A simple differential production method of silicon utilizing organisms for future use in lunar settlements

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Abstract:
Silicon utilizing organisms are well known to tolerate different stress. A classification of these organisms indicates important groups of diatoms, horsetails, lichens and many species of protozoa, sponges, monocotyledons and gram-positive bacteria. Role of silicon in living creatures is still not clearly understood, but recent experiments revealed distinct pathways of silicon utilization including demonstration of silicon transporter genes in them. Some of these organisms particularly specific stress tolerated diatoms can be confined from environment in 0.5% and 2.0% sodium metasilicate solutions and produced in a large scale by photo induced growth activation after a long nutritional and water stress for use in lunar settlements. An orderly arrangement of these organisms for serial applications on the Moon will help in terraforming on it. A planned follow up of this venture is necessary to understand the complex microenvironment, which will be produced by them.

Key words: Silicon utilizing organisms, lunar settlements, diatoms

1. Introduction

Silicon utilizing organisms may be defined as organisms with high silicon content (≥ 1% dry weight) and they can metabolize silicon with or without demonstrable silicon transporter genes (SIT) in them. It is well known that organisms with high silicon content can survive in extremes of temperature, pressure, radiation, pH, salinity and nutrient conditions. In fact, Reynolds described temperature tolerance of silicon compounds in living creatures as early as in 1893. Thus organisms with high silicon content can aptly be utilized within compromised artificial environments in extraterrestrial situations. There are distinct silicon accumulator plants like Equisetaceae, Cyperaceae, Gramineae, Juncaceae, and organisms like diatoms, and porifera contain enough silicon, in the range of 6 – 43.7% dry weight. A classification of silicon utilizing organisms is given in Table 1.

There are some similarities between carbon and silicon as they both belong to period IV of the periodic table. Although carbon compounds are abundantly found in living creatures on earth and they are the basis of evolution of life on it, there

was at least a minor role of silicon compounds in the development of the primitive forms of life when earth was quite inhospitable for the development of carbon based life [1]. Even today silicon maintains a “nutrient profile” similar to nitrate and phosphate in ocean.

Only few minerals are found more than 1% in plants- mainly N, K, Si, Ca and Na. While ecological aspects of N, K, Ca, Na are well known, but role of silica in terrestrial ecosystems is not sufficiently understood. It was found that silicon-utilizing organisms can adapt after repeated subcultures and sometimes after blind passages on silicate medium with carbon free constituents having little nitrogen and phosphates, probably with the help of a trace amount of carry-over carbon during inoculation procedures. When silicon level was studied in such grown up cells on carbon “free” silicate medium by electron prove microanalyser following thorough washing procedures, it was found that silicon in cells grown on carbon “free” silicate medium was much higher (24.9%) than those grown on conventional carbon based medium (0.84%) [2]. In many cases appearance of growths of different microorganisms was earlier on silicate medium in comparison to their growths on conventional carbon based media (Table 2, unpublished), however, further growth was always less on silicate media [3,4]. On carbon “free” silicate medium fungi produced altered morphological patterns (unpublished).

Silica is glassy and transparent to visible light but impermeable to radiations. Silicon utilizing organisms can tolerate different types of radiations and it was found that although there are metabolic changes in increased radiation, it gives a positive impact on the nutritional quality owing to reduction of C:P ratio. Silica can provide resistance against all other physical stress and it helps in tolerating water stress [5]. Silicon utilizing organisms can grow in anaerobic condition.

Microgravity situation may induce less lignin formation in plants without affecting their growth significantly. Commercial gardening experiment in international space stations indicated that seed to seed life cycle is possible in space. Plants may help in bioregenerative life support system not only by releasing precious oxygen but they also help in recycle drinking water.

The Moon contains oxygen (42.6%), magnesium (20.8%), silicon (20.5%), iron (9.9%), calcium (2.31%), aluminium (2.04%), nickel (0.472%), chromium (0.314%), manganese (0.131%), titanium (0.122%), many chemicals in trace amounts and possible presence of water in polar craters. The dark colored lunar basalts, which are igneous rocks, are mainly silicates and they also contain iron, titanium, zirconium, uranium and lanthanum. Lunar sedimentary rocks are equivalent to regolith, which also contains large amounts of silicon. Titanium present on the Moon may enhance growth of silicon utilizing organisms on solid surfaces.

Silicon biomineralization occurs as ‘biologically controlled biomineralization’ where silicon is precipitated to serve some physiological purpose and it may also occur as ‘biologically induced biomineralization’ [6] as a byproduct of cell’s metabolic activity or through its interactions with the surrounding chemicals.

In biologically controlled mineralization, there is an overwhelming control of the

microorganism on nucleation and mineral growth stage [7]. There is delineation of space as intracellular silica deposition vesicle (SDV) for the locus of mineralization, which is sealed off from external environment. After this silicate is sequestered and transferred to the mineralization site by energy driven (energy may be derived from photosynthesis in daylight and from glucose metabolism in absence of daylight) pump mechanism in presence of specific transporter protein [8].
In biologically induced biomineralization at first there is silicon nucleation in which there is spontaneous growth of some critical nuclei which are resistant to rapid dissolution. Then growth of these critical silicon nuclei occurs if the ions are same or there will be precipitation over the critical nuclei if the ions are different. Ultimately the initial amorphous phase will be converted into crystalline phase.

Silicon deposition may also occur due to Ostwald ripening. If silica concentration is more than the solubility of amorphous silica (at 100°C ~ 380 mg L^{-1}), monomeric silica [$Si(OH)_4$] is formed which is converted into oligomers (dimers, trimers and tetramers) by polymerization. Ultimately large polymers of silanol (-Si-OH-) and siloxane (-Si-O-Si-) will be formed. Silicification occurs by hydrogen bonding with neutrally charged polysaccharides [9], by cation bridging where cell wall is the outermost layer [10] or by direct electrostatic interactions with cationic amino groups present in protein-rich biofilms [11].

Diatom silicon transporters (SITs) are membrane-associated proteins that directly transport silicic acid [8]. Specific transport enzymes will promote silicification in a supersaturated state of silicon, thus rate of silicification within diatoms is 10^6 times higher than abiological formation from supersaturated solution [12]. There are five SIT genes – cfSIT1-5 having 10 transmembrane segments, one intracellular N terminus, and one intracellular C-terminal coiled –coil motif in *Cylindrotheca fusiformis*. SIT genes of other diatoms are basically same, although coiled-coil motif may be absent [13] . Slicon transporter gene of rice is also described recently [14]. SDV membrane or the Silicalemma contains different proteins and when external silica is low they are increased in amount [15]. Different types of polypeptides known as silaffins and long-chain polyamines (LCPA) are found in embedded proteins of silica matrix after dissolving it with hydrofluoric acid from purified frustules of diatoms. Silaffins 1A, 1B, 2, 1H, 1L, and LCPA can promote rapid precipitation of silica [15, 16,17,18]. Some native silaffins (Nat Sil-1A and 2), which are regulatory molecules of LCPA, are also obtained after treatment of frustules with ammonium fluoride.

It is very difficult to explain the reason for this silicification; probably it was developed in more silica rich hydrosphere during the Cambrian (110 mg L^{-1} at <25°C which declined gradually to 5 mg L^{-1} in recent times) [19] mainly to construct cell walls with less energy consumption and helping photosynthesis by forming CO_2 from bicarbonates [20], besides acting as armour against predation by zooplankton [21].

Considering all above-mentioned facts it appears that silicon-utilizing organisms can be used in lunar settlements. Thus in this paper a production method of silicon utilizing organisms has been studied and their orderly use on the Moon has been described.

Table 1 Classification of silicon utilizing organisms:

Algae

Phylum Bacillariophyta
Class Centrobacillariophyceae
Cells in valve view circular, ovoid, triradiate or polygonal
Actinocyclus, Actinoptychus, Anthodiscus, Asteromphalus, Astrolampra, Altheya, Bacteriastrum, Bellerochea, Bergonia, Biddulphia, Cerataulina, Chaetoceros, Climacodium, Corethron, Coscinodiscus, Cyclotella, Ditylum, Entogonia, Eucampia, Guinardia, Hemidiscus, Lauderia, Leptocylindrus, Melosira, Planktoniella, Pyrgodiscus, Pyxidicula, Rhizosolenia, Roperia, Sceletonema, Stephanodiscus, Stephanopyxis, Syndetocysis, Thalassiosira, Triceratium.
Class Pennatibacillariophyceae
Cells elongate, bilaterally symmetrical in valve and girdle view
Order Pennales (Pennate diatoms)
Araphidineae : Pseudoraphe present
Amphicampa, Asterionella, Campylosira, Cerotoneis, Cymatosira, Diatoma, Fragilaria, Grammatophora, Liemophora, Meridion, Peronia, Pseudoeunotia, Raphoneis, Rhabdonema, Striatella, Synedra, Tabellaria, Thalassionema, Thalassiothrix, Tetracyclus.
Raphidioidineae : Rudimentary raphe at cell ends.
Actinella, Eunotia
Monoraphidineae : Raphe on one valve and pseudoraphe on other
Achnanthes, Cocconeis, Eucocconeis, Rhoicosphenia.
Biraphidineae : Raphe on both valves
Amphipleura, Amphiprora, Amphora, Anomoeoneis, Bacillaria, Caloneis, Campylodiscus, Cylindrotheca, Cymatopleura, Cymbella, Denticula, Didymosphenia, Diploneis, Epithemia, Fragilariopsis, Frustulia, Gomphonema, Gyrosigma, Hantzschia, Mastogloia, Navicula, Neidium, Nitzschia, Phaeodactylum, Pinnularia, Pleurosigma, Rhopalodia, Scoliopleura, Strauroneis, Stenopterobia, Surirella, Tropidoneis.
Phylum Chrysophyta
Class Chrysophyceae : Cell wall often contain siliceous plates, Endogenous siliceous cysts formed in some of them.

Protozoa

Flagellated protozoa
Phylum Heterokonta : In classification of animal kingdom Chrysophyta and many members of Bacillariophyta are included in the phylum Heterokonta
Phylum Choanoflagellida-Siliceous theca
Ameboid protozoa
Phylum Actinopoda
Class Polycystinea: Marine radiolarians with a siliceous skeleton – *Thassicola, Collozoum, Sphaerozoum*
Class Phaeodarea: Marine radiolarians with a siliceous skeleton - *Aulacantha*
Class Heliozoea : Some contains siliceous scales and spines

Sponges

Class Hexactinellida (Hyalospongiae, glass sponges): Siliceous fibers
Euplectella (Venus's flower basket) belonging to this group. These are the dominant sponges in the Antarctic.
Class Demospongiae : 90% sponge species are belonging to this group. Many of them contain siliceous spicules
Class Sclerospongiae : Siliceous spicules

Plants

Kingdom: Plantae,
Division: Pteridophyta, Class: Equisetopsida, Order: Equisetales, Family: Equisetaceae, Genus: *Equisetum*
Division: Magnoliophyta
Class: Liliopsida,
Order: Poales,
Family: Juncaceae, Genera: *Andesia, Distichia, Juncus* , *Luzula* , *Marsippospermum, Oxychloe, Prionium, Rostkovia.*
Family: Poaceae, Subfamilies: Arundinoideae, Bambusoideae (91 genera, about 1000 species), Centothecoideae, Chloridoideae, Panicoideae, Pooideae(Genus *Triticum,*Genus *Secale*, *S. cereale*), Stipoideae, There are about 600 genera and 10,000 species of grasses; Genus *Saccharum* and Genus *Oryza* are within this family.
Family: Bromeliaceae, Genus: *Aechmea,* Species: *A. fasciata*
Family: Cyperaceae (About 70 genera and 4,000 species)
Order: Zingiberales
Family: Marantaceae, Genus: *Calathea* (13 species)
Order: Alismatales
Family: Araceae, Genus: *Spathiphyllum* (40 species), Genus: *Anthurium* (About 800 species)
Order: Asparagales
Family: Agavaceae, Genus: *Chlorophytum,*Species: *C. comosum*
Family: Laxmanniaceae, Genus: *Cordyline* (15 species).
Family: Ruscaceae, Genus: *Deacaena*
Class: Magnoliopsida
Order: Apiales
Family: Araliaceae, Genus: *Hedera,* Species: *H. helix,* Genus: *Schefflera* (About 700 species)
Order: Rosales
Family: Rosaceae, Genus: *Rosa* (About 150 species), Genus: *Fragaria* (About 20 species)
Order: Sapindales
Family: Rutaceae, Genus: *Citrus*
Order: Cucurbitales
Family: Cucurbitaceae, Genus: *Cucumis,* Species: *C. sativus*
(Monocotyledons contain 10-20 times as much Silicon as dicotyledons.)

Fungi

Kingdom: Fungi
Phylum: Ascomycota
Class: Eurotiomycetes
Order: Eurotiales
Family: Trichocomaceae, Genus: *Aspergillus (*200 species)
Order: Moniliales
Family: Moniliaceae, Genus*: Penicillium* (14 species)
Family: Dematiaceae, Genus: *Cladosporium*
Class: Dothideomycetes
Order: Pleosporales, Genus: *Alternaria* (44 species)
Lichens: Crustose, Filamentose, Foliose, Fructicose, Leprose, Squamulose, Gelatinose.
Gram positive bacteria: Almost all gram positive bacteria can utilize silicon; however, it is very prominent in *Mycobacterium* spp.

Table 2: Appearance of growth of some microorganisms on carbon "free" silicate medium (average differences in days from conventional media are given in parenthesis)

Growth on silicate medium is earlier than that on conventional media : *Mycobacterium scrofulaceum* (7), *M. flavescens*(2), *M. avium*(7), *M. xenopi*(2), *M. tuberculosis*(4).

Growth on silicate medium is similar to that on conventional media : *M. marinum*, *M. gordonae*, *M. intracellulare*, *M. terrae*, *M. triviale*, *M. fortuitum*, *Bacillus subtilis*, *B. pumilus*, *Lactobacillus casei*, *Aspergillus* spp., *Penicillium notatum.*

Growth on silicate medium is delayed than that on conventional media : *M. smegmatis*(1), *Streptomyces rimosus* (4), *S. venezuale*(6), *Nocardia asteroides*(1), *N. brasiliensis*(2), *N. caviae*(2), *Rhizopus* spp. (9), *Trichophyton rubrum*(2), *T. violaceum*(2), *T. tonsurans*(2), *T. mentagrophytes*(2).

2. General methods

2.1. Stress adaptive "enrichment" of silicon-utilizing organisms in sodium metasilicate solutions

Silicon utilizing organisms can thrive in sodium metasilicate, Fluka (SM) solution as high as up to 4% concentration (personal observation). To confine common silicon utilizing organisms from the environment for future use in lunar settlements SM solutions of four different concentrations- 0.5%, 1%, 2% and 4% were prepared in tap water. SM solutions and control water were kept in wide mouth plastic (polyethylene, transparent, high density, permeability of CO_2/H_2O low; 0.6/5.4 mmol mm $s^{-1}m^{-2}Pa^{-1}$; water absorption 0 mmol $kg^{-1}day^{-1}$) containers in several lots (10 each), they were exposed to air for 24 hours then they were closed with airtight caps and kept in dim lighted areas for as long as 5 years in ambient temperature for "enrichment" of a selective group of stress adaptive silicon utilizing organisms. This five-year "enrichment" was calculated to allow proper adaptation in a long period of stress following my experiences in previous works.

2.2 Rapid photo induced growth

After five years of stress the organisms present in different solutions were exposed to daylight (~ 500 µmol $m^{-2}s^{-1}$ PPF 12 hours each day, PPF-photosynthetic photon flux) for one month following diurnal rhythm.

2.3 Morphological and cultural examinations of the photo induced growth

After exposure to sunlight macroscopic and microscopic studies of the growths were done directly and after standard staining and cultural procedures for different microorganisms as described elsewhere [22,23,24,25].

2.4. Analysis of different SM solutions and control water after photo induced growth

All the procedures of chemical analysis were adopted from the standard method for examination of the water and waste water (APHA-AWWA-WPCF) published by American Public Health Association. Arsenic estimation was done according

to the Indian Standard 3025:1986 (Third Revision) and bacteriological test-total coliform count was done following the guidelines of All India Hygiene & Public Health, Kolkata, India. Test for hardness could not be performed due to interference of high alkalinity and metal ions in silicate solutions.

2.5. Recovery following prolonged dry stress

The silicon utilizing organisms in 0.5% and 2.0% SM solutions (organisms of 1% and 4% SM solutions were not considered, see below) after their photo induced growth were allowed to dehydrate preventing any contamination from outside, in sterile laminar flow chamber, and loosened caps were covered with sterile “micro pore membrane”. After complete dehydration 5 lots were kept for 6 months and another 5 lots were kept for one year in shades and then sterile distilled water was added in each container up to their original levels, and then they were again exposed in sunlight with diurnal rhythm and the growths were studied as before.

3. Results

3.1 Growths in different SM solutions and in control water

Different varieties of organisms grew in different concentrations of SM solutions- from a dense deep green color growth in control medium to light green color growth in 0.5% SM solution (Fig.1), yellow color growth in 1% SM solution, orange color growth in 2% SM solution (Fig.3) and a scanty whitish color growth in 4% SM solution. In comparison to the control medium diatoms were markedly increased (~4 times) in both 0.5% and 2.0% silicate solutions but in 0.5% silicate solution bigger sized diatom (Fig.2) species grew selectively than those (Fig.4) in 2.0% silicate solutions. Phytoplanktons other than diatoms were more in the control medium than that in silicate solutions (Graph-1). Gram positive bacteria particularly the acid-fast variety grew abundantly in 1.0% silicate solutions. They were mostly *Mycobacteria* belonging to Runyon Group II (scotochromogen) and saprophytic group. Coliforms were less in all the silicate solutions. Scanty green biofilms were present in 0.5% and 1.0% silicate solutions while it was very prominent in the control medium. *Aspergillus* spp. predominates in 0.5%, 1.0% and 2.0% silicate solutions while *Rhizopus* spp.was the predominant one in the control medium and scanty fungi of diverse varieties were set up in 4% SM solution.

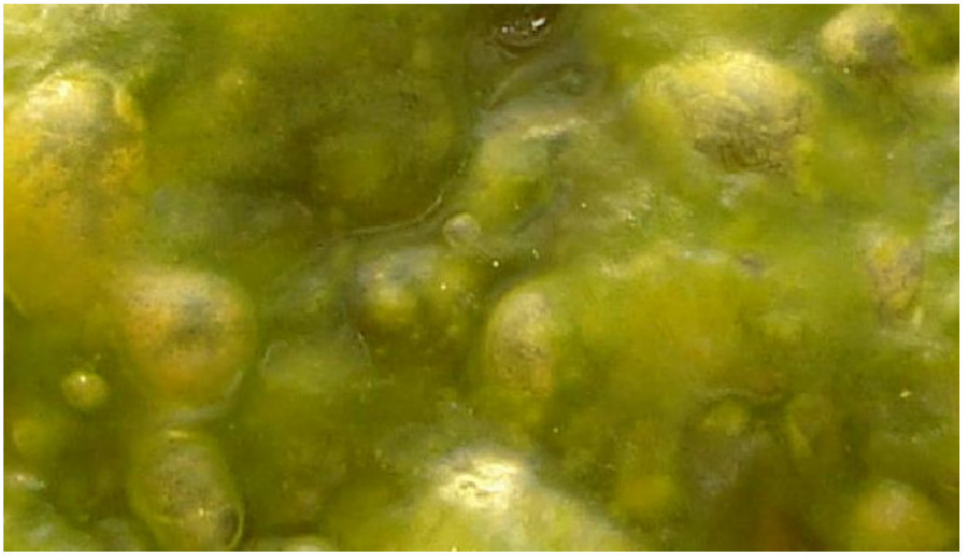


Fig. 1. Photo induced growth in 0.5% SM solution.

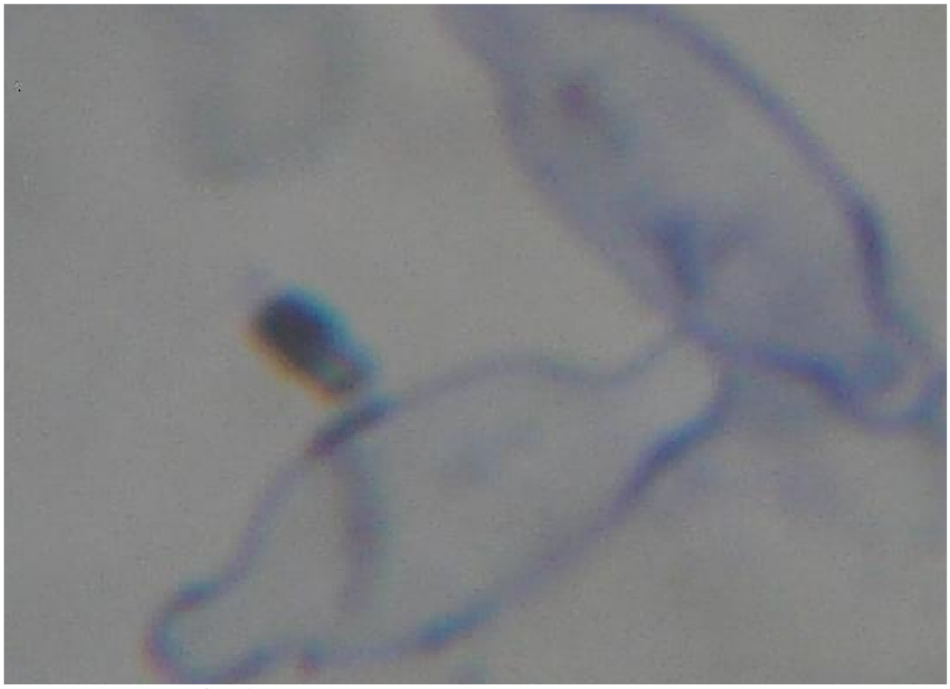


Fig. 2. Diatoms of 0.5% SM solution

Fig. 3. Photo induced growth in 2.0% SM solution.

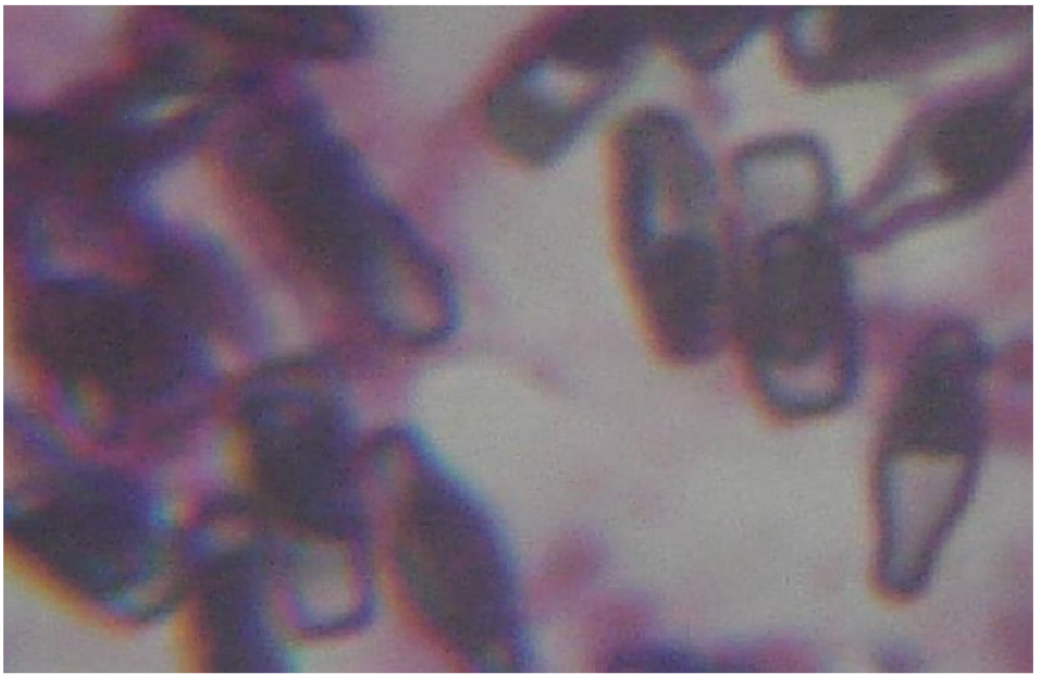
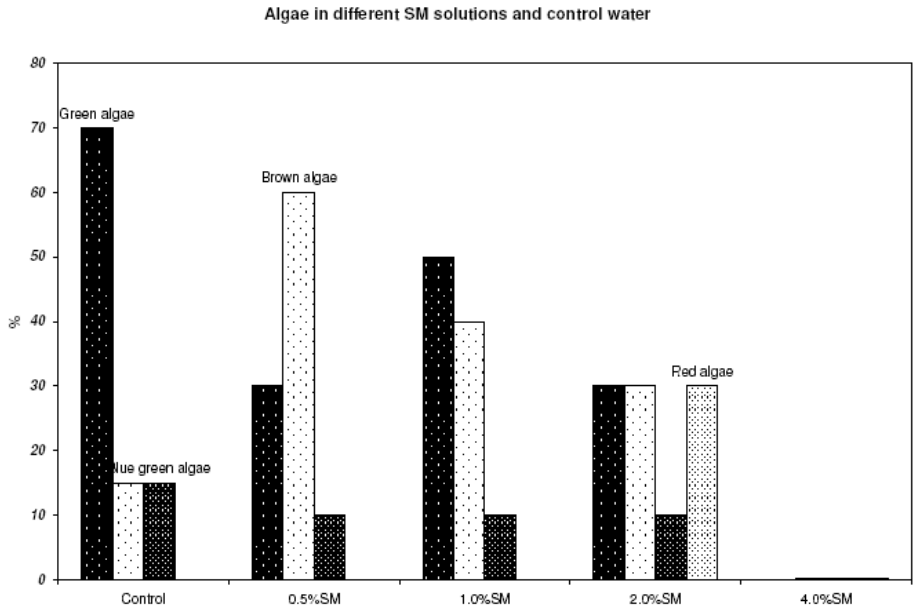


Fig. 4. Diatoms of 2.0 % SM solution



Graph 1 : Algae in different SM solutions

3.*2 Analysis of different SM solutions and control water*

Analysis of different SM solutions after photo induced growth is given in Table-2. While hardness of water could not be measured in silicate solutions, pH was found almost normal in 2% solution, but it was still higher even after 5 years in other silicate solutions, Chlorides were more or less normal in all solutions except in 4% solution where it was very high. There was about 50% increase of sulfate in all the SM solutions, 25-50% increase of nitrate, normal iron levels in 0.5% and 1% solutions, markedly increased and markedly decreased (both about 4 times) amount of iron in 2% and 4% solutions respectively.

3.3 Recovery after prolonged dry stress

In the study of recovery of silicon utilizing organisms after prolonged dry stress, it was found that all the previously isolated organisms could be recovered from dried 0.5% and 2% SM solutions after photo induced growth.

Table 3: Chemical analysis and coliform count of different silicate solutions and the control fluid after photo-induced growth

	Control	0.5% Si Sol.	1.0% Si Sol.	2.0% Si Sol.	4.0% Si Sol.
pH	7.1	7.7	12.5	7.9	13.2
Hardness (as CaCO3), mg/L	08	-[a]	-	-	-
Chloride (as Cl), mg/L	164.94	158.6	154.97	184.9	669.79
Sulfate (as SO4), mg/L	43	66	68	61	71
Fluoride (as F), mg/L	-[b]	-	-	-	-
Nitrate-Nitrogen (as NO3-N), mg/L	18	22	21	31	28
Iron (as Fe), mg/L	1.06	2.10	1.25	4.01	0.34
Arsenic (as As), mg/L	<DL[c]	<DL	<DL	<DL	<DL
Total coliform organism (MPN/dL)	>PL[d]	>PL	>PL	>PL	>PL

[a]for hardness results see the description in the text, [b]Fluoride was not detected, [c]<DL : below detection limit ; [d]>PL : above permissible limit, <10 MPN/dL.

4. Discussion

4.1 Necessity of production of silicon utilizing organisms for lunar settlements

In this study silicon utilizing organisms adapted to nutritional and water stress were produced in a large scale following a 'simple' isolation technique from the environment for their possible use on lunar surface or elsewhere. Silicon utililizing organisms were selected for micro- and macro-terraforming on the Moon as they were found suitable to tolerate environmental stress and their ecotypes have already undergone various mutations against different kinds of stress throughout their stay on earth for millions of years.

Constantly changing environment in recent times resulting severe storms, floods, fires, earthquakes, volcanic eruptions, toxic chemicals etc. in earth are probably the 'symptoms' of sickness in coordination with the 'Gaia theory', indicating that our world may be a hostile place to us in coming years. A change in sea level is also now critical as was found before occurrence of all mass extinctions on earth in the Phanerozoic [26]. Again there are possibilities of a major catastrophe if there is an impact with a materiorite in near future. Considering all these aspects it is obvious that mankind should find out other areas away from earth for habitations. This is also true that without terraforming mankind will perish in extraterrestrial habitations, as total dependency on artificial production systems

will lead to extinction. Similarly genetically manipulated biological support system, usually being unstable, mankind should also not depend on them.

4.2 Protocol for use of silicon utilizing organisms in lunar settlements

A simple protocol may be followed to use these silicate-utilizing organisms in lunar settlements. After providing minimum essential requirements for life in lunar extraterrestrial situation, these organisms may be utilized. Otherwise the protocol may be followed directly in a lunar crater to allow the organisms to find out a suitable zone for their growth.

4.2.1 Lunar crater protocol

Initial study may be done in any terraced or concentric crater where growth of scattered organisms is expected to occur on some parts of the terraces and on some areas of the rim or on ropy crest. As our primary settlement will be located on Malapert Mountain on the rim of Shackleton crater, one may think of scattering silicon utilizing organisms on the rim of Shackleton, but preferably initial experiments may be done on other craters.

4.2.1.1: Microterraforming on moon

In the initial venture antibiosis between various species should be prevented. Thus phytoplankton should be used before zooplanktons. Diatoms of Eu-eurytherm variety of *Nitzschia* and *Chaetoceros* group may be selected initially. Then red algae grown in 2% silicate solution and then yellow green algae grown in 0.5% silicate solutions may be scattered to boost up the algal inhabitants. In each step, before use, the organisms should be tested for pathogenicity and toxigenecity by standard tests. Silicon utilizing organisms of 1% SM and 4% SM solutions were not considered for terraforming as they contain mainly *Mycobacteria* and scanty fungi respectively.

Selection of diatoms for the initiation of microterraforming process in my opinion is quite justified. There were five periods of mass extinctions on earth namely end-Ordovician, late Devonian, end-Permian (96% extinction), end-Triassic and K-T (30-70% extinction) interspaced with short periods of extinctions [27]. In the K-T event, only 20-25% extinction occurred in planktonic diatoms (probably survived by forming resting spores) while 80% and 90% extinctions occurred in radiolarians and in foraminifera respectively [28].

4.2.1.1 .1 Known Eu-eurytherm phase 3-12 months (The durations were calculated to allow growth under a stressful condition on lunar surface)

Nitzschia subcurvata, N. curta, N. cylindrus, N. prolongatoides, N. pneudonana ,Chaetoceros dichaeta, C. neglectus may be used in this phase.

4.2.1.1 .2: High silicon habitat algal phase 3-12 months

In this phase algae grown in 2.0% silicate solution may be used, and if step 4.2.1.1.1 fails these algae will initiate the process of terraforming.

4.2.1.1 .3: Low silicon habitat algal phase 3-12 months

In this phase algae grown in 0.5% silicate solution may be used.

4.2.1.1 .4: Lichen phase 3-12 months

In this phase different lichens may be used.

Sub cultivations even blind passages may be done if necessary in between steps. This is because active and passive dispersal mechanism will be less on lunar surface. After successful completion of 4.2.1.1.4 step an array of irregular-

three-dimensional cell aggregates almost similar to microbial mats will be developed on lunar surface.

4.2.1.2. Macroterraforming of moon

Important silicon utilizing plants and following that organisms (only extremophile variety) like rotifers, tardigrades, nematodes, protozoa, fungi and bacteria may be added which will live in close association of silicon utilizing plants and this process may continue.

4.2.1.2.1 High Silicon metabolizing plants phase 1-5 years

In this phase dryland grasses , *Bambusa glaucesscens, Chlorophytum comosum*, *Anthurium scherzerianum*, *Calathea makoyana* , *Aechmea fasciata*, *Spathipyllum*, *Equisetum arvense* , *Schefflera actinophylla*, *Hedera helix* , *Cordyline terminalis*, *Dracaena deremensis* may be used.

4.2.1.2.2: Food related silicon accumulator plant phase1-5 years

This is a continued phase in close association with all previously applied organisms. In this phase, species of *Oryza, Saccharum, Triticum, Citrus, Fragaria, Cucumis, Rosa* etc may be applied.

4.2.1.2.3 Animal introduction phase1-5 years

In this step there may be introduction of rotifers, tardigrades, nematodes, protozoa etc.

4.3 Artificial support protocol

In artificial support system on lunar surface silicon utilizing organisms may be used to support growth of non silicon-utilizing organisms and to produce a biosphere in such systems as it is not practicable to carry all essential nutrients for lunar settlements from earth.

4.4 Follow up protocol after terraforming

After initial terraforming one should monitor the biomass on the lunar surface. A follow-up analysis of microclimate in the terraforming is necessary to understand the complex variables including temperature, radiation, humidity, ‘wind’ etc. Although there are various methods of measurement of biomass e.g. drying, ashing, determination of energy content, carbon analysis etc., remote sensing data combined with ground based measurements (‘ground truth’) is important. For this purpose the size of the smallest picture element or pixel is important for good resolution in satellite remote sensing and modeling of the microterraforming. Carbon partitioning will be highly effective on lunar surface. Micrometeorological techniques like aerodynamic and eddy covariance methods may also be tried. One can also measure Adenylate Energy Charge (AEC) ratio. This measurement is simple to perform and is extremely sensitive [29]. However, in all types of measurements only *in situ* growth on lunar surface should be considered as experiments on earth may give wrong interpretations. As there is no evidence of life in any of the materials, which were brought from the moon, there is also no chance of obliteration or interference of any *in situ* ‘biosphere’ on it.

5. Conclusion

A simple method for production of stress tolerated silicon utilizing organisms for use in lunar settlements has been described along with a classification of such organisms. A protocol consisting of orderly arrangement of these organisms for use on lunar surface has also been described. Although the protocol is apparently simple but in practice it may not be so simple due to presence of multiple variables. It is not easy to understand the kinetics of such artificial microenvironment on the Moon. However, scientific progress should overcome such difficulties during terraforming.

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