. (2020) who displayed an excess in the soil pH via the application of compost from 7.82 to 7.99. In most cases, a significant increase in the soil pH was observed with adding B. megaterium with apatite at both levels to the soil compared to the control meanwhile a remarkable variation was recorded at the 2<sup>nd</sup> period because of the amplification of bacterial population with more apatite degradation which leads to raising pH. From the abovementioned results, the soil pH as affected by apatite either added alone or combined with organic amendments could be ranked in increasing order of apatite level 1 + citric > apatite level 1alone > apatite level 1 + B. *megaterium* > apatite level 2 + citric > apatite level 2 alone > apatite level 1 + compost  $\approx$  apatite level 2 + *B. megaterium* > apatite level 2 + compost.

A	Combination	Period				Maar
Apatite level		1	2	3	4	Mean
Control		8.04	8.04	8.05	8.05	8.05
Recommended dos	e	8.70	8.72	8.73	8.73	8.72
2 g/pot	Apatite alone	8.11	8.20	8.24	8.31	8.22
	Apatite+Citric acid	7.44	7.49	7.64	7.71	7.57
	Apatite+Compost	8.27	8.44	8.51	8.53	8.44
	Apatite+B. megaterium	8.15	8.27	8.29	8.32	8.26
Mean		7.99	8.10	8.17	8.22	8.12
4 g/pot	Apatite alone	8.22	8.34	8.39	8.44	8.32
	Apatite+Citric acid	7.52	7.56	7.69	7.75	8.28
	Apatite+Compost	8.35	8.53	8.63	8.66	8.55
	Apatite+B. megaterium	8.29	8.39	8.43	8.44	8.44
Mean		8.10	8.21	8.29	8.32	8.40
Periods mean		8.04	8.15	8.23	8.27	8.26
$I SD of T (0.11) P (0.08) and P \times T (0.29)$						

Table (4): The effect of apatite alone or combined with citric acid, compost and phosphate solubilizing bacteria on the soil reaction (pH) of the clay loam soil.

T= treatments, P= periods,  $P \times T$ = periods × treatments.

Also, all treatments of apatite at level 2 (4 g/pot) showed a higher increase in the soil pH compared to that at level 1 (2g/pot) as a direct contribution of the rock amount. Most of the previous results are in agreement with those of Ali (2020) who demonstrated that the inoculation of apatite with bio-fertilizer leads to a slight increase in the pH from 8.15 to 8.23.

#### 3.3 Soil available P

Generally, a positive impact on P availability was obtained from all investigated treatments and the incubation periods (Table 5). Meanwhile, the highest available P values were obtained with the NPK recommended dose treatment due to its high solubility rate. These findings are in a harmony with those obtained by Ahmed et al. (2021) who found an increase in the soil available P from 9.81 to 30.14 mg kg<sup>-1</sup> as a result of NPK chemical fertilizer application. Also, a significant available P increase was pronounced between the 1<sup>st</sup> and 2<sup>nd</sup> periods while it was nonsignificant between the 3<sup>rd</sup> and 4<sup>th</sup> periods. Moreover, a significant increase in the available P was observed with applying apatite alone at the 3<sup>rd</sup> and 4<sup>th</sup> incubation periods compared to the control. Similar results were revealed by Taalab et al. (2019) who found an augmentation effect on the available that increased from 16.0 to 18.6 mg/kg via the addition of rock phosphate to some clay soils at El-Kanater area, Egypt. Concerning the citric acid addition combined with the apatite, a slight 46

increase in the available P was recorded compared to applying the apatite alone.

A (* 1 1	Combination		м			
Apatite level		1	2	3	4	Mean
Control		8.75	8.79	8.85	8.94	8.83
Recommended dose		18.82	19.46	19.64	19.75	19.42
2 g/pot	9.45	9.77	11.52	11.95	10.67	8.22
	9.63	9.81	11.76	12.04	10.81	7.57
	11.62	12.32	13.92	14.18	13.01	8.44
	11.51	12.45	12.59	12.62	12.29	8.26
Mean		10.55	11.09	12.45	12.70	11.70
4 g/pot	11.85	11.96	12.82	13.09	12.43	8.32
	12.07	12.19	13.11	13.16	12.63	8.28
	14.08	14.57	15.28	15.44	14.84	8.55
	13.95	14.71	14.77	14.81	14.56	8.44
Mean		12.99	13.36	14.00	14.13	13.62
Periods mean		11.77	12.22	13.22	13.41	12.66
LSD of T (1.09), P (0.63) and P×T (2.41)						

Table (5): The addition effect of apatite alone or combined with citric acid, compost and phosphate solubilizing bacteria on the available phosphorus (mg/kg) of the clay loam soil.

T= treatments, P= periods,  $P \times T$ = periods × treatments.

However, this increase in the available P was highly significant compared to the control. It could be due to the preservation of phosphorous from the fixation on the surfaces of clay minerals through the chelation process; apatite degradation is also contributed, but to a lesser extent. These results are in an agreement with those demonstrated by Basak (2019) who ranked some organic acids (according to its capability on apatite decomposition) in a descending order of oxalic acid > citric acid > tartaric acid > formic acid > malic acid > succinic acid > acetic acid. Meanwhile, the apatite+compost treatment showed a significant increase in the available P at all incubation periods which it may be attributed to the pH reduction via the released organic acids as well as its initial content of microorganisms (Figure 2). Similarly, Akhtar *et al.* (2019) mentioned that the combination between inorganic P fertilizer and farmyard manure could represent an adequate approach to improve the P availability. In addition, B. megaterium have high strength to release phosphorus from apatite. Also, an interesting observation was noticed that their effect on P release was less than the compost; this could be because of insufficient organic matter. The same trend was found by Taalab et al. (2019) who stated that an increasing effect of available P from 16.0 to 51.9 mg/kg was recorded through the integrated addition of RP and phosphorus solubilizing bacteria after 70 days of Regarding the level 2 incubation. (4g/pot) of apatite addition, it was found that the apatite at level 2 combined with the compost had the highest capability to

increase phosphorus availability while the *B. megaterium* came the second. So, the available P of the periods mean of level 2 was higher than that of level 1 and that of the mean of treatments. Meanwhile, the maximum available P increase rate in this study was recorded at the  $2^{nd}$  period of the soil incubation.



Figure (2): The relative increases in the available P induced by the addition of apatite alone or combined with some organic amendments to the clay loam soil.

#### 5. Conclusion

From the above-mentioned results, it could be concluded that the citric acid and compost combined with rock phosphate resulted in increases in the available P. However, the  $2^{nd}$  incubation period revealed the highest significant impact on the phosphorus releasing.

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