Eurasian Journal of Soil Science 2022, 11(3), 198 - 205

Eurasian Journal of Soil Science

Journal homepage : http://ejss.fesss.org

Effect of *Bacillus megaterium* var. *phosphaticum* applied together with rock phosphate on wheat yield and some soil properties in a calcareous soil

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Article Info

Received : 19.06.2021
Accepted : 11.01.2022
Available online : 14.01.2022

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Abstract

This study aims to determine the effect of *Bacillus megaterium var. phosphaticum* applied together with rock phosphate on the yield of wheat grown in calcareous soil, some biological properties of soils and phosphorus fractions in the soil under greenhouse conditions. Considering the P fixation capacity of the soil used in the experiments and the amount of P present in the soil, the trial subjects were created based on randomized block designs with 3 replications, depending on whether 0, 25, 50, 75 and 100% of the P required to be given to the wheat plant was met from rock phosphate and whether it was bacterial or not, and finally wheat was grown. In the harvested plants, grain and stem weights were determined, grain and stem P contents were analysed and the amounts removed with grain and stem were calculated. Dehydrogenase (DHA) and phosphatase (PA) enzyme activities were performed in the soil samples taken after harvest. Soluble and loosely bound-P, Calcium-bound-P (Ca-P), Reductant soluble-P (RS-P) fractions and Olsen-P were determined in soil samples taken before planting and after harvest. The percent reduction in the fractions was calculated by using the pre-sowing and post-harvest values of these samples. According to the results, *Bacillus megaterium* DSM 3228 strain inoculated with rock phosphate increased grain and stem yield, grain and stem P content, and P amount removed by grain and stem of wheat. These parameters were found to be higher at high doses of P applied as rock phosphate. Inoculation increased the DHA and PA values of the soils. A decrease in P fraction forms with low solubility was determined by inoculation, some of this phosphorus was removed by plants and some of it was retained in the soil in different forms.

Keywords: *Bacillus mageterium* var. *phosphaticum*, enzyme activity, inoculation, inorganic P fraction, wheat.

Introduction

Phosphorus (light-bearing) is the second major macronutrient essential for plant growth and development and plays a role in basic biological functions such as cell division, synthesis of nucleic acids, photosynthesis and respiration, as well as energy transfer, fat, sugar and starch formation. Naturally, the amount of phosphorus in various soils generally varies between 0.01-0.15%, but not all of this is in a form suitable for plant use. Phosphorus requirement in vegetative organs for normal plant development varies between 0.3-0.5% in dry matter content, and in the case that the phosphorus content of plants is usually 0.1% or less, the plant suffers from phosphorus deficiency.

All over the world, phosphorus chemical fertilizers are used in traditional agriculture to eliminate phosphorus deficiency and to obtain the highest yield in the product. Due to the difference in the amount,

: https://doi.org/10.18393/ejss.1057950
: http://ejss.fesss.org/10.18393/ejss.1057950
Publisher : Federation of Eurasian Soil Science Societies
e-ISSN : 2147-4249

type and application times of the applied fertilizers and the lack of knowledge of the practitioners in this field, the health of living things and the environment can be adversely affected by phosphorus fertilizer applications. Phosphates were put in the same class as nitrate as an important pollutant, particularly in the last quarter of the 20th century. Regardless of its source, most of the dissolved phosphates are retained in the soil, and the part that can reach the sea due to the reasons such as erosion is deposited and imprisoned for millions of years (Correll, 1998; Daniel et al., 1998). In addition, the use of phosphorus fertilizers causes some heavy metals such as Cd, Cr, Pb and Ni in the structure of fertilizers to permeate into the soil and plant structure, and may have negative effects on soil and environmental health (Huang and Jin, 2008; Atafar, 2010). Due to the high costs arising from raw materials and intermediate inputs in fertilizer production in our country in recent years, fertilizer production has decreased and its import has increased. Further research is needed to ensure less use of these fertilizers and increase their effectiveness in order to reduce the import of raw materials, intermediate inputs and fertilizers in fertilizer production and to minimize the negative effects of chemical fertilizers on the environmental health.

Due to the negative effects and costs of chemical fertilizers on living things and the environment, the need for the use of more natural resources such as rock phosphate, which can be an alternative to these fertilizers, has arisen. However, the limited solubility of rock phosphate and the low rate of release limit the use of this material in agriculture. There are various factors affecting the utilization of raw phosphates by cultivated plants. In particular, soil reaction is the most important soil feature in the solubility of rock phosphate (Kanabo and Gilkes, 1987; Bolan and Hedley, 1990). Studies have shown that there has been an increase in crop yield with the application of raw rock phosphate in acid-reaction soils (Chien and Menon, 1995). On the other hand, it was determined that the raw rock phosphate application on calcareous alkaline reaction soils did not have a significant effect on the phosphorus nutrition of the plant (Çağatay et al., 1973).

It is known that microorganisms dissolve insoluble phosphate by producing organic acids (malic acid, acetic acid, indoleacetic acid) and by chelating oxoacids from sugar (Dawwam et al., 2013; Khan et al., 2016; Behera et al., 2017; Pande et al., 2017) and many microorganisms with the ability to dissolve phosphorus have been identified by various researchers (Chunga et al., 2005; Fernández et al., 2007; Iyer et al. 2017). It has been demonstrated by studies that inoculation of seeds or soil with phosphate-solving bacteria (PSB) increases the solubility of fixed soil phosphorus and phosphates applied as fertilizers, resulting in higher crop yields (Batool and Iqbal, 2019). In addition, it has been pointed out that the use of rock phosphate as phosphorus fertilizer and its solubility with microorganisms can be an alternative to costly chemical fertilizers (Kaur and Reddy, 2015).

The current study aims to determine the effect of *Bacillus mageterium var. phosphaticum* applied together with rock phosphate on the yield of wheat grown in calcareous soil, some biological properties of soil and phosphorus fractions in the soil under greenhouse conditions.

Material and Methods

Material

In this study, bacteria that dissolve phosphorus as material, rock phosphate as phosphorus source and wheat as plant were used. The microorganism *Bacillus megaterium var. phosphaticum* is isolate of DSM 3228, obtained from DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Mascheroder Weg 1b 38124 Braunschweig, Germany). Rock phosphate was obtained from domestic sources in Turkey and rock phosphates from Mardin Mazı Mountain were used for this purpose. Altındane variety of wheat (Triticum aestium) was used as the test plant in the experiments. A soil with high lime content and low phosphorus content was used in the experiments.

Soil used in the trials was determined as silty loam (Sand: 38.28%; Clay: 11.5%; Silt: 50.21%) and bulk density was 1.25 gr.cm^{-3}, field capacity was 32.21% and wilting point was 16.21%. In addition, its pH (1:1, Soil : Water suspension) was 7.80; Electrical conductivity was (1:1, Soil : Water suspension) 0.502 dSm^{-1}; Lime content was determined as 42.6%, organic Matter was 0.90% and available P content was 3.04 mg kg^{-1}. The pH value of the rock phosphate used as a phosphorus source was 8.22, the total phosphorus content was 27%, the water-soluble phosphorus was 0.08%, and the water + citrate-soluble phosphorus values were 0.081%.

The trial subjects were created based on randomized block designs with 3 replications, depending on whether 0, 25, 50, 75 and 100% of the P required to be given to the wheat plant was met from rock phosphate and whether it was bacterial or not considering the P fixation capacity of the soil used in the experiments and the amount of P present in the soil.

For the application of bacteria in the experiment, lyophilized cultures of the mentioned bacteria were activated under completely aseptic conditions, and liquid culture of bacteria was formed in Nutrient agar (peptone 5 g, meat extract 3 g, 10 mg $MnSO_4.H_2O$ L^{-1}, pH=7). The greenhouse experiment was established and carried out in the light, temperature etc controlled research greenhouse in the Black Sea Agricultural Research Institute (at 25^0C, 350 ppm CO_2, 8 hours dark and 16 hours light conditions). In the greenhouse experiment, different doses of rock phosphate placed in pots of 5 kg soil (<4 mm) over the dry weight and bacterial applications were applied to each pot separately according to the trial subjects. Bacteria inoculation level was applied to the soil at 5 ml (10^8 CFU. mL^{-1}) per seed. Wheat seeds were sown by hand, 15 in each pot. After the first emergence of the seeds from the soil, thinning was made to have 10 plants in each pot. Nitrogen and potassium required for plants without any phosphorus fertilization during the experiment were added in liquid form according to the results of soil analysis. The moisture content of the soil was kept at the field capacity level; for this purpose, the water lost from the soil by various ways was completed with pure water by taking the weights every day.

Analysis Methods

Grain and stem weights were determined by harvesting the plants that reached grain maturity, P contents of grain and stem were analysed, and the amounts removed with grain and stem were calculated. Dehydrogenase and phosphatase enzyme activities were performed in the soil samples taken after the harvest. Soluble and loosely bound-P, Calcium bound P (Ca-P), Reductant soluble-P(RS-P) fractions and Olsen-P were determined in the soil samples taken before planting and after harvest (Table 1). The % reduction in fractions was calculated with the following formula using the pre-planting and post-harvest values of these samples:

$$\Delta Pi = (C_{\text{preplanting}} - C_{\text{postharvest}}) \times 100 / C_{\text{preplanting}}$$

Table 1. Analyses applied to soil samples taken at the end of the trials

Plant Analyses	Method
Grain weight	Gravimetrically (Jones, 2001)
Stem weight	Gravimetrically (Jones, 2001)
Grain and Stem P Amount	The total P amount in dry-burned soil samples was determined by the vanadomolybdophosphoric yellow photometric method (Jones, 2001)
Soil Biological Analyses	
Dehydrogenase activity	Spectrophotometric determination of the colour formed as a result of the conversion of TTC entering the cell into TPF with the effect of the dehydrogenase enzyme in the cell (Pepper et al., 1995)
Phosphatase enzyme activity	Decomposition of disodium *p*-nitrophenylphosphate with the effect of phosphatase enzymes and spectrophotometric determination of the released *p*-nitrophenol (Tabatabai and Bremner,1969)
Inorganic Phosphorus fractions	
Soluble and loosely bound P	Extraction with 1M NH_4Cl solution (Self-Davis et al., 2009)
Calcium-bound P (Ca-P)	Extraction with 0.25 M H_2SO_4 solution (Self-Davis et al., 2009)
Reductant soluble P(RS-P)	Extraction Na-dithionite- Na-citrate solution (Self-Davis et al., 2009)
Olsen P	0.5 N $NaHCO_3$ extraction (Olsen et.al., 1954)

Inorganic phosphorus fractions were done according to the method proposed by Kuo (1996). The procedure used for calcareous soils has been described in detail by Self-Davis et al. (2009). In the first step, soluble and weak Al and Fe bound phosphates are separated or labile phosphates (NaOH/NaCl) are extracted. In the second step, it is extracted in soluble reductant (occluded phosphorus bound to Fe and Al oxides and pedogenic Ca-phosphates with reduced availability)/(Na citrate-bicarbonate-dithionite-extracted-CDB). In the third step, the fraction of phosphates bound to calcium of primary minerals -apatite group, hardly soluble phosphates (HCl extracted) are extracted.

Evaluation of Obtained Results and Statistical Analysis

The trials were analysed in the ANOVA statistical program according to the split plot trial design in the randomized plot experimental design, and the differences were classified according to Duncan and LDS (0,05).

Results and Discussion

Effect of treatments on grain and stem yield and removed P amount

Table 2 shows the changes in grain yield, P content and P amount removed with grain of wheat by *Bacillus megaterium* DSM 3228 strain inoculated with rock phosphate. Both the application of rock phosphate and inoculation affected the grain yield of the wheat statistically. The highest yield was obtained from the application of 75% of the P required to be given to the wheat in the subjects with and without inoculation. Microorganism inoculation was the subject that increased the grain yield more than the subject without inoculation. Rock phosphate application affected the grain P content of wheat statistically and the highest grain P value was obtained from 100% application of P required to be given to the wheat plant. However the inoculation application, did not cause a statistical difference in the grain P values of the plant. Both the rock phosphate application and inoculation caused a statistical difference in the P content removed by the plant grain. The highest P value removed with the grain was obtained from the 75% dose application of the P required to be given to the wheat. The P value removed by grain was found to be higher in the inoculated subjects than in the non-inoculated subjects.

Table 2. Grain yield, grain P content and removed P amount by grain of wheat

	Grain yield (gr pot $^{-1}$)			Grain P (%)			P removed by grain (mg pot $^{-1}$)		
	-DSM 3228	+DSM 3228	Mean	-DSM 3228	+DSM 3228	Mean	-DSM 3228	+DSM 3228	Mean
0	3.088	4.050	3.733 B	0.129	0.129	0.129 C	3.971	5.212	4.592 C
25	3.087	4.683	3.887 B	0.193	0.175	0.185 B	5.967	8.193	7.080 B
50	3.487	4.360	3.923 B	0.195	0.195	0.197 AB	6.801	8.482	7.642 B
75	4.313	5.230	4.771 A	0.189	0.202	0.195 B	8.163	10.51	9.337 A
100	3.330	4.151	3.740 B	0.206	0.209	0.208 A	6.851	8.690	7.770 B
Mean	3.461 B	4.495 A		0.182	0182		6.351 B	8.218 A	
		$LSD_{0.05}$			$LSD_{0.05}$			$LSD_{0.05}$	
Inoculation (I)		0.284*						0.650*	
P doses (P)		0.378*			0.0126*			0.879*	
I*P									

Changes caused by *Bacillus megaterium* DSM 3228 strain inoculated with rock phosphate in stem yield, stem P content and the amount of P removed by stem of wheat are given in Table 3. Both rock phosphate application and inoculation affected the stem yield of wheat statistically. The highest yield was obtained from the application of 100% of the P required to be given to the wheat in the subjects with and without inoculation. Microorganism inoculation was the subject that increased the stem yield more than the non-inoculated subject. Rock phosphate application did not affect the stem P content of wheat statistically, whereas inoculation resulted in a statistically significant difference. Inoculation was the application that increased the stem P content of the plant more. Both the rock phosphate application and inoculation caused a statistical difference in the P content removed by the plant stem. The highest P value removed by the stem was obtained from the 100% application of the P required to be given to the wheat. The P value removed by the stem was found to be higher in the inoculated subjects than in the non-inoculated subjects. Many studies have shown that the application of phosphorus-solving microorganisms alone or in combination with any source of phosphorus provides increases in the development, growth parameters and P removal of various plants (Mamta et al., 2010; Singh and Reddy, 2011; Gupta et al., 2012; Hussain et al., 2019).

Table 3. Stem yield, Stem P content and removed P amount by stem of wheat

	Stem Yield, gr pot^{-1}			Stem P, %			P removed by stem, mg pot^{-1}		
	-DSM 3228	+DSM 3228	Mean	-DSM 3228	+DSM 3228	Mean	-DSM 3228	+DSM 3228	Mean
0	2.843	3.658	3.250 C	0.022	0.036	0.0267	0.633	1.300	0.967 B
25	3.127	3.975	3.550 BC	0.025	0.026	0.0267	0.796	1.018	0.908 B
50	2.920	4.044	3.483 BC	0.025	0.025	0.0267	0.733	1.004	0.870 B
75	3.680	4.138	3.908 B	0.023	0.027	0.0250	0.835	1.137	0.987 B
100	4.293	4.526	4.410 A	0.026	0.029	0.0283	1.103	1.307	1.207 A
Mean	3.373 B	4.068 A		0.024 B	0.028 A		0.820 B	1.153 A	
		$LSD_{0.05}$			$LSD_{0.05}$			$LSD_{0.05}$	
Inoculation (I)		0.379*			0.0033*			0.121*	
P doses(P)		0.463*						0.156*	
I*P									

Table 4 shows the change caused by the *Bacillus megaterium* DSM 3228 strain inoculated with rock phosphate in the DHA and PA of the soil samples taken after harvest. Inoculation affected DHA statistically, and rock phosphate doses were found to be statistically ineffective. The subjects with inoculation were the application that increased the DHA activity of the soils more than the subjects that were not inoculated. Both the application of rock phosphate and inoculation affected the PA of the soils taken after the harvest of the wheat statistically. Phosphatase activity was found to be higher in inoculated subjects compared to non-inoculated subjects. The highest phosphatase activity was obtained from 75% application of P required to be given to the wheat plant. This was followed by 25% and 50% applications, and the lowest PA value was obtained from the subject in which no fertilizer was applied.

Dehydrogenase activity is not independent of the host microbial cell; therefore it is considered a good indicator of total microbial activity (Masciandaro et al., 2000; Kızılkaya, 2008). Phosphatase activity is an extracellular enzyme, that is, it is synthesized by microorganisms and accumulates in the soil, where it is synthesized. This enzyme is effective in organic and inorganic phosphorus availability in the soil and is widely used in the evaluation of the biological activity of soils (Amador et al., 1997; Kızılkaya and Hepşen, 2004). It has been demonstrated by previous studies that the inoculation of microorganisms into the soil or seed that promote plant growth, alone or together with any P source, causes changes in the biological properties of the soil (Kızılkaya and Bayraklı, 2005; Singh and Reddy, 2011; Kaur and Reddy, 2014; Chaya and Bijoy, 2015).

Table 4. Dehydrogenase activity (DHA) and Phosphatase activity (PA) activity of soil samples taken after harvest

	Dehydrogenase activity (DHA) μg TPF g^{-1} soil 24h 25^0C			Phosphatase activity (PA) μg p-nitrophenol g^{-1} soil		
	-DSM 3228	+DSM 3228	Mean	-DSM 3228	+DSM 3228	Mean
0	2.551	2.952	2.751	111.9	120.4	116.1 C
25	2.750	3.499	3.123	131.8	123.2	127.5 AB
50	2.702	2.864	2.783	123.5	130.9	127.2 AB
75	3.204	2.990	3.095	124.9	144.6	134.7 A
100	2.321	2.757	2.541	112.9	138.6	125.7 B
Mean	2.706	3.012		121.0	131.5	
	$LSD_{0.05}$			$LSD_{0.05}$		
Inoculation (I)		0.268*			7.509*	
Phosphor doses(P)					8.204*	
I*P						

In this study, P fractions the sequential analysis of inorganic phosphorus proposed by Kuo (1996) were modified for application to calcareous soils (Self-Davis et al., 2009). In this approach, fractionation procedures are based on the different solubility of various inorganic P forms in various extracts. With NaOH/NaCl extraction, some of both Al-P and Fe-P as well as the soluble loosely bound P were extracted. Reductant soluble P retained in the matrices of aggregates/minerals was extracted by CDB (Na citrate-bicarbonate-dithionite) extraction, and finally, poorly soluble phosphates and especially the Ca-P fraction were extracted using HCl (Milić, 2019).

P added to the soils as rock phosphate increased the inorganic phosphorus fractions of the soils compared to the control (Table 5). Studies have shown that phosphorus added to soils in different forms increases the amount of phosphorus in the soils (Wang et al., 2010; Audette et al., 2016; Mahmoud et al., 2018). The highest phosphorus amounts were determined as the Ca-P fraction both in the pre-planting and post-harvest periods. The order of the P fractions in the soil were as Ca-P > reductant-P > Soluble and loosely bound-P. In the studies, it has stated that the dominant form in calcareous soils is Ca-P (Solis and Torrent, 1989; Shen et al., 2004; Kızılkaya et al., 2007). P fractions in the pre-planting soil samples were higher than the amount of P fractions in the post-harvest soil samples (except reductant-P) in the inoculated and non-inoculated treatments. The P values in the post-harvest soil samples may have been lower due to some chemical reactions and/or plant uptake in the soil. The relationships between plant P uptake and inorganic P forms in calcareous soils are not clearly defined. Plant P uptake is most closely associated with Ca-P (Kamprath and Watson, 1980), resin extractable P (Yang et al., 1990), and citrate-bicarbonate P (RS-P) (Solis and Torrent, 1989). Samadi (2006) reported significant positive relationships between Ca_2-P, Al-P, Fe-P and Ca10-P fractions and plant P uptake. Compared with the pre-planting P fractions, the Ca-P fraction had lower values in the inoculated subjects than being in the non-inoculated. When the reduction rates in the fraction are examined, it is seen that the percentage of decrease in the inoculated subjects is higher. On the other hand,

there was an increase in reductant-P values when compared to the pre-planting P fractions, and this increase was higher in the inoculated subjects. The ability of plants to utilize acid-soluble P (Ca-P) fractions has been attributed to acidification of the rhizosphere (Grinsted et al., 1982; Ahmad et al., 2018). Phosphorus-solving microorganisms can secrete various acids and provide the dissolution of Ca-P by lowering the pH of the soil and rhizosphere region. *Bacillus mageterium var. phosphaticum* is a very important phosphorus dissolving bacterium, especially capable of dissolving calcium phosphate. It is known that *Bacillus* strains produce lactic, isovaleric, isobutyric and acetic acid mixtures and these play an important role in phosphorus solubility. Other organic acids as glycolic, oxalic, maleic, and succinic acids have also been identified as phosphate solvents and are produced by bacteria that dissolve phosphorus (Banik and Dey 1982, Illmer and Schinner, 1992). Bacteria secrete these organic acids and solubilize insoluble inorganic phosphate in the form of tricalcium, dicalcium, rock phosphate and hydroxy apatite (Goldstein 1986, 1995; Güneş et al., 2013). According to Oberson et al. (2001), microorganisms constitute highly important dynamic reserves of potentially useful nutrients for plants. Microorganisms play an important role in the conversion of organic phosphorus in the soil by secreting phosphatase enzyme and they also allow moderately unstable forms from phosphate compounds to pass into solution. Gong et.al (2014) reported that *Penicillium oxalicum* I1 (PI1) isolate, a fungus with high phosphorus dissolving ability, converted a wide variety of insoluble phosphates such as $Ca_8H_2(PO_4)_6.5H_2O$, $AlPO_4$, $FePO_4$ and $Ca_{10}(PO_4)_6(OH)_2$ in soil into soluble $CaHPO_4$ and this isolate prevented the conversion of $CaHPO_4$ into insoluble form.

Table 5. P fractions of soils taken before planting and after harvest, % decrease in Olsen-P values

			Post-harvest	Reduction in Fraction (%)	
	Pre-planting	+DSM 3228	- DSM 3228	+DSM 3228	- DSM 3228
			Soluble and loosely bound P (mg kg^{-1})		
0	2.595	2.539	2.031	2	22
25	2.595	2.539	2.116	2	18
50	2.883	1.354	1.947	53	32
75	3.325	1.524	2.285	54	31
100	3.748	1.778	2.539	53	32
			Ca-P (mg kg^{-1})		
0	186	156	159	16	15
25	193	170	171	12	11
50	199	180	185	10	7
75	225	196	200	13	11
100	235	210	215	11	9
			Reductant-P (mg kg^{-1})		
0	9.625	10.70	9.513	-11	1
25	12.70	13.38	11.59	-5	9
50	6.930	9.513	7.135	-37	-3
75	8.855	8.918	10.11	-1	-14
100	8.085	17.540	16.35	-117	-102
			Olsen-P (mg kg^{-1})		
0	3.104	2.324	2.462	25	21
25	3.235	2.324	2.321	28	28
50	3.133	2.380	2.480	24	21
75	4.040	3.201	3.447	21	15
100	3.635	2.954	3.242	19	11

Conclusion

In this study, *Bacillus megaterium* DSM 3228 strain inoculated with rock phosphate increased grain and stem yield, grain and stem P content, and P amount removed by grain and stem of wheat. These parameters were found to be higher at higher doses of P applied as rock phosphate. Inoculation increased the DHA and PA values of the soils. A decrease was determined in P fraction forms with low solubility by inoculation, some of this phosphorus was removed by plants and some remained in the soil as other forms. It is considered that inoculated strain and some acids secreted by the plant roots are effective in this conversion. In calcareous soils, the solubility of more natural resources such as rock phosphate can be achieved by applying the *Bacillus megaterium* DSM 3228 strain. Conducting these trials with different phosphorus-solving bacteria and for many years will provide us more information about the behaviour of P fractions in the soil.

Acknowledgment

This study was supported by TAGEM (General Directorate of Agricultural Research and Policies). Project number is TAGEM–BS-05/04-02/02-13.

References

Ahmad, M,, Ahmad, M,, El-Naggar, A.H., Usman, A.R.A., Abduljabbar, A., Vithanage, M, Elfaki, J., Al-Faraj, A., Al-Wabel, M.I., 2018. Aging effects of organic and inorganic fertilizers on phosphorus fractionation in a calcareous sandy loam soil. *Pedosphere 28*(6): 873-883.

Amador, J.A., Glucksman, A.M., Lyons, J.B., Görres, J.H., 1997. Spatial distribution of soil phosphatase activity within a riparian forest1. *Soil Science* 162(11): 808-825.

Atafar, Z., Mesdaghinia, A., Nouri, J., Homaee, M., Yunesian, M., Ahmadimoghaddam, M., Mahvi, A.H., 2010. Effect of fertilizer application on soil heavy metal concentration. *Environmental Monitoring and Assessment* 160: 83–89.

Audette, Y., O'Halloran, I.P., Evans. L.J., Martin, R.C., Voroney, R.P., 2016. Kinetics of phosphorus forms applied as inorganic and organic amendments to a calcareous soil II: effects of plant growth on plant available and uptake phosphorus. *Geoderma 279*: 70-76.

Banik, S., Dey, B.K., 1982. Available phosphate content of an alluvial soil as influenced by inoculation of some isolated phosphate-solubilizing micro-organisms. *Plant and Soil* 69: 353-364.

Batool, S., Iqbal, A., 2018. Phosphate solubilizing rhizobacteria as alternative of chemical fertilizer for growth and yield of Triticum aestivum (Var. Galaxy 2013). *Saudi Journal of Biological Sciences* 26(7): 1400-1410.

Behera, B.C., Yadav, H., Singh, S.K., Mishra, R.R., Sethi, B.K., Dutta, S.K., Thatoi, H.N., 2017. Phosphate solubilization and acid phosphatase activity of Serratia sp. isolated from mangrove soil of Mahanadi river delta, Odisha, India. *Journal of Genetic Engineering and Biotechnology* 15(1): 169–178.

Bolan, N.S., Hedley, M.J., 1990. Dissolution of phosphate rocks in soils. 2. Effect of pH on the dissolution and plant availability of phosphate rock in soil with pH dependent charge. *Fertilizer Research* 24: 125-134.

Chien, S.H., Menon, R.G., 1995. Factors affecting the agronomic effectiveness of phosphate rock for direct application. *Fertilizer Research* 41: 227-234.

Chunga, H., Parka, M., Madhaiyana, M., Seshadria, S., Songb, J., Chob, H., Saa, T., 2005. Isolation and characterization of phosphate solubilizing bacteria from the rhizosphere of crop plants of Korea. *Soil Biology and Biochemistry* 37: 1970–1974.

Correll, D.L., 1998. The role of phosphorus in the eutrophication of receiving waters: A review. *Journal of Environmental Quality* 27(2): 261-266.

Çağatay, M., Kacar, B., Ülgen, N., Turan, C., 1973. Türkiye şartlarında Türkiye ham fosfatlarının ziraate faydalılık nispetlerinin tayini üzerine bir araştırma. TÜBİTAK Tarım Orman Araştırma Grubu Yay. Sayı: 25. [in Turkish]

Daniel, T.C., Sharpley, A.N., Lemunyon, J.L., 1998. Agricultural phosphorus and eutrophication: A Symposium overview. *Journal of Environmental Quality* 27:251-257.

Dawwam, G.E., Elbeltagy, A., Emara, H.M., Abbas, I.H., Hassan, M.M., 2013. Beneficial effect of plant growth promoting bacteria isolated from the roots of potato plant. *Annals of Agricultural Science* 58(2): 195–201.

Chaya, D.S., Bijoy, N., 2015. Effects of AM Fungi and plant growth-promoting Rhizobacteria on enzymatic activities of soil under Turmeric (*Curcuma longa* L.) cultivation. *Journal of the Indian Society of Soil Science* 63(4): 442-448.

Fernández, L.A., Zalba, P., Gómez, M.A., Sagardoy, M.A., 2007. Phosphate-solubilization activity of bacterial strains in soil and their effect on soybean growth under greenhouse conditions. *Biology and Fertility of Soils 43*(6): 805-809.

Goldstein, A.H., 1986. Bacterial solubilization of mineral phosphates: historical perspective and future prospects. *American Journal of Alternative Agriculture 1*(2): 51-57.

Goldstein, A.H., 1995. Recent progress in understanding the molecular genetics and biochemistry of calcium phosphate solubilization by gram negative bacteria. *Biological Agriculture and Horticulture* 12(2): 185-193.

Gong, M., Du. P., Liu, X., Zhu, C., 2014. Transformation of inorganic P fractions of soil and plant growth promotion by phosphate-solubilizing ability of *Penicillium oxalicum* I1. *Journal of Microbiology 52*(12): 1012-1019.

Grinsted, M.J., Hedley, M.J., White, R.E., Nye, P.H., 1982. Plant-induced changes in the rhizosphere of rape (*Brassica napus* var. emerald) seedlings: I. pH change and the increase in P concentration in the soil solution. *New Phytologist* 91(1): 19-29.

Gupta, M.M., Kiran, S., Gulati. A., Singh, B., Tewaria, R., 2012. Isolation and identification of phosphate solubilizing bacteria able to enhance the growth and aloin-A biosynthesis of *Aloe barbadensis*. *Microbiological Research* 167(6): 358-363.

Güneş, A., Turan, M., Güllüce, M., Şahin, F., Karaman, M.R., 2013. Effects of different bacteria application on solubility of rock phosphate. *Toprak Su Dergisi* 2(1): 53-61. [in Turkish]

Huang, S.W., Jin, J.Y., 2008. Status of heavy metals in agricultural soils as affected by different patterns of land use. *Environmental Monitoring and Assessment* 139(1–3): 317–327.

Hussain, A., Adnan, M., Iqbal, S., Fahad, S., Saeed, M., Mian, I. A., Muhammad, M.W., …… Andaleeb, S., 2019. Combining phosphorus (P) with phosphate solubilizing bacteria (PSB) improved wheat yield and P uptake in alkaline soil. *Pure and Applied Biology* 8(2): 1809-1817.

Illmer, P., Schinner, F., 1992. Solubilization of inorganic phosphates by microorganisms isolated from forest soils. *Soil Biology and Biochemistry* 24(4): 389-395.

Iyer, B., Rajput, M.S., Rajkumar, S., 2017. Effect of succinate on phosphate solubilization in nitrogen fixing bacteria harbouring chick pea and their effect on plant growth. *Microbiological Research* 202: 43-50.

Jones, J.B., 2001. Laboratory guide for conducting soil tests and plant analyses. CRC Press, New York, USA. 363p.

Kamprath, E.J., Watson, M.E., 1980. Conventional soil and tissue tests for assessing the phosphorus status of soils. In: The role of phosphorus in agriculture. Khasawneh, F.E. (Ed.) ASA, Madison, WI, pp. 433-469.

Kanabo, I.A.K., Gilkes, R.J., 1987. The role of soil pH in the dissolution of phosphate rock fertilizers. *Fertilizer Research* 12: 165-173.

Kaur, G., Reddy, M.S., 2014. Influence of P-solubilizing bacteria on crop yield and soil fertility at multi locational sites. *European Journal of Soil Biology* 61: 35-40.

Kaur, G., Reddy, M.S., 2015. Effects of phosphate-solubilizing bacteria, rock phosphate and chemical fertilizers on maize-wheat cropping cycle and economics. *Pedosphere* 25(3): 428–437.

Khan, A.L., Halo, B.A., Elyassi, A., Ali, S., Al-Hosni, K., Hussain, J., Al-Harrasi, A., Lee, I. J., 2016. Indole acetic acid and ACC deaminase from endophytic bacteria improves the growth of Solanum lycopersicum. *Electronic Journal of Biotechnology* 21: 58-64.

Kızılkaya, R., 2008. Dehydrogenase activity in Lumbricus terrestris casts and surrounding soil affected by addition of different organic wastes and Zn. *Bioresource Technology* 99(5): 946–953.

Kızılkaya, R., Bayraklı, B., 2005. Effects of N-enriched sewage sludge on soil enzyme activities. *Applied Soil Ecology* 30(3): 192-202.

Kızılkaya, R., Hepşen, Ş., 2004. Effect of biosolid amendment on enzyme activities in earthworm (Lumbricus terrestris) casts. *Journal of Plant Nutrition and Soil Science* 167(2): 202-208.

Kızılkaya, R., Bayraklı, F., Sürücü, A., 2007. Relationships between phosphatase activity and phosphorus fractions in agricultural soils. *International Journal of Soil Science* 2(2): 107-118.

Mahmoud, E., Ibrahim, M., Abd El-Rahman, L. Khader, A., 2018. Effects of biochar and phosphorus fertilizers on phosphorus fractions, wheat yield and microbial biomass carbon in vertic torrifluvents. *Communications in Soil Science and Plant Analysis* 50(3): 362-372.

Mamta, B., Rahic, P., Pathaniad, V., Gulatic, A., Singhd, B., Bhanwrae, R.K., Tewaria, R., 2010. Stimulatory effect of phosphate-solubilizing bacteria on plant growth, stevioside and rebaudioside-A contents of Stevia rebaudiana. *Applied Soil Ecology* 46(2): 222–229.

Masciandaro, G., Ceccanti, B., Ronchi, V., Bauer, C., 2000. Kinetic parameters of dehydrogenase in the assessment of the response of soil to vermicompost and inorganic fertilisers. *Biology and Fertility of Soils* 32(6): 479–483.

Milić, S., Ninkov, J., Zeremski, T., Latković, D., Šeremešić, S., Radovanović, V. & Žarković, B., 2019. Soil fertility and phosphorus fractions in a calcareous chernozem after a long-term field experiment. *Geoderma 339*: 9-19.

Oberson, A., Friesen, D.K., Rao, I.M., Bühler, S., Frossard, E., 2001. Phosphorus transformations in an Oxisol under contrasting land-use systems: The role of the soil microbial biomass. *Plant and Soil* 237(2): 197-210.

Olsen, S.R., Cole, C.V., Watanabe, F.S., Dean, L.A., 1954. Estimation of available phosphorus in soils by extraction with sodium bicarbonate. U.S. Department of Agriculture, Circular No 939, USA, 19p.

Pande, A., Pandey, P., Mehra, S., Singh, M., Kaushik, S., 2017. Phenotypic and genotypic characterization of phosphate solubilizing bacteria and their efficiency on the growth of maize. *Journal of Genetic Engineering and Biotechnology* 15: 379–391.

Pepper, I.L., Gerba, C.P., Brendecke, J.W., 1995. Environmental microbiology: a laboratory manual. Academic Press. New York, USA. 197p.

Samadi, A., 2006. Contribution of inorganic phosphorus fractions to plant nutrition in alkaline-calcareous soils. *Journal of Agricultural Science and Technology* 8: 77-89.

Self-Davis, M.L., Moore, P.A., Joern, B.C., 2009. Water- or Dilute Salt-Extractable Phosphorus in Soil. In: Methods for Phosphorus Analysis for Soils, Sediments, Residuals, and Waters. Kovar, J.L., Pierzynski, G.M. (Eds.). Southern Cooperative Series Bulletin, Virginia Tech University, Blacksburg, pp. 22-24.

Shen, J., Li, R., Zhang, F., Fan, J., Tang, C., Rengel, Z., 2004. Crop yields, soil fertility and phosphorus fractions in response to long-term fertilization under the rice monoculture system on a calcareous soil. *Field Crops Research* 86(2-3): 225-238.

Singh, H., Reddy, M.S., 2011. Effect of inoculation with phosphate solubilizing fungus on growth and nutrient uptake of wheat and maize plants fertilized with rock phosphate in alkaline soils. *European Journal of Soil Biology* 47:30-34.

Solis, P., Torrent, J., 1989. Phosphate fractions in calcareous Vertisols and Inceptisols of Spain. *Soil Science Society of America Journal* 53(2): 462-466.

Tabatabai, M.A., Bremner, J.M., 1969. Use of p-nitrophenyl phosphate for assay of soil phosphatase activity. *Soil Biology and Biochemistry* 1(4): 301-307.

Wang, J., Liu, W.Z., Mu, H.F., Dang, T.H., 2010. Inorganic phosphorus fractions and phosphorus availability in a calcareous soil receiving 21-year superphosphate application. *Pedosphere* 20(3): 304-310.

Yang, J.E., Jacobsen, J.S., 1990. Soil inorganic phosphorus fractions and their uptake relationships in calcareous soils. *Soil Science Society of America Journal* 54(6): 1666-1669.