USE OF SOIL MICROBIAL CULTURES TO INCREASE THE SOLUBILITY OF PHOSPHORUS IN EPPAWALA ROCK PHOSPHATE

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ABSTRACT

At present, Sri Lanka imports phosphorus fertilizers incurring a lot of foreign exchange even though there is a high-quality apatite deposit at Eppawala. Eppawala rock phosphate (ERP) can be utilized as a Phosphorus fertilizer having increased the water solubility of rock phosphate through chemical or biological processes. However, biological processes are more cost-effective and ecofriendly. Thus, the study focused to increase the solubility of ERP using the selected soil microbes. Soil samples which contain different microbes were collected using a simple random sampling method (n-30) from five forested areas (Badagamuwa, Sinharaja, Diyathalawa Turpentine, Girandurukotte Teak, and Nonperial Pinus plantations) considering the phosphorus level in the soil, distribution in Sri Lanka, and microbial count in the soil. Microbial rich cultures were prepared by using the obtained soil samples and applied to the ERP. A randomized complete block design (RCBD) was used for the experiment with 5 replicates for each treatment and only ERP was used as a control. Treated samples were tested for the available phosphorus percentage using the Ascorbic acid method during 8 weeks within a week interval. The results were statistically analyzed using two-way ANOVA and Tukey pairwise comparison test (95% confidence interval). The available phosphorus percentages of all treated samples were significantly higher ($p<0.05$) than the controlled samples in every week. The highest available phosphorus percentages of all treated ERP samples were in 0.75% - 0.81% range while controlled (ERP) was 0.42%, which significantly recorded ($p<0.05$) in 6th week. Selected soil microbial cultures increased the solubility of ERP and therefore ERP can be utilized as a phosphorus fertilizer with applying the further improved microbial cultures.

Keywords: *Eppawala rock phosphate, phosphorus solubility, soil microbes*

1. INTRODUCTION

1.1. Phosphorus for Crops

Phosphorus is one of the most essential nutrients for crops. Phosphorus is essential to efficient photosynthesis (Chaudhary et al., 2008), to proper germination, to increase the fertility of yield (Mitran et al., 2018), to enhance growth and maturation of plants (Chaudhary et al., 2008), to enhance the fertilization of some other nutrients like nitrogen (Suzaki et al., 2015), to produce the organic components like protein, DNA, RNA and, Vitamin (Chaudhary et al., 2008), to results proper root system (Niu et al., 2013) and, to enhance the strength of crops resilience (Kaul et al., 2019). However, phosphorus is one of the most limiting elements in the Sri Lankan soil, because the soil contains a high amount of total phosphorus, but water-soluble phosphorus content is low (Wijewardena, 1994). Therefore, phosphorus fertilizers are used widely in Agriculture. There are many types of commercially available phosphorus fertilizers. Rock phosphate (RP), Triple super phosphate (TSP) and Single super phosphate (SSP) derived from phosphoric acid, calcium orthophosphates, ammonium phosphates, ammonium polyphosphate and nitric phosphates are few of them. Many countries import phosphorus fertilizers while Sri Lanka expends billions for importing Phosphorus fertilizer. Sri Lanka was in number 95 place for phosphate fertilizer imports (9,407.74 Metric *Tons*) in 2018 while placed in number 103th place in 2017 (Nation master- FAO, 2018). Imports and fertilizer costs were increased over the last few years and it is still increasing (Wijewardena, 2005).

1.2. Eppawala Rock Phosphate

There is a huge apatite deposit at Eppawala, North - Central province of Sri Lanka which has 60 million metric tons of estimated rock phosphates. Mother rock contains mainly apatite, dolomite, and calcites (Pitawala et al., 2003). This Rock contains 33-40 % of phosphate forms (P_2O_5). Eppawala Rock phosphate consider as one of the unique, high quality, less impure, phosphorus deposits that contains Calcium phosphate with Aluminum, chlorine, Florine, and hydroxide ions [$Ca_5(PO_4)_3Cl$/ 3 $Ca_3(PO_4)_2Ca(F,Cl)_2$].

Lanka Phosphate limited is the responsible authority for this ore and they are excavating and processing rock phosphate to sell as an inorganic fertilizer for only perennial agricultural crops like tea, coconut and, rubber (Ministry of Agriculture- Sri Lanka, 2018). They produce Eppawala Rock Phosphate (ERP) and High-Grade Eppawala Rock phosphate (HERP) while maintaining the P_2O_5 content availability of HERP at 28-30 % and 40-43 % respectively. Although Eppawala Rock phosphate creates many advantages as a fertilizer it not practically effectively utilizes as a fertilizer for all crops due to its less water solubility which recorded as <0.5%. Significant amount of foreign exchange will be saved by optimum utilization of the Eppawala Apatite deposit (Udawatte et al, 2020).

1.3. Increasing Water Solubility of ERP with Biological Process

Chemical processes and Biological processes can be used to increase the water solubility of phosphors in Eppawala rock phosphate. In the chemical process, hazardous acids (like Sulphuric) are commonly used in order to produce more soluble forms of phosphate fertilizer. However; it is not an economically feasible and environmentally friendly method in a country like Sri Lanka.

Therefore, the biological process is recommended to produce environmentally friendly fertilizer. Hence the purpose of this study was focused to increase the solubility of ERP using the selected soil microbes which contains a mixture of microbial species.

2. LITERATURE REVIEW

2.1. Dissolution of Rock Phosphate

Two major types of phosphate rocks namely sedimentary (carbonate Apatite) and igneous (fluor-chlor-hydroxyl-apatite) phosphate rocks (Subasinghe, 2013). About 210 million tons of worldwide rock phosphate usage is recorded in 2010. (Van Kauwenbergh, 2010). Crops absorb phosphorus in only the form of PO_4^{3-}, HPO_4^{2-} and H_2PO_4-. Calcium apatite is soluble in acids to produce such bioavailable forms (Dorozhkin, 2012). According to the chemical model, phosphate dissolution is explained with the [1] AND [2] equations (Dorozhkin, 2011a) and (Dorozhkin, 2011b)

$Ca_5(PO_4)_3(F, OH) + 7H^+ = 5Ca^{2+} + 3H_2PO_4^- + HF, H_2O$ ----------------------[1]

$Ca_5(PO_4)_3(F, OH) \rightarrow 5Ca^{2+} + 3PO_4^{3-} + F^-, OH$----------------------------------[2]

2.2. Mineral Phosphate Solubilizing Microorganisms

Many studies have reported that the several bacteria and fungi species that dissolve mineral phosphates. *Pseudomonas, Bacillus, Rhizobium, Burkholderia, Achromobacter, Agrobacterium, Microccocus, Aereobacter, Flavobacterium, and Erwinia* have identified as common mineral phosphate solubilizing bacteria (Rodríguez & Fraga, 1999). *Penicillium, Aspergillus* have identified as mineral phosphate solubilizing fungus (Whitelaw, 1999). Bacteria and Fungus show different mechanisms for solubilizing mineral phosphates. Bacteria release organic acids like glycolic, oxalic, acetic which cause a decrease in pH(Banik & Dey, 1982). Fungus directly release the H+ ions to drop pH(Illmer & Schinner, 1992). Other mechanisms are releasing the chelating ions (Spebbeb, 1958) and inorganic acids such as nitric acid, carbonic acids to drop pH (Rudolfs, 1922).

Naturally occurring microbes were previously used to dissolve the rock phosphate (Masimbula et al, 2016). The Microbial culture which was collected from "Surathalee Falls" pine forest showed the highest releasement of phosphorus with 0.86% after 20 days. Isolated microorganisms also previously used to dissolve the rock phosphate (Saeid et al., 2018). Worldwide, Phosphate solubilizing microbes have used to increase the water solubility of phosphates However, in Sri Lanka, it not properly commercializes because of practical issues are occurring in isolation and preservation of the individual species of microorganisms.

3. METHODOLOGY

3.1. Collection of Soil Samples

Top Soil samples witch contain different microbes were collected using a simple random sampling method (n-30) from five forested areas (Badagamuwa (B), Sinharaja (S), Diyathalawa Turpentine (D), Girandurukotte Teak (G), and Nonperial Pinus plantations (N)) considering phosphorus level in soil, distribution in Sri Lanka (Figure1), and microbial count in soil.

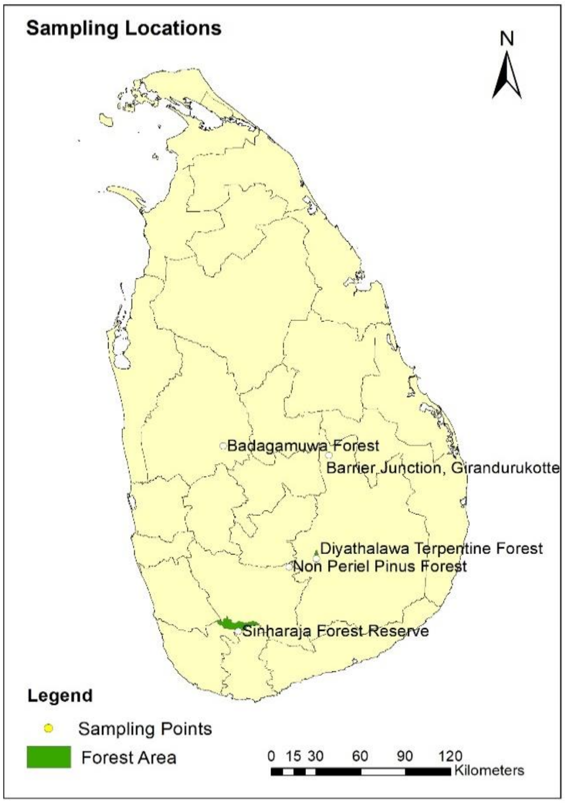

Figure 1: GIS-based map for site selection

3.2. Preparation of Microbial Rich Cultures

Five microbial cultures were prepared using 3 L–of natural water, 60 g of Gliricidia leaves, 75 g of brown sugar, 30 g of fresh cow dung and 3 g of collected soil sample. Those were aerated with an electric motor and allowed to 10 days to increase the population of microorganisms.

3.3. Preparation of Treatments

Eppawala Rock phosphate was dried at 110 ^{0}C for four hours and sieved with a 180 μm aperture size sieve. Brown sugar crushed and sieved with a 180 μm aperture size sieve. Five treatments were prepared according to the following order by using different microbial cultures, crushed brown sugar, Eppawala Rock phosphate, and natural water.

Treatment 1: 30 ml Microbial culture of S+ 0.5 g Dextrose + 60 g Eppawala Rock Phosphate

Treatment 2: 30 ml Microbial culture of P+ 0.5 g Dextrose + 60 g Eppawala Rock Phosphate

Treatment 3: 30 ml Microbial culture of G+ 0.5 g Dextrose + 60 g Eppawala Rock Phosphate

Treatment 4: 30 ml Microbial culture of B+ 0.5 g Dextrose + 60 g Eppawala Rock Phosphate

Treatment 5: 30 ml Microbial culture of T+ 0.5 g Dextrose + 60 g Eppawala Rock Phosphate

Treatment 6: Natural water + 0.5 g Dextrose + 60 g Eppawala Rock Phosphate (Reference)

The experiment was conducted in order to a randomized complete block design (RCBD) with preparing 5 replicates for each treatment. A drip irrigation system was applied in order to avoid drying the treated samples.

3.4. Colorimetric Determination for Available Phosphate Content

Treated samples were tested for the available phosphorus percentages using the Ascorbic acid method (Baillie et al., 1990) in every weekly intervals for 8 weeks. Absorbance was detected from UV- Spectrophotometer in 880 nm wavelengths.

4. DATA ANALYSIS & RESULTS

The data were statistically analyzed using two-way ANOVA and the Tukey pairwise comparison test (95% confidence interval).

Table 1: Available phosphurus percentage (%) with the time (p value<0.05)

Treatment		Time (Weeks)						
		1	2	3	4	5	6	7
1	Mean	0.36	0.29	0.58	0.65	0.61	0.75	0.58
	SD	±0.02	±0.04	±0.02	±0.01	±0.02	±0.00	±0.04
2	Mean	0.44	0.31	0.64	0.56	0.64	0.81	0.52
	SD	±0.01	±0.04	±0.00	±0.02	±0.04	±0.00	±0.01
3	Mean	0.42	0.28	0.57	0.6	0.68	0.80	0.57
	SD	±0.02	±0.05	±0.05	±0.03	±0.02	±0.01	±0.02
4	Mean	0.44	0.32	0.62	0.6	0.65	0.81	0.6
	SD	±0.01	±0.06	±0.05	±0.03	±0.02	±0.01	±0.02
5	Mean	0.43	0.3	0.6	0.58	0.63	0.75	0.52
	SD	±0.02	±0.06	±0.01	±0.00	±0.01	±0.02	±0.03
6	Mean	0.28	0.28	0.32	0.34	0.35	0.42	0.35
	SD	±0.01	±0.01	±0.01	±0.01	±0.02	±0.00	±0.01

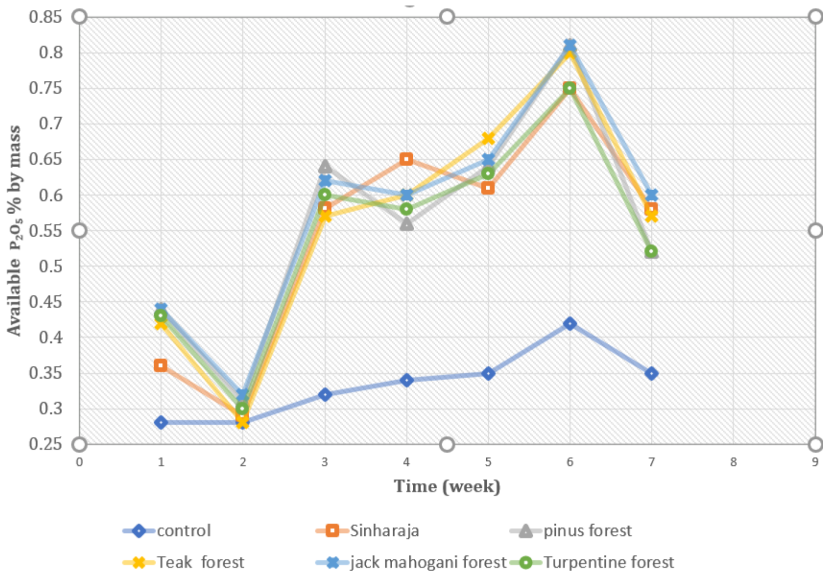

Figure 2: Available P_2O_5 % for 7 weeks

Table 2: Grouping information between treatments using the Tukey method and 95% confidence level

Treatment	Number of Replicates	Mean	Grouping
4	21	0.580288	A
3	21	0.566140	A B
2	21	0.563652	A B
1	21	0.550128	B
5	21	0.548360	B
6	21	0.338338	C

Table 3: Grouping information between weeks using the Tukey method and 95% confidence level

Weeks	Number of Replicates	Mean	Grouping
6	18	0.727041	A
5	18	0.597827	B
3	18	0.559792	C
4	18	0.559656	C
7	18	0.529295	C
1	18	0.396327	D
2	18	0.301453	E

Means that do not share a letter are significantly different.

5. DISCUSION OF THE FINDINGS

In the preparation of microbial culture, components act as follows Cow dung provides nutrients and microorganisms. It acts as a catalyst in the aerobic fermentation process (Srivastava et al., 2010). Brown sugar acts as an energy source (Gunina & Kuzyakov, 2015). Gliricidia leaves gives the other main nutrients, especially nitrogen. Natural water was collected from Surathali Falls in Belihuloya area to avoid being hazard towards the added microorganisms. Microbial mixture was used because some microorganisms show better activity when they are in the symbiotic with other microorganisms (Gore & Sreenivasa, 2011).

Soil microorganisms might differ in place to place. According to the results, the addition of soil microorganisms affects positively on Rock phosphate. The available phosphorus percentages of all treated samples were significantly increased ($p<0.05$) than the controlled samples every week. The highest available phosphorus percentages of all treated ERP samples were in 75% - 81%

range while controlled (ERP) was 0.42%, which significantly recorded ($p<0.05$) in the 6th week.

The pattern of the available P_2O_5 % in treatments almost the same growth curve of the microorganisms. The first two weeks indicate the Lag phase which their stability time in the new environment. In this period P_2O_5% was decreased due to phosphorus utilized by microorganisms for stable there. In the Exponential phase (2-6 Weeks) cells are dividing and number of microorganisms were increased. Therefore, in this period dissolve P_2O_5% was increased due to increase microbial metabolism. In the 6 - 7th week again P_2O_5% was decreased due to reach the death phase of the population.

6. CONCLUSION & CONTRIBUTION

Selected soil microbial cultures increased the solubility of ERP by two times and therefore ERP can be utilized as a phosphorus fertilizer with applying the further improved microbial cultures. It will be a great opportunity to bring down the import fertilizer amounts, and it will gain huge benefits to the Sri Lankan economy.

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