COMPARATIVE EFFECT OF LUNAR FINES AND TERRESTRIAL ASH ON THE GROWTH OF A

BLUE-GREEN ALGA AND GERMINATING RADISH SEEDS

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ABSTRACT

Although it is understood that photosynthetic organisms will be required as components of a closed ecological life support system (CELSS) for a manned lunar base, a basic problem is to identify organisms "best" capable of utilizing lunar regolith materials. Also, there is need to determine what nutrient supplements have to be added to lunar soils, and at what levels in order to promote high bio-mass production.

This research compares the growth of Anabaena flos-aqua, UTEX1444, when cultured in a water leachate prepared from Apollo 14 sample number 14148-6 to the alga's growth when cultured in a water leachate of terrestrial ash. The measure of chlorophyll absorption at 650 nm was used as the index of growth. Additionally, mean average root length is reported for radish seedlings at 24, 36, and 48 hours after seeds were allowed to germinate on leachate moistened filter paper or on lunar or terrestrial soil particulates.

Results showed that during the first 9 days, the growth of Anabaena flos-aqua in both lunar and terrestrial leachates was much less than that of alga growing on enriched culture medium. The addition of enriched medium to all cultures on day 9 resulted in dramatic increase in growth in all, and by day 15 the level of growth in lunar leachates surpassed that of control and terrestrial leachate cultures. Radish seedlings showed the greatest mean average root length at 24, 36, and 48 hours for those plants treated with terrestrial leachates or terrestrial soil particulates. After 24 hours, root growth of control plants exceeded that of plants treated with lunar leachate or lunar fines. However, the plants treated with lunar materials showed greater root growth at 36 and 48 hours than did the controls.

The findings indicate that the lunar material has an initial inhibitory effect followed by one that is stimulatory on the growth of both the al and the radish seedlings. The stimulatory effect coupled with the addition of oef cient nutrients to the algal cultures appeared to promote increased bio-mass production. More controlled experiments are required before definitive conclusions can be made.

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INTRODUCTION

Among the major problems in the development of a closed ecological life support system (CELSS) for human habitation of the moon is that of a choice of biological components capable of utilizing lunar materials and man's metabolic waste products to regenerate into the system those materials required to support human life. Materials absolutely essential for human life are oxygen, water, and food. To repeatedly transport these materials from earth to a lunar base is generally recognized as infeasible. Photosynthesizing organisms produce the food required to support life on planet earth by utilizing solar energy, soil minerals, water, and carbon dioxide with concomitant evolution of oxygen and replacement of water into the biosphere. How efficient can these organisms be on the moon? Although at the present time the availability of water on the moon has not been determined, there is abundant solar energy, and the lunar regolith though lacking organic compounds contains all of the minerals required for plant growth with the exception of nitrogen, zinc, boron, and molybdenum (NASA 44-005-114, 1972). Supplies of nitrogen and carbon will become available from human waste products in the lunar base, while the last three of the foregoing nutrients are required in trace amounts only.

Early experiments with Apollo 11, 12, and 14 materials have shown that several species of higher plants demonstrated increased growth after treatment with lunar fines (Walkinshaw et al., 1970, and Walkinshaw and Johnson, 1971). Also, higher plants subjected to simulated lunar photoperiods were shown to develop high quality crops (Terskov et al., 1978). In a number of experiments directed toward the development of a CELSS, representative forms of green algae

have been used. Outstanding among these experiments is the work of Gitelson et al. (1975), and Fong and Funkhouser (1982). However, there is an apparent absence of data on blue-green algae as a component of CELSS.

The tenacious ability of blue-green algae to exist in the harshest as well as the milder of earth's environments for well over three billion years (Young, 1981) would suggest that this group of plants may include some species worthy of choice as components of a CELSS for a manned lunar base. Blue-green algae, known scientifically as cyanopnyceae, are photosynthesizing plants widely distributed in and on soils and in both marine and freshwater habitats. Additionally, they are successfully maintained in the laboratory by agar and hydroponic techniques. Recently, blue-green algae were discovered growing 82 feet below the surface in ice-covered lakes in the Dry Valleys of Antarctica. Also, these algae were found growing inside of rocks collected in the Dry Valleys (Young, 1981).

The blue-greens exist as unicellular, colonial, and filamentous forms. All forms have been described as pro-karyotic because they lack a formed nucleus and other cellular organelles. Research on a number of species of blue-green algae has shown that these plants have remarkable adaptive capability, and as photosynthesizers they have a significant role in the regeneration of oxygen and the utilization of carbon dioxide as well as the recycling of mineral nutrients in the biosphere.

Outstanding examples of the adaptive capability of the blue-green algae are the unique physiological and ecological behavior of some species in response to environmental stresses. These behavioral responses include: (1) the ability to fix molecular nitrogen when inorganic nitrogen supplies are low; (2) the ability

to withstand pH values as low as 2 or as high as 10 (Lewin, 1962); (3) the ability to develop specialized cells for surviving phosphorus deficiency; and (4) the ability to grow in both aerobic and anaerobic conditions (Trainor, 1978). More unusual than the foregoing is the behavior of the blue-green algae in the lakes of the Dry Valleys of Antarctica referred to earlier. There the plants grow 82 feet below the surface, receiving only 0.1 of 1.0 percent of the surface light, and for one-third of the year they receive no light. Researchers theorize that during photosynthesis, the algae produce glycollate along with other short-chain carbon compounds that remain in the water and are used as an energy source when there is no light (Young, 1981). If indeed this explanation is correct, it is interesting to compare this mechanism of adaptation to that in earlier reports of uptake of phosphates by blue-green algae in excess of requirements and the storage of this nutrient for utilization when the external supply is depleted (Lewin, 1962).

Both algae and higher plants have specific requirements for light, moisture, hydrogen ion concentration, a carbon source, and macro- and micronutrients for continued growth and reproduction. The required macronutrients have been identified as Na, Ca, Mg, Cl, SO_4, PO_4, and N, and the micronutrients as Mn, Zn, Fe, Co, and Mo. Each species has optimum, minimum, and maximum levels for each factor or substance required. Deficiency or toxic levels of these requirements result in a broad spectrum of manifestations of morphological and/or physiological anomalies too numerous to be outlined here. Extreme deficiencies may result in the death of plants.

The ultimate source of light for all life forms is solar energy, while the earth's abundant water bodies supply the needed mositure. Temperature variations and fluctuations in hydrogen ion concentration are influenced in large part by geographical location and metabolic activity of all life forms in a given area. Terrestrial photosynthesizers depend for the most part on carbon from the atmosphere in the form of CO_2, while soil and aquatic forms utilize carbonates from the growth medium. Terrestrial and soil, as well as aquatic forms, depend upon the soil as the ultimate source of the micro- and macronutrients.

Algal plant bodies absorb the required nutrients directly from the growth media, while the seeds of higher plants contain sufficient amounts of nuturients to permit seed germination and growth of the primary root and shoot systems under proper conditions of light, moisture, temperature, and available oxygen. As the seed stores are depleted, root hairs on the epidermal cells absorb nutrients from the growth medium to accommodate the further nutrient requirements of the seedling. Numerous culture studies have been done to establish the absolute nutrient requirements for a variety of species, and these are available in the literature.

THEORY

The purpose of the study reported here was to test the hypothesis that variations in inorganic content of lunar and terrestrial soil leachates will result in corresponding variations in the support of blue-green algal growth. Additionally, variations comparable to lunar and terrestrial soil nutrient variations will be demonstrated in the root growth of germinating radish seedlings exposed to lunar and terrestrial leachates, and to particulates of lunar fines and terrestrial ash.

Lunar mare surface material is described as unweathered basalt rock fragments similar to silts in particle size (2-60 m), and it has a permeability similar to silt. The minerals of the lunar soils contain all of the elements found in terrestrial soils with the exception of Mo, Zn, Co, and N (NASA 44-005-114, 1972).

For this experiment, trench surface sample number 14148-5 from the Apollo 14 collection was used. The relative amounts of major and trace elements in this sample were determined by Philpotts et al. (1972) and Lindstrom et al. (1972). The sample of terrestrial soil used was collected on the JSC site just outside of Building 31. The terrestrial soil was ashed at $800^{\circ}C$ for four hours in order to degrade the organic matter.

Leachate Preparation

Lunar and terrestrial leachates were each prepared in two separate batches. Lunar 1 (L1) and Terrestrial 1 (T1) were prepared by adding 49.59 mg and 50 mg respectively to two separate 50 ml Erlenmeyer flasks containing 50 ml of glass

distilled H_2O. Both samples were covered and magnetically stirred for four hours per day for three days at room temperature. The second batch, Lunar 2 (L2) and Terrestrial 2 (T2) was prepared by adding 41.40 mg and 50 mg respectively to two separate Erlenmeyer flasks, each containing 10 ml of glass distilled H_2O. The flasks were covered and stirred magnetically for six hours per day for five days. All samples were vacuum pumped through a membrane filter. The undissolved particles were stored for future reference. Determination of pH values were made on the supernatants and each was adjusted to pH 7.4.

Alga Culture Preparation

An agar culture of the blue-green alga, Anabaena flos-aqua UTEX 1444 was obtained from the University of Texas algae collection. To 50 ml of an aqueous culture medium for bacteria free axenic culture of blue-green algae (Aaronson, 1970), five 1-mm-square slices of agar were added. The flask was stoppered with a cotton plug containing a glass tube cotton filter. This flask was maintained under continuous light as a stock culture, and the pH was adjusted periodically by addition of culture medium.

Experimental Procedures

The design of the experiment is shown in figure 1, Scheme for Testing the Viability of Lunar Soil.

The algal bio-assay was done with L1 and T1 leachates only because of the small volume of L2 and T2 leachates.

Nine ml of an undiluted, a 1:2 dilution, and a 1:10 dilution of L1 and T1 leachates were added to six separate 50-ml flasks. In a seventh flask, 9 ml of

SCHEME FOR TESTING VIABILITY
OF LUNAR SOIL

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                    ┌─────────────────────┐
                    │ SOIL LEACHATE IN    │
                    │ GLASS DISTILLED H₂O │
                    └─────────────────────┘
                              │
                    ┌─────────────────────┐
                    │   MINERAL ASSAY     │
                    └─────────────────────┘
                              │
                    ┌─────────────────────┐
                    │     DILUTION        │
                    └─────────────────────┘

                         ╱─────────╲
                        │   ALGAL   │
                        │ BIO-ASSAY │
                         ╲─────────╱
      NON-TOXIC,                          TOXIC, OR NOT
   LIFE SUPPORTIVE                       LIFE SUPPORTIVE

  ┌──────────────────┐              ┌──────────────────┐
  │     COMPARE      │              │  DILUTE FURTHER  │
  │ GROWTH TO CONTROL│              │    OR ENRICH     │
  └──────────────────┘              └──────────────────┘
                                            │
  GROWTH EQUAL TO    LESS GROWTH     ┌──────────────────┐
   OR GREATER                        │     REPEAT       │
                                     │    BIO-ASSAY     │
  ┌──────────────┐  ┌──────────────┐ └──────────────────┘
  │ ADD LUNAR DUST│ │    ENRICH    │
  │  PARTICLES    │ │   LEACHATE   │
  └──────────────┘  └──────────────┘
  ┌──────────────┐  ┌──────────────┐
  │ TEST GROWTH  │  │    REPEAT    │
  │  OF ROOTS    │  │    ASSAY     │
  └──────────────┘  └──────────────┘

                ┌──────────────────┐
                │     COMPARE      │
                │ GROWTH TO CONTROL│
                └──────────────────┘
   GROWTH EQUAL TO              GROWTH LESS
    OR GREATER

  ┌──────────────────┐        ┌──────────────────┐
  │ LEACHATE IS LIFE │        │    CONTINUE      │
  │   SUPPORTIVE     │        │   EXPERIMENT     │
  └──────────────────┘        └──────────────────┘
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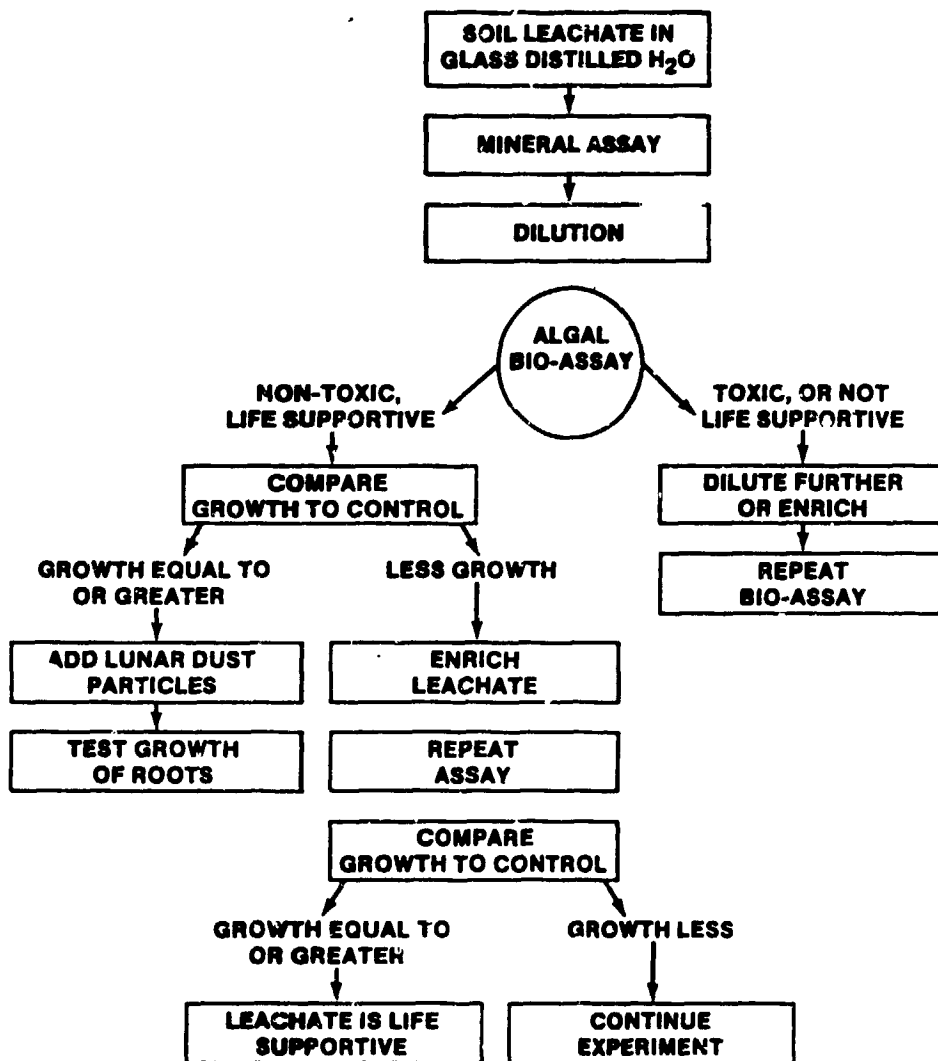
Figure 1.

algal culture medium was placed. This flask served as the control culture. All flasks were inoculated with a 1-mm-square slice of the agar culture of the alga. The flasks were stoppered with cotton plugs containing glass tube cotton filters, and maintained under continuous light at room temperature.

The cultures were allowed to stabilize for three days. Absorption at 650 nm on the spectrophotometer was recorded for the control and each experimental culture on day 4, 7, 8, and 9. After the reading on day 9, each flask was inoculated with 1 ml of the algal culture medium. Thereafter, readings were made on day 10, 11, 14, 16, and 18.

Radish seed germination tests were run with L1, T1, L2, and T2 leachates. Germination tests were also run with lunar fines particulates and terrestrial ash. To six separate filter paper-lined petri dishes, 1 ml of an undiluted, a 1:2 dilution, and a 1:10 dilution of L1 and T1 leachates was added. Glass distilled H_2O was added to a seventh petri dish which served as a control. Five radish seeds were placed in each dish approximately 2.5-3.0 cm apart. The dishes were covered and maintained in the dark. Measurements of root length were made at 24, 36, and 48 hours. During the 3-day period, the filter paper was kept moist with the respective diluents. The experiment was repeated with a parallel run of L2 and T2 leachates.

Additionally, to each of two filter-lined petri dishes 1 ml of distilled H_2O was added. Five radish seeds were placed approximately 2.5-3.0 cm apart in each dish. The seeds in one dish were sprinkled with 5 mg of lunar fines. Those in the other dish were sprinkled with 5 mg of terrestrial ash. The dishes were covered and maintained in the dark. Measurements of root length were made at 24,

36, and 48 hours. During the three-day period, the dishes were kept moist with glass-distilled H_2O.

HETEROCYST STRUCTURE IN ANABAENA

A. CONTROL - IN ENRICHED CULTURE MEDIUM

B. EXPERIMENTAL - IN LUNAR UNDILUTED LEACHATE
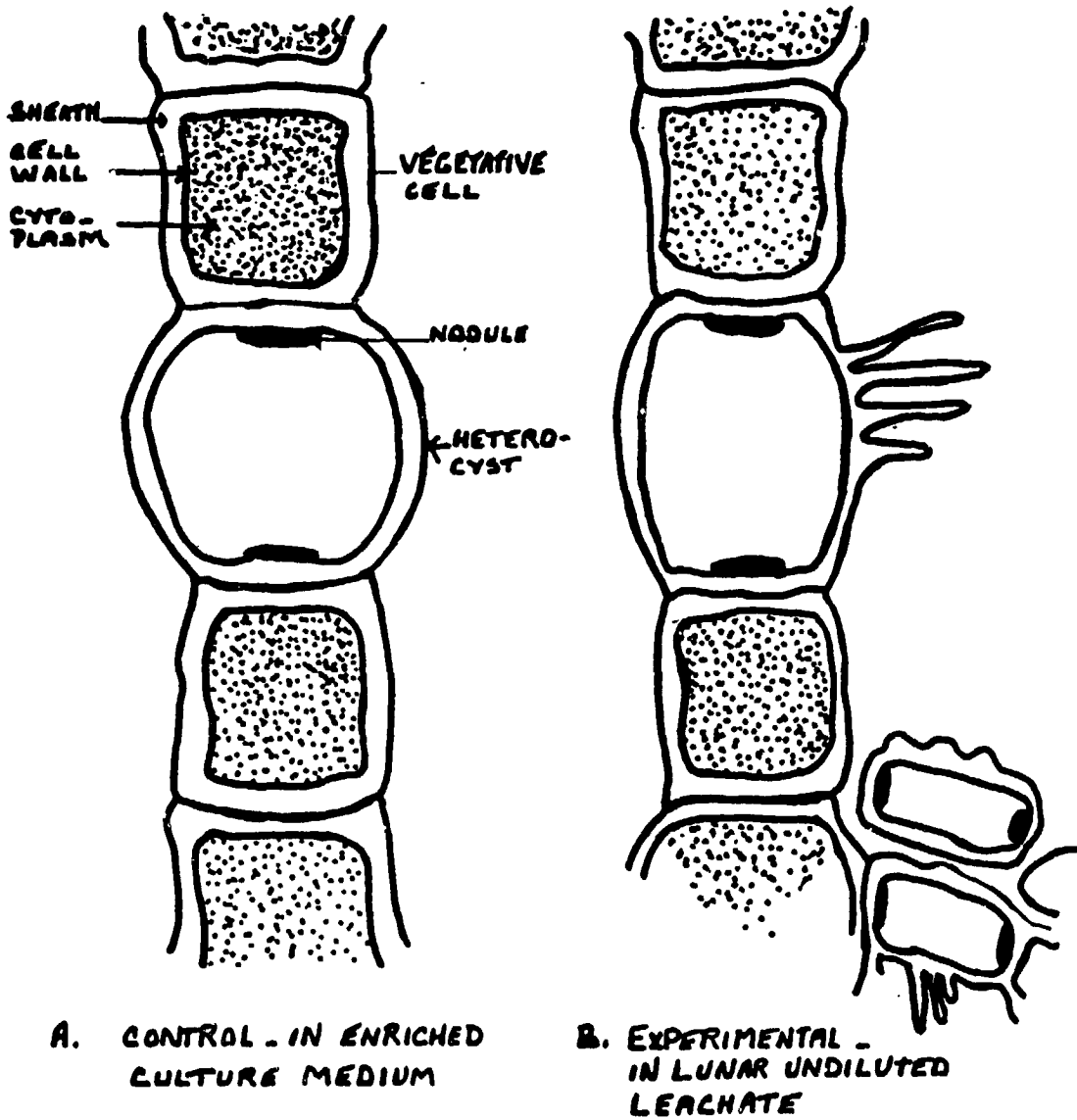
RESULTS

Anabaena flos-aqua cultured in a medium specific for blue-green algae and maintained under continuous fluorescent light demonstrated a four-day lag phase followed by exponential growth over a four-day period. Inoculation with .additional culture medium after a slight decline in growth on the ninth day resulted in exponential increase to the 14th day.

Alga cells cultured in leachates from lunar fines or terrestrial ash did not demonstrate growth comparable to that of the control. Comparison of growth in the algal cultures is shown in figure 2. At all concentrations of the lunar leachates, the algal cultures were essentially still in lag phase at day 4 and there was decline of growth to day 9. Inoculation with culture medium at that time resulted in exponential growth but at a much slower rate than the control culture. The undiluted terrestrial leachate culture demonstrated a growth pattern approximating that of the control. However, the rate of growth was much less. At day 14, undiluted leachate cultures of both lunar and terrestrial samples showed comparable levels of growth. On day 15, the level of growth in the three lunar leachate samples was greater than that in the control culture as well as that of the terrestrial leachate samples.

The mean average root length of control radish seedlings exceeded that of all experimental samples at 24 hours of growth, with the exception of T1 and T2 undiluted, and the T1 1:10 dilution samples. At 36 hours, T1 and T2 undiluted, T2 1:2 dilution, and the terrestrial fines samples surpassed the control by 1-3 mm while at 48 hours the mean average root length of all experimental samples was greater than that of controls (figure 3). Root growth in undiluted terrestrial

leachate and the terrestrial ash samples was much greater than all other samples at 48 hours.

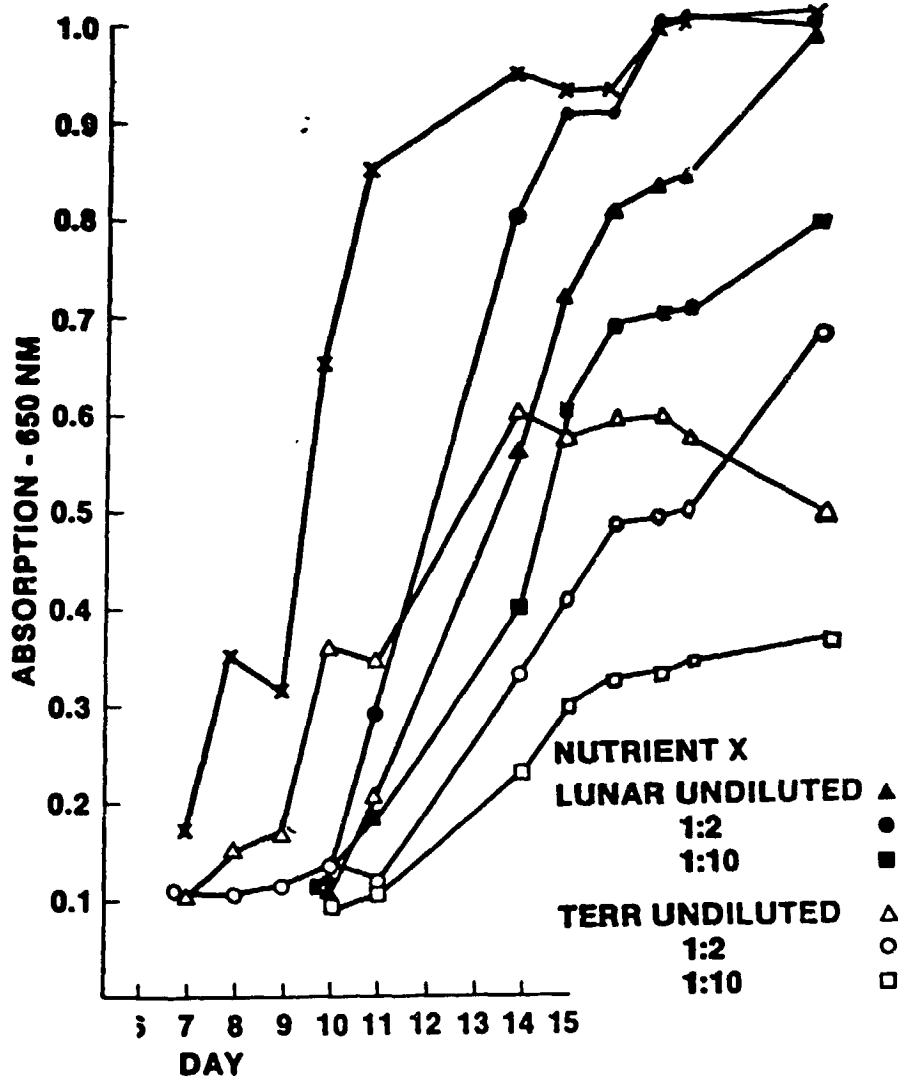# CHLOROPHYLL CONTENT
AS INDEX OF ALGAL GROWTH

NUTRIENT X
LUNAR UNDILUTED ▲
1:2 ●
1:10 ■

TERR UNDILUTED △
1:2 ○
1:10 □

Figure 2.
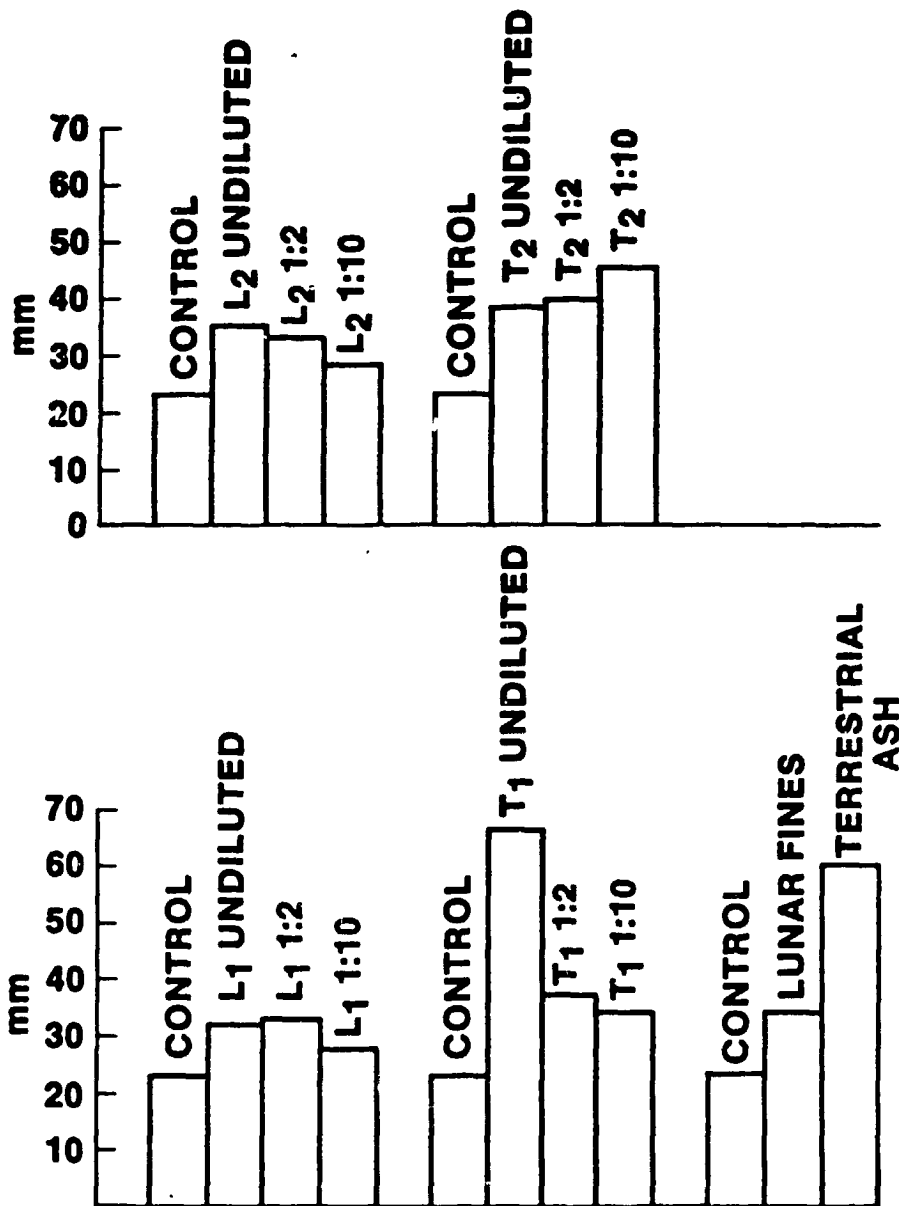
MEAN AVERAGE
ROOT LENGTH AT 48 HOURS

Figure 3.

CONCLUSIONS

Preliminary findings presented here suggest that amounts of nutrients sufficient to interact with the growth of plants are H_2O soluble from Apollo 14 sample number 14148-6. There is indication that the lunar material has an initial inhibitory effect followed by a stimulatory effect. However, there is evidence that addition of some nutrients may be required to permit optimal growth. These nutrients may be Bo, Mo, and Zn. In the case of nitrogen fixing blue-green algae such as Anabaena flos-aqua, additions of nitrogen may not be required since these organisms can utilize molecular nitrogen when Bo, Mo, and Zn are available. It is not possible to arrive at definitive conclusions based on the data obtained here. It is recommended that more controlled follow-up experiments be run. These controls should include:

(1) bubbling of a measured amount of CO_2 through the algal cultures;

(2) a measure of the incident light;

(3) constant shaking of the algal cultures to dispel the build-up of oxygen gas;

(4) microscopic study of algal cells for detection of morphological changes;

(5) measurement of bio-mass quantity in each culture;

(6) tests to determine whether the algal cells produce in the environment volatile substances that are toxic to higher plants;

(7) radish seedlings should be grown to maturity in hydroponic lunar leachate medium with additions of measured amounts of specific nutrients. Growth of root and shoot systems should be monitored during the growth period and measurement of final bio-mass production should be made.

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