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Release of phosphates in different types of soil by using Phosphate Solubilizing Microorganism (*Pseudomonas fluorescens)*

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Abstract

An experiment was conducted in the glasshouse of Soil Science Division under Nepal Agriculture Research Council (NARC), Khumaltar, Lalitpur from 16th July to 6th October, 2017 with the major objective to study the release of phosphates and change in pH of soil by Phosphate Solubilizing Microorganisms (PSM) in different types of soil. The experiment followed the principle of Completely Randomized Design with five treatments and three replications. The five treatments of the experiment were T_1 (Pine forest Soil), T_2 (Red Soil), T_3 (Swampy area Soil), T_4 (Clay Soil) and T_5 (Normal Cultivated Soil). 0.1% PSM was applied in each replication @ 5ml per seed with two seeds of French bean in each replication. Various parameters were observed and analysed using SPSS. Results of PSM application on different treatments revealed that the release of phosphates varied significantly among the treatments in different time interval. The highest phosphate release (390.57kg P_2O_5 ha^{-1}) was found in the treatment T_4 (Clay Soil) and the time of maximum release was recorded at 20 days after PSM application. Similarly, the change in pH after the PSM application was also found statistically significant between the treatments in different time interval. The maximum change in soil pH was recorded in treatment T_3 (Swampy Soil). Significant positive correlation was found between plant height and available phosphorus content of soil. Likewise, positive correlation was found between plant height and soil pH and between available phosphorus and soil pH but it was not significant. Concluding, application of PSM in soil can solubilize the insoluble phosphorus and make it available for plant uptake.

Keywords: Phosphate Solubilizing Microorganisms (PSM), phosphorus, pH, release

1. Introduction

Phosphorus is one of the 17 most essential major nutrients required for plant growth. It is frequently deficient for crop production and is required by crops in relatively larger amount. It plays an important role in major metabolic processes in plant including photosynthesis, energy transfer, (Khan, 2010) and nitrogen fixation in legumes (Saber *et al*. 2005). Plant available phosphorus is usually deficient in the soil because it is fixed in soil layers (Wang *et al.* 2009). This insoluble or fixed phosphorus is in different forms fixed with calcium $(Ca_3PO_4)_2$, aluminum (Al_3PO_4), and iron (Fe_3PO_4). Inorganic P occurs in soil, mostly in insoluble mineral complexes. These insoluble, precipitated forms cannot be absorbed by plants (Rengel & Maschner, 2005).

The availability of plant nutrients is highly affected by soil pH. It has been determined that most plant nutrients are optimally available to plants within this 6.5 to 7.5 pH range, plus this range of pH is generally very compatible to plant root growth. The amount and manner in which soluble phosphorus becomes fixed is very dependent on soil pH. Phosphorus becomes fixed either in the form of iron or aluminum phosphate in acidic soil (Norrish and Rosser, 1983) or in the form of calcium phosphate in neutral to alkaline soils (Lindsay *et al*. 1989). Phosphorus becomes most soluble at pH 6.5 (Narsian and Patel, 2009).

Soil microorganisms are involved in a wide range of biological processes including the transformation of insoluble plant nutrients (Babalola and Glick, 2012). Phosphate solubilizing micro-organisms refer to a group of soil microorganisms that are the component of phosphorus cycle and can release phosphorus from insoluble sources by different mechanisms of solubilization and mineralization. Thus, inoculating seeds/crops/soil with PSM is a promising strategy for the improvement of plant absorption of P and thereby reducing the use of chemical fertilizers (Alori *et al*. 2012). Several strains of bacterial and fungal species have phosphate solubilizing capabilities. Phosphate solubilizing bacteria (PSB) hold most important position in P-solubilizing soil microbes. Important genera of phosphate solubilizing bacteria include *Pseudomonas* and *Bacillus* (Illmer and Schinner, 1992).

95-99% of phosphorus present in the soil is insoluble and cannot be utilized by plants. Only

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0.1% of the total P exists in a soluble form available for plant uptake (Zhou *et al*. 1992). Efficiency of applied P fertilizer throughout the world is only around 10-25% (Isherword, 1998). The high input of chemical fertilizers not only represents a major cost of agricultural production but also impose adverse environmental impacts on overall soil health (Tilman *et al*. 2001). The repeated and injudicious applications of chemical P fertilizers, leads to the loss of soil fertility (Gyaneshwar *et al*. 2002) by disturbing microbial diversity, and consequently reducing yield of crops.

The environmental and economic concerns have led to the search for sustainable way of P nutrition of crops. For this, phosphate-solubilizing microorganisms (PSM) have been seen as best eco-friendly, economically sound alternative to the more expensive chemical fertilizers and as a means for P nutrition of crop as it solubilize the insoluble soil phosphorus.

The study was conducted with the following objectives:

- To evaluate the effect of phosphate solubilizing microorganisms on release of phosphates and change in pH in different type of soils.
- To study the release pattern of phosphates after the application of PSM.
- To study the change in pH of soil after the application of PSM.
- To find out optimum time of maximum release of phosphorus after inoculation of PSM.
- To know the relation between soil pH and phosphorus availability after the inoculation of PSM

2. Materials and Methods

The experimental soils were collected from two different locations. Soil under pine forest, red soil, swampy soil and normal cultivated soils were collected from Lamatar-09, Mahalaxmi municipality, Lalitpur and the clay soil was collected from Dadhikot, Surabinayak municipality, Bhaktapur. The experiment was laid out in the glasshouse of Soil Science Division (SSD), Nepal Agriculture Research Council (NARC), Khumaltar, Lalitpur for the period of three months.

The experiment was laid out in Completely Randomized Design (CRD) consisting of five treatments, each with three replications. The treatments were;

T_1: Soil under pine forest
T_2: Red Soil
T_3: Swampy area Soil
T_4: Clay Soil
T_5: Normal Cultivated Soil

The soils to be used as treatments were collected from different locations as mentioned above from 15-20 cm deep top soil but the clay soil was collected from 10-15 ft depth. The experiment was setup inside the glasshouse on 18th July, 2017 using five treatments, each with three replication. Small plastic bags were filled with 700gm of the prepared soils. Two bean seeds were placed in every unit at a depth of 3-5 cm and 5ml of 0.1% *Pseudomonas fluorescens* solution was then poured over the seeds and covered with the soil.

The observation parameters of this study mainly include soil chemical properties such as soil pH and available phosphorus of soil. Initial chemical properties of soil were analyzed. This includes total nitrogen, available phosphorus, available potassium, organic matter and pH. After the inoculation of PSM, soil available phosphorus and pH were measured at every 20 days interval for four times. The plant parameter includes plant height (in cm). Plant height was measured at 15 days interval for four times after the seed being sown on 18th July, 2017.

Soil sample of all treatments were collected, air dried, passed through 0.02 mm sieve and their chemical properties were analyzed in the laboratory of HICAST. The general characteristics of the soils under study are presented in Table 1.

Table 1: General characteristics of soils under study

S. N	Soil type	Soil collection area	Soil Parameters				
			pH	Available P_2O_5 Kgha^{-1}	Available K_2O Kgha^{-1}	Total N	OM
1.	Soil under pine forest	Lamatar, Lalitpur	5.38	82.94	302.55	0.31%	4.03%
2.	Red soil	Lamatar, Lalitpur	4.33	28.37	134.85	0.142%	1.70%
3.	Swampy area soil	Lamatar, Lalitpur	5.83	65.48	278.59	0.240%	5.48%
4.	Clay soil	Dadhikot, Bhaktapur	6.60	247.74	230.68	0.282%	5.71%
5.	Normal cultivated soil	Lamatar, Lalitpur	7.24	636.28	1141.05	1.409%	10.6%

For statistical analysis, the data were subjected to Analysis of Variance (ANOVA) using SPSS version 23.0. The significance of the data were analysed at 5% level of significance. P-value, F-value, grand mean, least significant difference (LSD), standard error of mean were carried out for significance test using Duncan's Multiple Range Test (DMRT) to compare the data.

3. Result and Discussion

3.1 Effect of PSM on release of phosphates

The release of phosphates or availability of phosphorus after the application of PSM on different types of soil had been observed at different time intervals and is tabulated in Table 2. The data regarding release of phosphorus after the application of PSM was found statistically significant in different types of soil at different time of 20 days interval. The P-value in all the cases was found to be less than 0.05.

Table 2: Effect of PSM on release of phosphates in different soils

Treatments	Average available P_2O_5 (Kgha^{-1}) at different days					Mean P_2O_5 (Kgha^{-1})
	Initial	Day 20	Day 40	Day 60	Day 80	
T_1 (Pine forest soil)	82.946	98.58^b	84.39^c	76.52^c	55.42^d	78.72
T_2 (Red Soil)	28.376	49.47^b	58.56^c	33.16^c	26.13^c	41.83
T_3 (Swampy Soil)	65.484	100.40^b	92.03^c	63.63^c	58.94^c	78.75
T_4 (Clay Soil)	247.74	568.61^a	492.58^b	219.50^b	281.61^b	390.57
T_5 (Normal Cultivated Soil)	636.28	610.09^a	926.59^a	422.24^a	384.74^a	585.91
Grand Mean		285	331	163	160	
CV %		22.3	19.9	25.5	9.6	
LSD		119.7	123.7	78.3	28.84	
SEM		36.7	37.9	24.0	8.84	
P-value		<.001	<.001	<.001	<.001	

From the soil samples analysed at different days, the mean value of available phosphorus was found highest in treatment T_5 (Normal Cultivated Soil) with the value of 585.91 P_2O_5 $Kgha^{-1}$ and the lowest value i.e. 41.83 P_2O_5 $Kgha^{-1}$ was obtained in treatment T_2 (Red Soil). The Duncan's test also showed the treatment T_5 (Normally Cultivated Soil) as superior in case of phosphate release by PSM at every sampling days followed by treatment T_4 (Clay Soil), T_3 (Swampy Soil) and T_2 (Red Soil). The treatment T_1 (Pine forest Soil) showed similar result as that of T_2 and T_3 in initial sampling days but at the latter stage the release of phosphorus by PSM was found to be low.

Critical observation of data in Table 2 shows that the mean level of available P_2O_5 for treatment T_4 (Clay Soil) was found much higher than its initial level. There is increase in mean value also in case of treatment T_2 (Red Soil) and T_3 (Swampy Soil) but the increment is low as compared to T_4. While in case of the treatments T_1 (Pine forest Soil) and T_5 (Normal cultivated Soil), the mean level of available P_2O_5 after the application of PSM was found to be quite low than its initial level.

Persual of the data in Table 2 also shows that with the application of PSM, the available phosphorus content tends to increase initially in most of the soils. This may be due to the solubilization of P from insoluble and fixed/adsorbed forms. Microbial biomass assimilates the soluble P and prevents it from adsorption or fixation (Khan and Joergensen, 2009). This result goes in accordance with Sundara and Natarajan (2004), who reported that PSM application increases plant available P status in the soil. The increment was found to be very high in 20th day after the PSM application in case of treatments T_1 (Pine forest Soil), T_2 (Red Soil), T_3 (Swampy Soil) and T_4 (Clay Soil). But in treatment T_5 (Normal Cultivated Soil), the available phosphorus decreases initially and was found to be high at 40th day after the application of PSM.

This data reveals that the solubilization of phosphorus by PSM is high at the initial stages of their application which may be due to the higher number of microbial population initially. The solubilization then decreases gradually with time as the microbial population decreases. This corresponds to the finding of Chuanquing *et al.* (2014), who reported that the population of PSM decreases over time and needed to be inoculated continuously for release of phosphates.

The drop in available phosphorus content from its initial level in the latter stages i.e. 60 and 80 days after the application of PSM in most of the soils as seen in the above figure may be due to the uptake of P by the crop planted in the soil.

3.2 Effect of PSM on change in soil pH

When the data was subjected to Analysis of Variance (ANOVA) at 5% level of significance, the effect of PSM was found statistically significant in terms of change in soil pH in each type of soils and at different time after the application of PSM. The P-value for all treatments and all cases were less than 0.05. The Duncan's Multiple Range test found the treatment T_5 to be most responsive towards change in pH after PSM application in all the observed time intervals. Red soil i. e. treatment T_2 is found to be least responsive towards change in pH.

Table 3: Effect of PSM on change in soil pH in different types of soil

Treatments	Average soil pH at different days					Mean pH
	Initial	Day 20	Day 40	Day 60	Day 80	
T_1 (Pine forest soil)	5.38	5.297^d	6.840^b	6.173^c	5.807^c	6.02
T_2 (Red Soil)	4.33	4.616^e	5.897^c	5.383^d	5.140^d	5.26
T_3 (Swampy Soil)	5.83	6.154^b	6.947^b	7.123^b	6.780^b	6.75
T_4 (Clay Soil)	6.60	5.597^c	5.953^c	6.197^c	5.693^c	5.77
T_5 (Normal Cultivated Soil)	7.24	7.269^a	7.797^a	7.883^a	7.723^a	7.67
Grand Mean		5.787	6.687	6.552	6.226	
CV %		2.8	1.8	1.3	1.8	
LSD		0.3083	0.2218	0.1628	0.2110	
SEM		0.0945	0.0680	0.0499	0.0647	
P-value		<.001	<.001	<.001	<.001	

As per the data presented in Table 3, the mean soil pH value for all the treatments was found to be increased than its initial pH value except for treatment T_4 (Clay Soil). The highest mean pH value was obtained in treatment T_5 followed by the treatments T_3, T_1, T_4 and T_2. In terms of change in soil pH after the application of PSM, the highest change was obtained in treatment T_3 (Swampy Soil) with a change from 5.83 to 6.75 and the lowest change was obtained in treatment T_5 (Normal cultivated Soil) with a change from 7.24 to 7.67.

There is a gradual increase in soil pH after the application of PSM in most of the treatments upto 60 days. The highest increase in soil pH was obtained in the 3rd sampling i.e. 'day 60' for treatments T_3 (Swampy Soil), T_4 (Clay Soil) and T_5 (Normal cultivated Soil) while treatments T_1 (Pine forest soil) and T_2 (Red Soil) has reached the highest soil pH at day 40. Once the pH has reached at its highest, it tends to decrease gradually thereafter in all the treatments.

The rhizospheric soil was taken from every replication for its pH measurement at every 20 days interval for four times. The result has showed that the rhizospheric soil pH has increased in most of the cases after the PSM application. This result was similar with the result of Namli *et al.* (2017), who also found the increment in the rhizospheric soil pH with the use of PSB.

3.3 Study of Plant Parameters

Among various plant parameters, only plant height was taken into observation in this experiment as the experiment mainly focuses on soil properties rather than the plant properties.

Plant showed varied responses to different types of soil as each type of soil has different physical and chemical properties which directly affected the growth of the plant.

Table 4: Response of plant height to different types of soil with PSM application

Treatments	Average plant height at different days (cm)			Mean Plant Height (cm)
	Day 30	Day 45	Day 60	
T_1 (Pine forest soil)	130.3^a	143.3^{ab}	148.0^{ab}	140.53
T_2 (Red Soil)	72.3^b	83.0^c	86.3^c	80.53
T_3 (Swampy Soil)	118.3^a	131.3^{bc}	136.7^{bc}	128.77
T_4 (Clay Soil)	146.7^a	197.3^a	202.8^a	182.27
T_5 (Normal Cultivated Soil)	162.5^a	180.6^{ab}	193.0^{ab}	178.7
Grand Mean	130.3	147.1	153.4	
CV %	20.0	18.8	18.5	
LSD	47.55	52.01	53.28	
SEM	14.58	15.95	16.34	
P-value	0.010	0.007	0.006	

The plant height is considered to be an important factor to judge the efficacy of Phosphate Solubilizing Microorganisms. The plant height was recorded at different growth periods i.e. at 30, 45 and 60 days after sowing (DAS). The data regarding response of plant height of French bean to different type of soil with PSM application was found statistically significant at 5% level of significance. The different treatments showed significant effect on plant height at 30 DAS with P-value 0.010, 45 DAS with P-value 0.007 and 60 DAS with P-value 0.006. This means that the difference in soil physical and chemical properties does matter significantly in the plant growth. The Duncan's Multiple Range Test (DMRT) showed the treatment T_4 to be more superior in terms of increase in plant height. This superiority is constant in every observation. After clay soil treatment T_5 was found to be most responsive to increase in plant height which is then followed by T_1, T_3 and T_2.
The mean plant height varied from 80.53 cm to 182.27 cm due to various treatments. The highest plant height was observed in treatment T_4 (Clay Soil) and the lowest in treatment T_2 (Red Soil).

Closer observation of Table 4 shows that the plant height increases gradually with the increase of growth period. This increase in height was rapid between day 30 to day 45 in all the cases, while between day 45 to day 60 the increment is slower. At day 30, the highest plant height was recorded in treatment T_5 (Normal cultivated Soil) having height of 162.5 cm followed by T_4 (Clay Soil), T_1 (Pine forest soil), T_3 (Swampy Soil) and T_2 (Red Soil). At day 45 and day 60, the highest plant height was recorded in treatment T_4 followed by treatments T_5, T_1, T_3 and T_2. Treatment T_2 i. e. Red Soil showed the shortest height throughout the experiment. In fact, the increase in height was very less or more or less restricted in this soil. It may be due to the adverse properties of red soil for plant growth.

3.4 Correlation matrix
Available phosphorus content in soil, soil pH and plant growth are the parameters which are correlated with each other very greatly. Phosphorus content in soil is critical determinant of plant growth. The availability of higher phosphorus content to plant can result in higher plant growth. Similarly, the availability of plant nutrients such as N, P, K etc is greatly affected by soil pH which in turn affect to the plant growth and development.
The average value of soil available phosphorus, soil pH and plant height of different types of soil were correlated to find out if there is positive or negative correlation. Correlation matrix among different observed parameters in the experiment as influenced by the treatments is presented in following sub headings.

3.4.1 Available Phosphorus and Soil pH
When the average value of soil available phosphorus and soil pH were correlated, the correlation coefficient obtained was 0.757 as seen in Figure 1. This correlation value shows a high degree of positive relationship between these soil available phosphorus and soil pH but the correlation is not significant. This result is in contrast with the finding of Chuan quing *et al*. (2014), who reported that there was no positive correlation between decrease of pH and increase of available phosphorus.

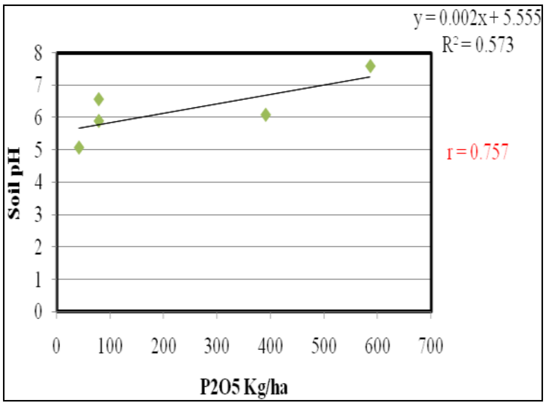
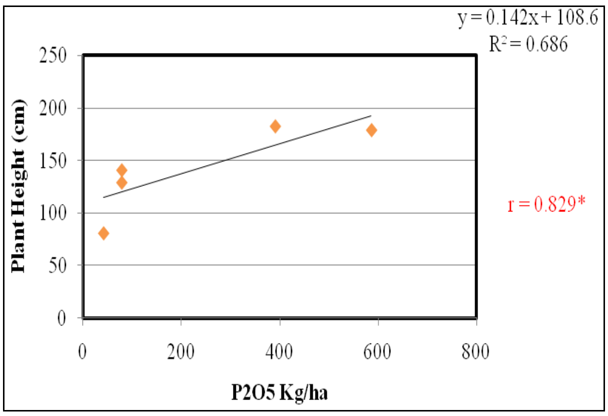


Fig 1: Correlation between Available Phosphorus and Soil pH

3.4.2 Available Phosphorus and Plant Height
The data presented in Figure 2 revealed that the average plant height has significant and high degree of positive correlation with average available phosphorus. The correlation value 0.829 indicated that the height of plant is highly dependent on the available phosphorus content of different soils. The lower the phosphorus content, the shortest is the plant height and vice-versa. The significant increase in plant height due to higher phosphorus availability was also reported by Poonia and Dhaka (2012) in Tomato.



(* Correlation is significant at the 0.05 level)

Fig 2: Correlation between Available Phosphorus and Plant Height

3.4.3 Soil pH and Plant Height

The plant height also has positive correlation with average soil pH having the correlation coefficient 0.721 as seen in Figure 3 but the correlation is not significant. The positive correlation indicates that soil pH has a direct effect on plant height. Hwan-E Joe (2008) reported that the soil pH has significant effect on plant height of cowpea. He found the growth parameters of cowpea at their maximum when the soil pH was within a range of 6.6-7.6.

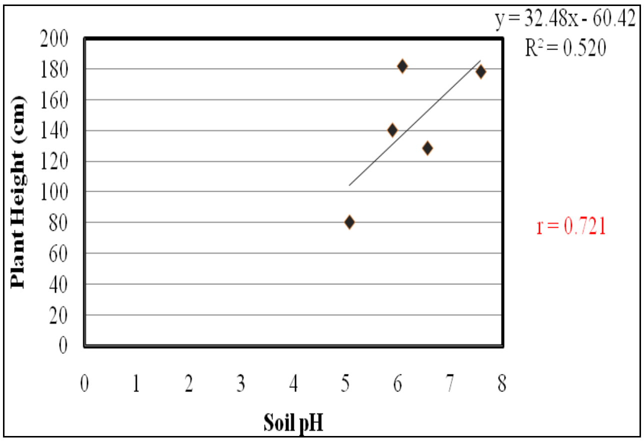


Fig 3: Correlation between Soil pH and Plant Height

4. Conclusion

Phosphorus being the key element in the nutrition of plants is required by plant in larger amount but its availability is limited in soil as it occurs mostly in insoluble forms in soil. The present study was initiated to assess the release of phosphate by PSM along with the change in soil pH in different types of soil. The study concluded that there is significant effect of PSM on release of phosphates in soil and change in soil pH. This release of phosphate and change in pH, however depends on the type of soil. The maximum phosphorus release was found in the Clay Soil and maximum change in pH was noticed on Swampy Soil. The phosphate release or availability of phosphorus was maximum after 20 days of PSM application. Similarly, the change in pH was maximum after 60 days of application. This study also found a positive correlation between soil pH and soil available phosphorus. Overall, the study concluded that the application of Phosphate Solubilizing Microorganisms in soil could be helpful to solubilize the insoluble phosphorus from soil and make it available for plant uptake.

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