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THE CONCEPTION OF GROWING FIRST GENERATION-PLANTS IN LUNAR GREENHOUSES

The ability to grow plants in greenhouses is a practical necessity for providing an advanced life support system for humans while inhabiting a permanently manned lunar base. Plants will provide fresh food, oxygen, and clean water for explorers living in lunar bases. The conception of first-generation plants growing in a lunar base anticipates them to play a main role in forming a protosoil of acceptable fertility needed for purposively growing second generation-plants (wheat, rice, etc.) at a low cost. The residues of the first generation-plants could be composted and transformed by microorganisms into a soil-like substrate within a loop of regenerative life support system. To reduce a cost of early missions to the Moon, it would be practical to use a local material such as the lunar regolith for plant growing in lunar greenhouses. The use of microorganisms for plant inoculation to leach nutritional elements from regolith, to alleviate lunar stressful conditions, to decompose both silicate rocks and plant straw needed for a protosoil formation is a key idea in a precursory scenario of growing pioneer plants for a lunar base.

INTRODUCTION

A lunar rock considered as a potential substrate for a plant growing. The European Space Agency and other space agencies have outlined new visions for solar system exploration that will include a series of lunar robotic missions to prepare for and support a human return to the Moon for future human exploration of Mars and other destinations [12]. In this connection, a permanently manned lunar base (PMLB) is a not-too distant future. A greenhouse within/outside the habitation will be a reality which is being planning nowadays. From the very beginning of an *in situ* Moon exploration, it seems to be practical and cost-effective to use a local material for a plant growing. This effort would need to be integrated closely with bioregenerative life support system. The biotechnological process to release some elements from regolith for propellant and food manufacture can be unified with a processing regolith for a fertile protosoil preparation.

A bioavailability of regolith for plant nutrition and its putative safety had to be tested at first. Various species of plants have been exposed to lunar materials delivered from the Moon to evaluate the biological impact of regolith. Botanical studies indicated that lunar material from Apollo 11 and 12 outposts could provide mineral nutrients for germinating seeds, for liverworts growth, and plant tissue cultures development [15, 36, 39]. The general conclusion was that lunar rock used as a substrate to grow plants was of a low bioavailability and needed some amendment. Moreover, regolith fines aggregated under watering, and the substrate aeration appeared to become worse [25, 37].

Under a scarcity of native lunar material, as a first step, terrestrial analogs may be evaluated in simulation experiments on a plant growing. In model experiments French marigold (*Tagetes patula* L.) could grow in a crushed rock, anorthosite, terrestrial counterpart of a lunar rock, however, a deficit of nutritional substances and probably plant intoxication by liberated ions of some elements did not allow marigold to grow normally [17]. When a consortium of well-defined plant-associated bacteria and mycorrhizal fungi were used for seed and substrate inoculation, a plant development was improved [17, 18, 22]. In the NASA-associated study Arabidopsis plants were grown in a JSC1a lunar simulant and natural terrestrial analogs. Evaluation of substrates for their potency to support plant growth with and without supplies showed that the native rock and soils required a variety of mitigation steps to support plant growth [11].

Another conception of growing plants in outposts anticipates to use the man-made soils delivered to the lunar surface from the Earth in ready-to-use form. This idea is proposed by A. Tikhomirov in cooperation with J.-B. Gros [9, 33—35] and developed by Liu et al. [20]. Basic characteristics of SLS is a similarity to organogenic fertile soils. In addition, it has high protective properties against phytopathogenic microorganisms, and a regenerative capacity of SLS makes it as element of BLSS. However, it seems costly to fly supplies there from the Earth, even on initial stages.

Terrestrial anorthosites as counterparts to lunar anorthosites. The most representative geomorphic and geologic structures on the Moon are lunar continents and mare which have differences in petrologic content of regolith. According to orbital data of Apollo-14, Apollo-16 and Luna-20, providing sampling of material, highlands are formed by most ancient rocks, described as the ANT association (anorthosite-norite-troctolite), and less distributed dunite and KREEP (K, Rare Earth Element, P) basalt. The main rock-forming minerals of anorthosites are calcium-containing plagioclase $Ca[Al_2Si_2O_8]$, pyroxene (Mg, Fe,Ca) $(Mg,Fe)[Si_2O_6]$, and olivine $(Mg,Fe)_2[SiO_4]$ [26]. The lunar mare are formed mainly gabrro. The variation in composition of Apollo-11 through 15 lunar materials used in botanical studies was described by P. H. Johnson et al. [14]; they contained practically all elements needed for plant growth except for C and N.

Ukrainian rocks from Korosten Pluton (Penizevitchi, Turchynka deposits, Zhytomyr oblast) provide suitable test-bed for modeling biomobilization of plant-essential elements from lunar-like rocks. The Penizevitchi anorthosite in addition to intermediate plagioclase, low-calcium pyroxene and olivine contains minor quantities of ilmenite $FeTiO_3$, orthoclase $K[AlSi_3O_8]$, biotite $K(Mg,Fe)_3[AlSi_3O_{10}](OH,F)_2$, and apatite $Ca_5[PO_4]_3(F,OH,Cl)$ [26]. The Turchynka type anorthosite is composed of plagioclase, pyroxene of low-calcium content, and olivine [26, 27]. As compared to average composition of lunar anorthosite, Ukrainian anorthosites contain a bit more SiO_2, Na_2O, FeO, MgO, TiO_2 and less CaO and Al_2O_3 that reflect more «alkaline» composition of plagioclase and a higher content of maffic minerals. There are other rock deposits on the Earth, like the Stillwater (Minessota, USA) and the Skaergaard Intrusion (East Greenland) complexes which are counterparts to lunar anorthosites [3].

Microorganisms as helpers to improve rocks bioavailability. Rocks contain nearly all the elements essential for growth except for organogenic ones, however, they are of low availability for plants. To use a local lunar material could be profitably, both as a substrate to implant seeds or seedlings and also as a source of essential nutritional elements for a plant growing, however, its availability should be improved. A rationally assembled consortium of microorganisms (RACM) could be applied to liberate plant-essential elements. According to the Committee on Space Research's (COSPAR's) planetary protection policy, decontamination procedures for the Moon are not anticipated because of absence conditions for a life.

Microorganisms are essential plant partners, and a plant innate immunity is suppressed without them [32]. There is a less chance for the plant to survive under stressful conditions and to resist both abiotic and biotic stressors without microbes [21, 40]. Plant growth-promoting rhizobacteria provide protection against soil-borne plant pathogens by antagonistic mechanisms [19]. Plant-associated bacteria, colonizing the root surface, can also trigger systemic resistance in aerial portions of the plant [24, 31]. It is known a capability of any bacteria to reduce accumulation of some heavy metals is known [6, 10]. With the aim to support the plant growth under hostile stressful conditions we propose to prime a plant-candidate with beneficial microbes to prevent suppression of plant immunity, to stimulate plant development, and to provide plants with a bio-factory for substrate leaching and releasing essential elements for plant growth [17, 22, 41]. In further programs helpful microbial communities will be used in biotechnologies to decompose the lunar material for biomining and extraction of needed elements [5].

The stable community of balanced populations of bacteria, cyanobacteria, algae, and fungi will be involved in a promoting plant biomass production and composting plant residuals, as well as in a biodegradation of the rocky substrate with the aim to form protosoil of acceptable fertility needed for purposively growing successive generations of plants like wheat, rice, soy, etc. A use of RACM to decompose a local lunar material will help reducing the need for supplies from the Earth. On either step, microbial community must be subjected to control a contamination of facilities etc.

Plants as candidates for protosoil preparation. In a long story of resistant plants selection for a fertile soil forming, plant responses on a hostile lunar environment will be studied in biological experiments on payloads [12, 30]. Later selected resistant plants will be involved in different programs at PMBL either to support human beings at habitation or to exploit them for *in situ* resource utilization (ISRU) purposes. The program of industrial growing plants begins from protosoil forming. The first plants must produce acceptable biomass for converting into a fertile protosoil. One of such a plant candidate is marigold. In the future stages of lunar agroindustry, this plant species may be also applied to recover a tired plant-growing substrate by producing secondary metabolites (allelochemicals) [23]. Marigold is an ideal harbor of arbuscular mycorrhizal fungi and could be used as industrial accumulator of fungi needed for «green» agro-industry. Beside the pragmatic side, the marigold could perform a role in esthetic decoration of the hostile environment of PMBL: the beautiful image of marigold cultivars, familiar to everybody and lovely, would remind an earthly spirit to habitants of PMLB and improve the emotions of lunar explorers.

***In situ* soil forming.** The idea of composting lunar fines with waste products from a manned base was proposed by Walkinshaw and Galliano [37]. Formation of a protosoil by microorganisms, composting crop residues, for purposively growing plants is being studied [9, 33—35]. Nowadays it seems to be practical to integrate *in situ* terraforming and waste recycling systems with ISRU to accommodate PMLB and to reduce the demand for energy, transfer mass and cost of future exploration [17, 18]. What microorganisms could be involved in «a lunar» biotechnology of a protosoil forming? It is known that a subset of lithotrophic *Bacteria* and *Archaea* derives their metabolic energy from inorganic substrates (e.g., Mn^{+2}, Fe^{+2}, S etc) and so far associates closely with minerals [4, 7, 16, 29]. Beside them, a wide variety of microorganisms and low eukaryotes interact with mineral surfaces, and among the most familiar are lichens [1], cyanobacteria [5, 8, 13], and fungi [28]. Dissolution of minerals results in releases of ions essential for plant nutrition. A study of a role of microbial communities in degradation of the structural integrity of lunar analogs is the first step to a protosoil forming. Microorganisms modify rates of chemical and physical weathering and clay forming which play a fundamental role in a soil like substrate forming. Terrestrial rocks provide a suitable test-bench for modeling biodegradation of lunar rock counterparts, using epi- and endolythic microorganisms. First our results on biocorrosion of the Penizevichi type anorthosite in a contact with *Paenibacillus* sp. were published [22]. Bio-signatures in the calciferous rock have been observed after removing the lichen *Xanthoria elegans* (J.-P. de Vera, personal communication). Results on a model study of the lunar simulant bioweathering demonstrated the physical decrease of the size of a Minnesota basalt grain after dissolution by a cyanobacterium [5]. From examining a biodegradation of a lunar-like rock in a concert with a biocomposting of plant biomass under assistance of microbial community, we will get some valuable information on first signs in terraforming.

Simulative study of biomobolization from a lunar rock by plants and microorganisms. There are evidences that plants mobilize both plant-essential and toxic elements from rocks. For example, seeds germinated in the Apollo-14 lunar material accumulated large quantities of iron, titanium, and other elements [38]. Further studies revealed that lettuce seedlings exposed to neutron-activated Apollo-11 and Apollo-14 lunar fines absorbed scandium, cobalt, manganese, and a variety of elements that included rare earth elements [2]. Apollo-16 investigations showed that cabbage seedlings grown in contact with lunar material accumulated high concentrations of aluminum. Our studies exhibited accumulation of Zn, Fe, Ni, Cr by French marigold grown in the terrestrial anorthosite [41]. It is clear that plants cannot consume elements from a substrate selectively, and therefore there is concern on human health associated with biomobilization of heavy metals from a rocky substrate for growing plants. The regolith must be tested for ion-exchange capacity and toxicity in simulative model ground-based experiments. The purpose of our study was to examine bioleaching capacity of both bacteria and a plant in contact with anorthosite of the Turchynka deposit and accumulation released elements by plants in model plant microcosms under controlled conditions. In model experiments, the presence of bacteria on the marigold roots improved antioxidant systems of the plant-host and so far protected marigold against toxic doses of accumulated heavy metals. The rationally assembled consortium of bacterial strains promoted a growth of marigold and supported the plant development under growth-limiting conditions by bioleaching and delivering essential nutritional elements to the plant. Due to the bacterial consortium, the model plant was supplied with an additional amount of some basic macro- and microelements. Bacteria corrected Ca/Mg and Fe/Mn-ratio in marigold, approaching them to physiologically optimal.

CONCLUSIONS

Growing first-generation plants such as the French marigold in the presence of a community of microorganisms, including eubacteria, cyanobacteria, mycorrhizal fungi, etc and converting the plant residues by microbes into a soil-like substrate may give the beginning of agro-industry at PMBL. However, bioaugmentation strategy of growing plants for lunar bases needs comprehensive study and a wider body of evidences. The shift in a planetary protection policy from that of absolute sterilization to a probabilistic approach opens the possibility for plant inoculation in green houses, as well as ISRU under assistance of microbial communities.

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КОНЦЕПЦІЯ ВИРОЩУВАННЯ ПЕРШОЇ ГЕНЕРАЦІЇ РОСЛИН У МІСЯЧНИХ ОРАНЖЕРЕЯХ

Можливість вирощувати рослини в оранжереях є необхідною умовою для забезпечення досконалою системою життєзабезпечення для людей, які чергують на місячній базі. Рослини надаватимуть свіжу їжу, кисень і чисту воду для дослідників, які живуть на місячній базі. Концепція першого покоління рослин, вирощених на місячній базі, передбачає, що рослини будуть відігравати основну роль у формуванні протогрунту належної родючості, необхідної для вирощування рослин наступних поколінь (пшениця, рис тощо) за низьких витрат. У циклі регенеративних систем життєзабезпечення залишки рослини першого покоління може бути перетворено мікроорганізмами в компост. Для зниження витрат у ранніх місіях на Місяць на рослинництво було б доцільно в місячних оранжереях використовувати місцевий матеріал, місячний реголіт. Використання мікроорганізмів для інокуляції рослин з метою вилучення елементів з реголіту для живлення рослин, послаблення дії місячних стресових умов, компостування рослин та силікатних порід, необхідного для формування протогрунту, є ключовою ідеєю у попередньому сценарії вирощування рослин-піонерів для місячної бази.