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Research Article

Extra-terrestrial Phosphate: the sole source of nourishment for Terrestrial Microbes

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Abstract

Phosphate solubilizing bacteria has the ability to convert extraterrestrial phosphorous into an available form. In this study, two types of soil sample (top and bottom soil) were collected from Chandannagar area (West Bengal, India).The actual experiment was performed in Pikovskaya's agar medium, pH 7.2 devoid of tricalcium phosphate and having rainwater and hail separately as the supposed phosphate source. The control experiment was performed in Pikovskaya's agar containing tricalcium phosphate. Accordingly, the different serial dilutions of topsoil were plated on Pikovskaya's agar medium. Also, anaerobic deep tube Pikovskaya's medium was prepared with bottom soil dilutions. The control for both the experiments was also prepared. After incubation at 37 °C for 5 days growth was observed in all the culture plates. Out of the 31 isolates, 15 were found to have good phosphate-solubilizing ability based on the phosphate solubility index. Pikovskaya's agar plates without phosphate were prepared and inoculated with the soil sample. No growth was observed in these suggesting that rainwater and hail and not soil phosphate served as the phosphate source for microbial growth. The anaerobic deep tubes also showed significant growth of facultative anaerobes and some obligate anaerobes. In addition, statistical analysis of phosphate solubility indices indicated microbes could utilize rainwater and hail as the potential phosphate source. This might be a proof that hail and rainwater were the sources of phosphorous for primitive life on earth.

INTRODUCTION

Phosphorous is an essential nutrient for the growth of terrestrial microbes. Though soil is rich in insoluble mineral and organic phosphates it generally has a lesser amount of phosphate in the available form that is, orthophosphate or Pi (Yadav *et al.*, 1997). Phosphorus is required for the formation of ATP, GTP or any other nucleotide triphosphate which serve as the energy currencies of the cell. The microbes in the soil may solubilize P_i from insoluble organic and mineral phosphates to notionally available form. It is also believed that they can solubilize phosphate present in rainwater and hail into usable forms. This phosphate may be then used by plants or the microbes themselves. In this study soil samples were serially diluted, plated onto Pikovskaya's agar (PVK agar) plates and isolation of mixed cultures of the microbes was done from both the surface and subsurface levels. These cultures were identified on the basis of their morphological characters and also by staining. The microbes from surface level were plated onto PVK agar plates and those from the subsurface level were inoculated into deep PVK agar tubes. Both of these setups had control and experimental test sets, in which the former had tricalcium phosphate as the phosphate source but the latter lacked this. Rainwater and hail were however added to the experimental sets as the supposed phosphate source. It was observed that microbes could grow in both the control and experimental sets, thus suggesting that the microbes could utilize the phosphate present in rainwater and hail. This serves as a proof that microbes can, indeed, solubilize the phosphate present in rainwater and hail and in the earlier times, during the origin of life, this extra-terrestrial phosphate was the sole source of nourishment for the terrestrial microbes. This is because no soil was known to form back then. Further, this work may be extrapolated to act as proof of the statement that life originally came to earth from outer space where the microbes had actually learned to solubilize phosphate which is an essential nutrient required for their survival. Thus, it can be said that, when meteorites, planetesimals and comet nucleus accelerated in the gravitational field of the earth, they reached hypervelocity and after they hit the surface of the planet, powerful blowouts of hot plasma generated in the form of a torch. They also created giant-size craters and dense dust clouds. These bodies were composed of all elements needed for the synthesis of organic compounds. Also, it is possible to show that, at the beginning of the formation of nucleons, nuclear stability is maximum for light atoms only (Seshavatharam UVS *et al.,* 2014). Thus, extra-terrestrial phosphate might have found their way into the storm clouds of earth. Interchanges of material between planets constituted essentially a cosmological primordial soup (Carl H. Gibson *et al.,* 2011). One aspect of this research program, is that interstellar dust and comets contain organic compounds, has been pursued by others as well. It is now widely accepted that space contains the "ingredients" of life. (Wainwright, 2010). This development could be the first hint of a huge paradigm shift to the establishment of the theory of cosmic ancestry of living organisms (Ray *et al.,* 2012). However, in our study the plates in which hail was given as microbes, the growth of microbes was far lesser as compared to those with rainwater as the phosphate source. This proves that majority of the bacteria residing in the soil are not resistant or adaptable to low temperatures. This can be extrapolated to conclude that the earliest microbes on earth were not accustomed to cold climates (Rauf K. *et al.,*2010). This falls in line with the fact that during the early years after the formation of the earth, the temperatures were very high. The temperature cooled down gradually and so the majority of the bacteria became adapted to either high or moderate temperature following the concept that primordial life began in the hot dilute soup of the oceanic waterbodies. (McCabe *et al.*, 2010). We confirmed that microbes like *Pseudomonas* spp. can solubilize phosphate which was also stated by Browne *et al* in 2009. Similarly, Chen *et al*., in 2006, proved the tricalcium phosphate solubilizing abilities of bacteria which was also observed by us since the microbes were able to grow in PVK agar containing the same compound. Finally, we could also verify the findings of (Christophe Migon *et al*., 1999) which claimed the availability of phosphate in rainwater. Thus, it can be said that extra-terrestrial phosphate still continues to be a contributor of phosphate for the terrestrial microbes as well as the plants. The hypotheses that early earth lacked soil and major nutrients such as phosphate came to earth from extra-terrestrial sources can also be verified to some extent from the conclusions of the present study. The extra-terrestrial origin of life and the claim that the first life forms on earth learned the methods of obtaining nutrition in their extra-terrestrial residence can also not be ignored.

MATERIALS AND METHODS

Soil sampling: Top and bottom soil and rainwater were collected from Chandannagar (22.8648° N, 88.3633° E), West Bengal, India. A part of rainwater was solidified by refrigeration to give hail.

Media and Chemicals used: Pikovskaya's agar (PVK agar) was used in two different composition- (a) with all the ingredients and (b) with all the ingredients except tricalcium phosphate which was the only phosphate source in the media. L-cysteine and thioglycolate were used in the PVK agar deep tubes to favor the growth of anaerobes.

Determining whether soil phosphate alone is enough for the growth of the microbes: In order to eliminate the possibility that phosphate might come from the soil (and hence may contradict the results of the experiment), a control experiment was performed. PVK agar plates without phosphate source were prepared and soil samples (from the same source as before) diluted up to 10^{-3}, 10^{-4} and 10^{-5} were prepared separately and were inoculated on the plates. The plates were checked for microbial growth after incubation.

Inoculation and characterization of the microbes: The serially diluted soil solutions (10^{-3}, 10^{-4} and 10^{-5}) were inoculated onto PVK agar plates some of which did not have any phosphate source while the other contained phosphate. Similarly, the subsurface colonies were inoculated in deep PVK agar tubes containing L-Cysteine, an oxygen scavenging agent which helped in maintaining an anaerobic environment in the deep tubes. The deep tubes were also of two types- those that had phosphate and those that didn't. Rainwater and hail were added separately to the plates and the tubes lacking phosphate source. For these, the phosphate solubilization index was also calculated (as given in tables 1, 2 and 3). Also, the fungal and bacterial colonies which were observed were then characterized by means of microscopic observation and biochemical tests.

Measurement of pH: Soil sample was prepared with dilutions of 10^{-3}, 10^{-4} and 10^{-5}. These were inoculated separately into three sets of test tubes (each set having five test tubes) containing Pikovskaya's broth in three different compositions: (a) having all the ingredients and rainwater as the phosphate source instead of tricalcium phosphate (b) having all the ingredients and hail as the phosphate source instead of tricalcium phosphate and (c) having all ingredients and tricalcium phosphate as the phosphate source (control set). For five consecutive days, one test tube from each of the three sets was taken, the pHs of the PVK broth inside them were measured and then discarded.

RESULTS AND DISCUSSION

PVK agar plates and in PVK agar deep tubes:

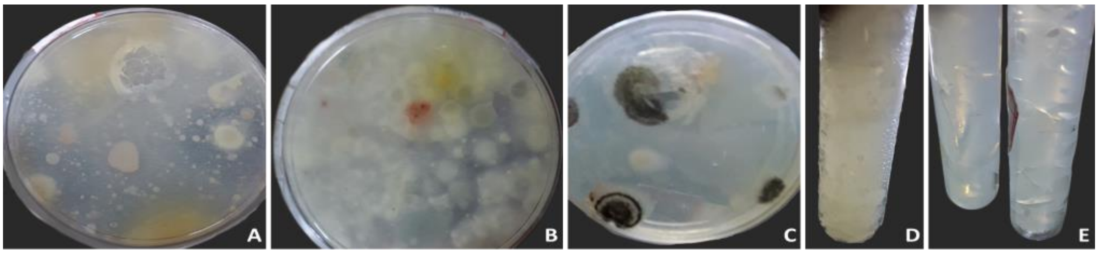


Fig 1. (A) Bacterial colony with surrounding halo (control experiment: tricalcium phosphate used as phosphate source) **(B)** Bacterial colony with surrounding halo (rainwater used as phosphate source) **(C)** Bacterial and fungal colony with surrounding halo (hail used as phosphate source) **(D)** Visible cracks in the deep tube PVK agar indicating the growth of anaerobes (control experiment: tricalcium phosphate used as phosphate source) **(E)** Visible cracks in the deep tube PVK agar indicating the growth of anaerobes (hail (on left) and rainwater (on right) used as phosphate source)

Characterization of the microbes: Both anaerobic and aerobic bacteria as also fungi were obtained. These are outlined in table 1. Some of the isolated microbes are presented in the figure below.

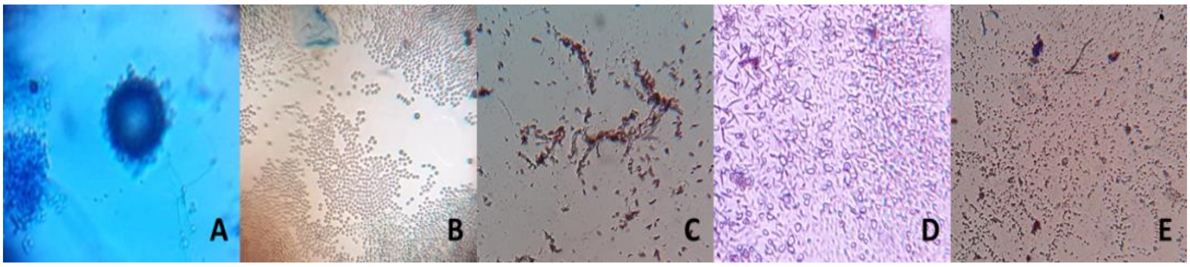


Fig 2. (A) *Aspergillus* spp. **(B)** *Saccharomyces* spp. **(C)** *E. coli* **(D)** *Actinomyces* spp. **(E)** *Pseudomonas* spp.

PVK plates with no phosphate source and soil inocula: The PVK plates without any phosphate source in which soil samples were inoculated did not show any microbial growth even after 5 days of incubation as shown below. This clearly indicates that soil phosphate alone is not enough to support microbial growth and rainwater and hail indeed provided phosphate to the microbes helping them to grow.

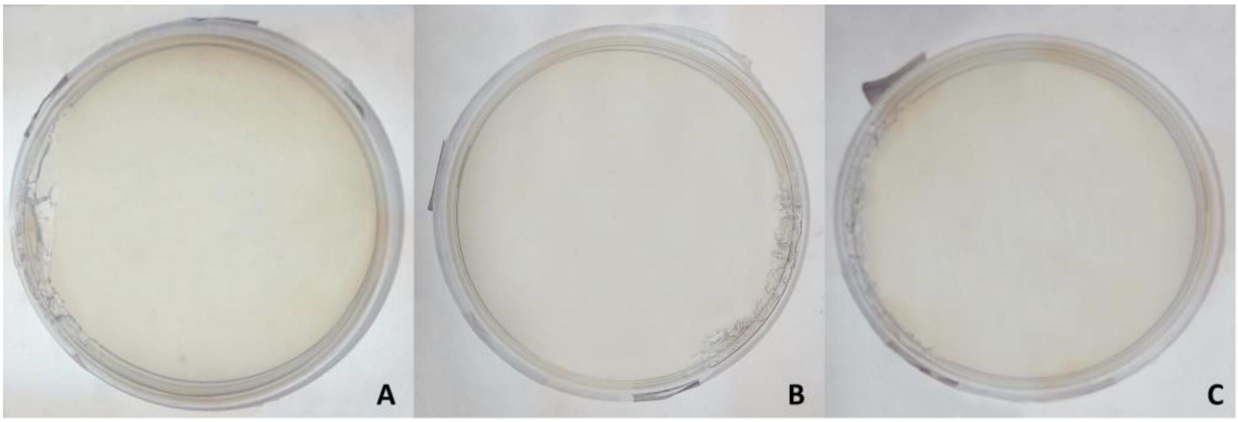


Fig 3. Pikovskaya's agar plates inoculated with: **(A)** Soil diluted to 10^{-3}, **(B)** Soil diluted to 10^{-4} and **(C)** Soil diluted to 10^{-5}

pH measurements of PVK broths with soil inocula: The pH of all the soil samples (that is, soil samples diluted to 10^{-3}, 10^{-4} and 10^{-5}) decreased over 5 days (as given in tables 4, 5 and 6) indicating the production of organic acid due to solubilization of phosphate by the microbes present in the soil samples. The comparisons are shown in graphs 2, 3 and 4. The PSI of bacteria for rainwater, hail and control experiment were compared as given in graph 1.

Table 1: calculation and comparison of Phosphate Solubilising Index (PSI) of bacteria growing on Pikovskaya's agar plates using rainwater as a phosphate source

Dilution of soil sample	Diameter of colony (CD in cm)	Diameter of halo zone (HD in cm)	PSI= (CD + HD) / CD*	Mean	Standard Deviation	Variance	Standard Error
10^{-3}	0.6	0.8	2.33	2.38	0.14	0.0208	2.38 ± 0.07
	0.5	0.6	2.20				
	0.5	0.7	2.40				
	0.5	0.8	2.60				
10^{-4}	0.7	1.0	2.43	2.88	0.49	0.2408	2.88 ± 0.22
	0.3	0.8	3.67				
	0.6	0.9	2.50				
	0.7	1.1	2.57				
	0.4	0.9	3.25				
10^{-5}	0.5	0.7	2.40	3.05	1.13	1.2683	3.05 ± 0.565
	0.3	1.2	5.00				
	0.9	1.3	2.44				
	1.1	1.5	2.36				

***CD = Colony Diameter; HD = Halo zone Diameter**

Table 2: Calculation and comparison of Phosphate Solubilising Index (PSI) of bacteria growing on Pikovskaya's agar plates using hail as a phosphate source

Dilution of soil sample	Diameter of colony (CD in cm)	Diameter of halo zone (HD in cm)	PSI= (CD + HD) / CD*	Mean	Standard Deviation	Variance	Standard Error
10^{-3}	0.4	0.9	3.25	3.1	0.24	0.0576	3.1 ± 0.12
	0.5	0.9	2.8				
	0.3	0.7	3.33				
	0.4	0.8	3				
10^{-4}	0.5	0.8	2.6	2.53	0.13	0.0169	2.52 ± 0.07
	0.7	1.0	2.43				
	0.3	0.5	2.67				
	0.5	0.7	2.4				
10^{-5}	0.5	0.9	2.8	3.03	0.32	0.1024	3.03 ± 0.16
	0.4	1.0	3.5				
	0.6	1.1	2.83				
	0.7	1.4	3				

***CD = Colony Diameter; HD = Halo zone Diameter**

Table 3: Calculation and comparison of Phosphate Solubilising Index (PSI) of bacteria growing on Pikovskaya's agar plates using tricalcium phosphate as phosphate source (control experiment)

Dilution of soil sample	Diameter of colony (CD in cm)	Diameter of halo zone (HD in cm)	PSI= (CD + HD) / CD*	Mean	Standard Deviation	Variance	Standard Error
10^{-3}	0.5	0.9	2.8	2.9	0.14	0.0196	2.9 ± 0.10
	0.4	0.8	3				
10^{-4}	0.5	0.7	2.4	2.37	0.05	0.0025	2.37 ± 0.04
	0.6	0.8	2.33				
10^{-5}	0.7	1.3	2.86	3.05	0.28	0.0784	3.05 ± 0.19
	0.4	0.9	3.25				

***CD = Colony Diameter; HD = Halo zone Diameter**
Note: Total 31 isolates were obtained

Table 4: pH measurement (for five consecutive days) of soil samples (of different dilutions) inoculated into Pikovskaya's broth where rainwater was used as the phosphate source

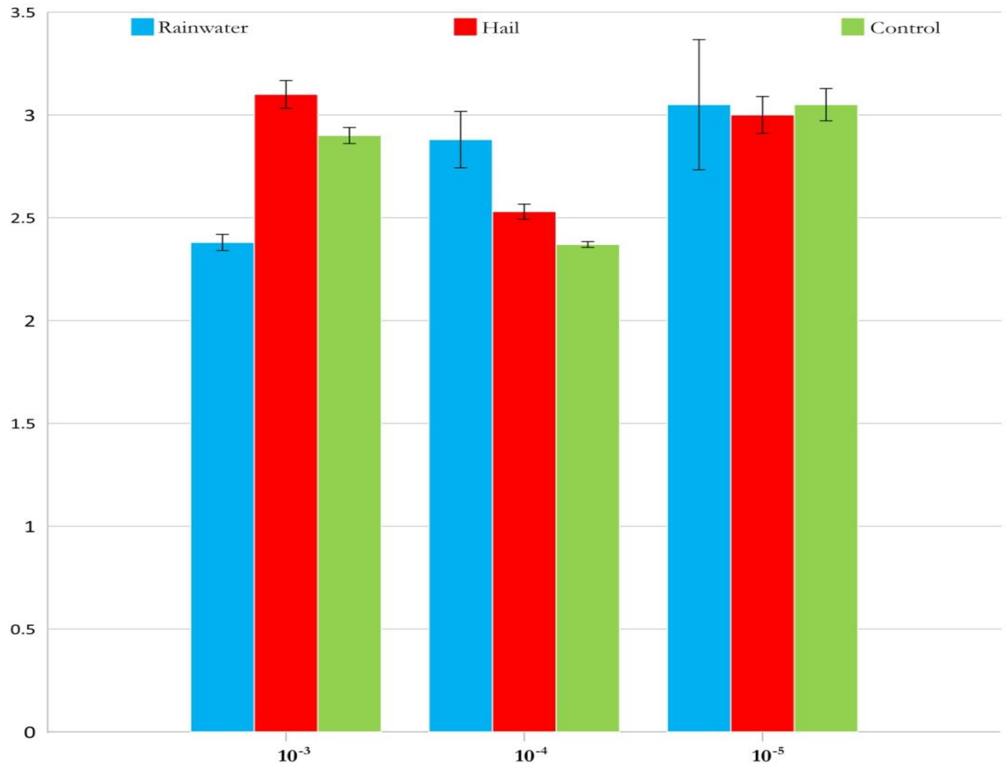
Dilution of the soil sample	Day	pH
10^{-3}	0	7.20
	1	6.12
	2	5.59
	3	5.22
	4	5.08
	5	4.85
10^{-4}	0	7.20
	1	6.77
	2	6.01
	3	5.63
	4	5.45
	5	5.23
10^{-5}	0	7.20
	1	6.84
	2	6.55
	3	6.29
	4	6.10
	5	5.98

Table 5: pH measurement (for five consecutive days) of soil samples (of different dilutions) inoculated into Pikovskaya's broth where hail was used as the phosphate source

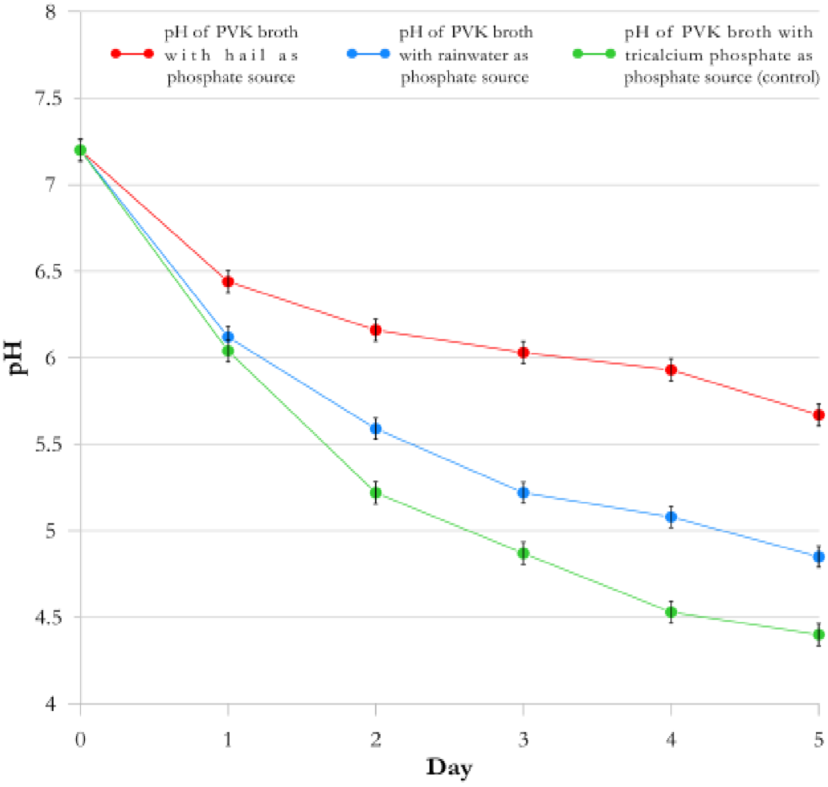
Dilution of the soil sample	Day	pH
10^{-3}	0	7.20
	1	6.44
	2	6.16
	3	6.03
	4	5.93
	5	5.67
10^{-4}	0	7.20
	1	6.87
	2	6.54
	3	6.28
	4	6.11
	5	5.96
10^{-5}	0	7.20
	1	6.90
	2	6.75
	3	6.56
	4	6.39
	5	6.24

Table 6: pH measurement (for five consecutive days) of soil samples (of different dilutions) inoculated into Pikovskaya's broth where tricalcium phosphate was used as the phosphate source (control experiment)

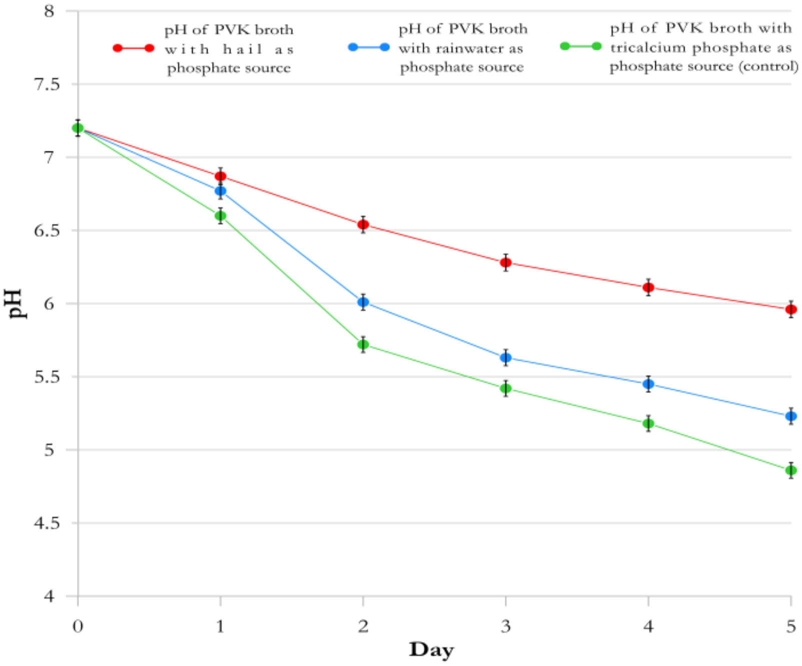
Dilution of the soil sample	Day	pH
10^{-3}	0	7.20
	1	6.04
	2	5.22
	3	4.87
	4	4.53
	5	4.40
10^{-4}	0	7.20
	1	6.60
	2	5.72
	3	5.42
	4	5.18
	5	4.86
10^{-5}	0	7.20
	1	6.70
	2	6.34
	3	6.10
	4	5.58
	5	5.32



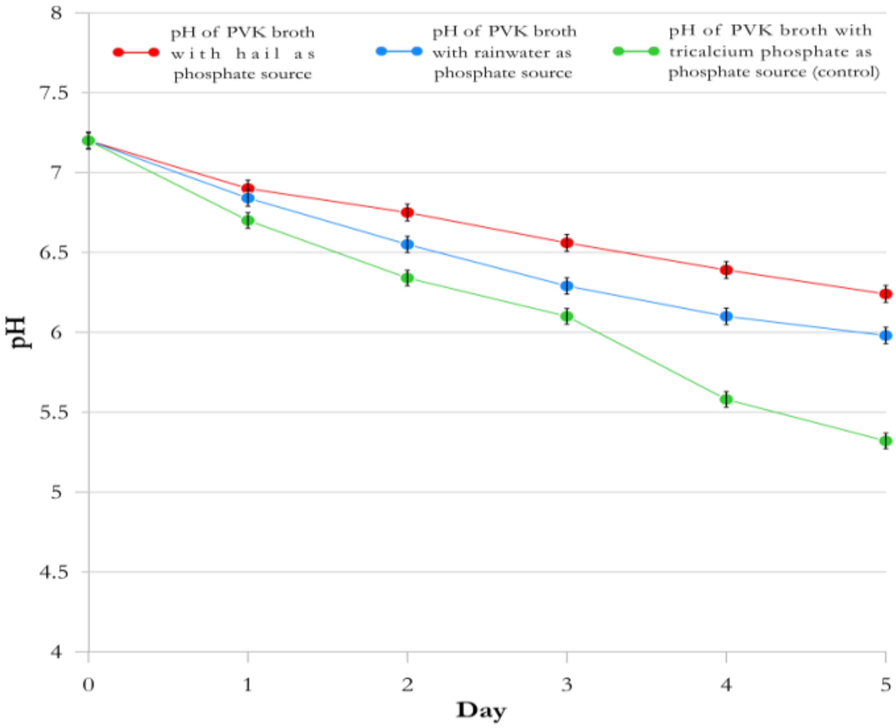
Graph 1: Comparison of Phosphate Solubility Indices (PSIs) of bacteria growing on Pikovskaya's agar plates using rainwater, hail, and tricalcium phosphate (control experiment) as phosphate source for different dilutions of soil sample (the vertical bars represent the standard deviation of the data)



Graph 2: Comparison of decrease of pH over five consecutive days for soil sample diluted to 10^{-3} and inoculated into three sets of Pikovskaya's broths containing rainwater, hail and tricalcium phosphate (control) as the phosphate source respectively (the vertical bars represent the standard deviation of thedata)



Graph 3: Comparison of decrease of pH over five consecutive days for soil sample diluted to 10^{-4} and inoculated into three sets of Pikovskaya's broths containing rainwater, hail and tricalcium phosphate (control) as the phosphate source respectively (the vertical bars represent the standard deviation of the data)



Graph 4: Comparison of decrease of pH over five consecutive days for soil sample diluted to 10^{-5} and inoculated into three sets of Pikovskaya's broths containing rainwater, hail and tricalcium phosphate (control) as the phosphate source respectively (the vertical bars represent the standard deviation of the data)

DISCUSSIONS

From the above results, we might conclude that the terrestrial microbes can solubilize phosphate from rainwater or hail into an available or usable form which is orthophosphate or P_i. Their unique ability to thrive on rain water and hail derived phosphate is similar to their ability to utilize artificially provided phosphate which is indeed a novel discovery. This idea of these microbes utilizing the rain water and hail derived phosphate is not only proved by their unique halo zone on the plates but also by the change in their pH over a specific period of time**.** From the above results, we can conclude that the terrestrial microbes can solubilize phosphate from rainwater or hail into an available or usable form which is orthophosphate or P_i. The hypotheses that early earth lacked soil and major nutrients such as phosphate came to earth from extra-terrestrial sources can also be verified to some extent from the conclusions of the present study. The extra-terrestrial origin of life and the claim that the first life forms on earth learned the methods of obtaining nutrition in their extra-terrestrial residence can also not be ignored. Our aim was to prove that if terrestrial bacteria can utilize phosphate from rainwater then this phosphate might have generated in extra-terrestrial space, given the fact that phosphorous undergoes a sedentary cycle and that no phosphorous is generated in the atmospheric form on earth. This can be extrapolated by stating that if terrestrial microorganisms can solubilize extra-terrestrial phosphate from rainfall, then they might have been utilizing it for long in extra-terrestrial space. In another way, it can be said that extra-terrestrial microorganisms capable of utilizing phosphate might have accelerated along with interplanetary dust particles into earth's gravitational field. Kani Rauf *et al*.,2010 stated that stratospheric particles are Interplanetary Dust Particles (IDPs) comprising an assortment of materials among which are included microfossil-like features in variable sizes and forms, such as coccoids, rods, and filaments. This is supported by the fact that naked plasmid DNA can remain intact after reaching earth from the space (Thiel CS *et al*., 2014 and Wainwright M *et al*., 2006). These microorganisms might have evolved along with the changing conditions of earth thus acquiring the ability to utilize terrestrial phosphate, but still retaining the ability to solubilize extra-terrestrial phosphate. Finally, we would like to add that this work may be further extended by collecting soil samples from different geographies and following the same procedure to check if the microbes in different types of soils have the same phosphate solubilizing capacity. Further research may be done regarding the crystal packing of hail structure that can trap phosphate efficiently and also better knowledge about the phosphate utilization system of the microorganisms is required to understand the pathway by which they can convert the phosphorus present in the rainwater and hail for utilization in their metabolic processes. Also, the relation between the types of soil the nutrients present in (particularly the natural phosphate content) can also be obtained by studying the phosphate-solubilizing ability of the microbes of that soil and also the total rainfall received by it.

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