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(Final Report)

LIFE IN EXTRATERRESTRIAL ENVIRONMENTS

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LIFE IN EXTRATERRESTRIAL ENVIRONMENTS

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FOREWORD

This final report summarizes the experimental work conducted from February 16, 1968 to July 14, 1969 under the National Aeronautics and Space Administration Contract No. NASr-22. By mutual agreement with Mr. Lawrence Hall, NASA Contract Officer, a summary of the work conducted during the overall contract period of April 16, 1965 to February 16, 1968 was not included in this report. This work was covered in detail in the following publications:

Survival and growth of potential microbial contaminants in severe environments. Life Sciences and Space Research, Vol. IV, 166-175, 1966.

Survival of Microorganisms in a Simulated Martian Environment. II. Moisture and Oxygen Requirements for Germination of Bacillus cereus and Bacillus subtilis var. niger spores. Appl. Microbiol. 15, 285-291, 1967.

Effect of reduced barometric pressure on water availability related to microbial growth. Life Sciences and Space Research, Vol. V., 174-186, 1967.

Ability of microorganisms to establish ecological niches in different soils and environments. Dev. in Industrial Microbiology, Vol. IX, 401-414, 1968.

Probability of growth of viable microorganisms in Martian environments. Life Sciences and Space Research, Vol. VI, 146-156, 1968.

A Martian surface simulation facility for bacterial studies. Proc. Trans. Inst. Environ. Sci. 327-332, 1968.

The studies were conducted with the advice of Dr. E. J. Hawrylewicz and with the technical assistance of Mr. B. T. Anderson, Mrs. M. L. Cephus, Mrs. B. J. Larson, and Miss V. K. Tolkacz.

Experimental data for the period of February 16, 1968 to July 14, 1969 are recorded in IITRI Logbooks C18169, C18459, C18586, C18591, C18833, C19044, and C19157.

Respectfully submitted,

IIT RESEARCH INSTITUTE

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LIFE IN EXTRATERRESTRIAL ENVIRONMENTS

I. INTRODUCTION

During the past year the experimental work concentrated on two subject areas, namely

a. The effect of ultraviolet radiation on the survival of airborne bacteria in simulated Martian dust clouds

b. The isolation of salt tolerant bacteria from soils that included soils obtained from desert and tundra regions, and the survival of these organisms in a simulated Martian environment.

A chamber was constructed to create simulated Martian dust storms and to study the survival of airborne microorganisms in the Martian environment, including ultraviolet irradiation. Representative types of sporeforming and nonsporeforming bacteria present in spacecraft assembly areas and indigenous to humans were studied. The organisms included Bacillus cereus, B. subtilis, Escherichia coli, Serratia marcescens, and Staphylococcus aureus.

It was found that daily ultraviolet irradiation of 2×10^7 to 9×10^7 ergs/cm^2 was not sufficient to sterilize the dust clouds. The soil particles apparently protected the organisms

from ultraviolet irradiation since the numbers of survivors from irradiated and unirradiated environments were similar. The results indicated that long term survival of organisms under the established environmental conditions was possible and that, pending further data, the contamination of Mars with terrestrial microorganisms would still be a distinct possibility.

Salt tolerant, facultative anaerobic bacilli, and cocci isolated from different soils were tested for their minimum a_w requirement and their survival in a simulated Martian environment. There appeared to be a greater correlation between the prevailing climatic conditions in the origin area of the soil and the ability of the organism to grow in a simulated Martian environment than between the minimum a_w requirement of the organism and survival. Larger numbers of bacillus and coccus species isolated from desert and tundra soils were able to grow in a simulated Martian environment than isolates from temperate climate soils.

The following papers summarizing the work performed under the contract were presented or submitted for publication during the report period:

1. Semiannual project report presented at the Spacecraft Sterilization Technology Seminar, Cape Kennedy, Florida, February 11 and 12, 1969.

2. "Effect of Ultraviolet on the Survival of Bacteria Airborne in Simulated Martian Dust Clouds," paper presented at the Annual COSPAR Meeting, Prague, Czechoslovakia, May 12 to 23, 1969.
3. Survival of Microorganisms in a Simulated Martian Environment. III. Effect of Soil Type and Moisture. Submitted for publication in Appl. Microbiol.
4. Survival of Microorganism in a Simulated Martian Environment. IV. Effect of Barometric Pressure, Gaseous Composition of Atmosphere, and Moisture. Submitted for publication in Appl. Microbiol.

II. EXPERIMENTAL METHODS

A. Rotating Drum Experiments

1. Chamber

The rotating chamber, 30 cm in diameter and 30 cm long, was constructed of aluminum. The chamber contained screen baffles that created dust clouds as the chamber rotated at approximately 20 rev/min. To enable the control of temperature and to shield personnel from the ultraviolet irradiation the rotating chamber was operated in an environmental chamber.

A suprasil quartz window was set in the face plate at one end of the chamber. Suprasil transmits 90% of the ultraviolet wavelengths in the 2000 to 3000 A region. Transmittance of suprasil drops off sharply at 1600 A. A sampling port was placed in the opposite face plate together with a port that permitted evacuation of the chamber to establish the desired partial pressures.

2. Ultraviolet Source

The ultraviolet source was a type A Hanover lamp No. 673A, 550 watts (Hanover Lamp Division, Newark, N. J.) with an 11.4 cm arc length. The emitted energy at different wavelengths in the ultraviolet was determined with an Eppley thermophile, a circular 12-junction, bismuth-silver instrument (Eppley Laboratory Equipment Co., Newport, Rhode Island). Approximately 50% of the lamp's emission was in the ultraviolet region between 2200 and 4000 A with about 10% in the 2400 to 2800 A region.

At a distance of 27 cm from the front of the chamber the ultraviolet intensity was 2.6×10^4 ergs/sec, cm^2 or approximately 8 times the ultraviolet intensity reported at the surface of Mars (3×10^3 ergs/sec, cm^2). The intensity at the back of the chamber, 59 cm from the ultraviolet source, was 5.3×10^3 ergs/sec, cm^2. To obtain the Martian equivalent for the respective irradiances the lamp would have to operate 55 min and 270 min.

Since bacteria were homogeneously distributed in the dust clouds and since there were no appreciable differences between bacterial counts of soil from the back or front portions of the chamber a 1 hr daily ultraviolet exposure was used with the intention to study the effect of increased durations of exposure in future experiments.

3. Preparation of Bacterial Cultures

Spores of B. cereus and B. subtilis were produced on trypticase soy agar (BBL). After incubation at 35°C for 6 to 7 days free spores were harvested in chilled 0.025 M phosphate buffer (pH 7.0) and were washed seven times before final suspension in the buffer. The suspensions were stored at 5°C and were used without heat shock since the germination of spores by soil particle abrasion was being investigated.

Stock cultures of E. coli, S. marcescens, and S. aureus were produced by growing the organisms on trypticase soy agar for 24 hr or 48 hr at 35°C. Harvesting and storage procedures were the same as described for B. cereus and B. subtilis.

4. Bacterial Inoculation and Recovery

Felsite-limonite or limonite coated soils were sterilized at 121°C for 2 hr on each of two succeeding days and dried for 30 min in a vacuum autoclave. After cooling, the soil was inoculated by spraying 4 ml of a phosphate buffer suspension of the organisms. This was calculated to give 10^6 viable cells/g of soil without introducing excessive amounts of water.

Two or three soil samples were aseptically removed at various time intervals, usually at 1 and 3 hr and at 1, 3, 7, 14, and 21 days post inoculation, through the sampling port while the chamber rotated. The samples were diluted with 0.1% peptone solution and the appropriate dilutions were plated in duplicate on trypticase soy agar for recovery of total viable bacteria count. Spore counts were performed by heating the soil suspension dilutions for 10 min at 80°C before plating on trypticase soy agar. The plates were incubated at 37°C for 24 to 48 hr.

When studying environments with partial pressures, the chamber was equilibrated to ambient pressure with the simulated Martian atmosphere (67% carbon dioxide, 30% nitrogen, and 3% argon) and continuously purged until samples were collected after which the desired partial pressure was reestablished.

B. Salt Tolerance Experiments

1. Recovery of Salt Tolerant Organisms

Salt tolerant organisms were isolated from soils obtained from the following locations.

1. Desert Soils
    a. Libyan desert, Libya
    b. Mecca Hills, California
    c. Sonoran Desert, Arizona
    d. White Sands National Monument, New Mexico.

2. Tundra Soils

   a. Rocky Mountain National Park, Colorado

   b. White Mountains, California.

3. Temperate Climate Soils

   a. Mollisol soil, Kane County, Illinois.

   b. Podzol soil, Kane County, Illinois.

The soil samples were suspended in 0.1% peptone solution and appropriate dilutions were plated on trypticase soy agar containing 7.5% (w/v) of sodium chloride. The plates were incubated at 25°C for 7 days. Simultaneously spore counts were performed by heating the soil suspensions for 20 min at 60°C before plating on trypticase soy agar containing 7.5% (w/v) of sodium chloride and incubating at 25°C for 7 days.

2. Determination of Minimum a_w Requirements

The isolates were further tested for their salt tolerance by determining their ability to grow in trypticase soy broth adjusted to an a_w of 0.90, 0.86, or 0.84. The a_w solutions were prepared by the method of Scott (Austral. J. Biol. Sci. 6, 549-564, 1953). The tubes were incubated at 25°C and observed for visible growth at 7, 28 and 56 days. The isolates were grouped according to their colonial and cellular morphology and minimum a_w requirements. A limited number of biochemical tests was conducted.

C. Simulated Martian Environment

The following conditions were used in experiments designed to study the effects of a simulated Martian environment on survival or growth of bacteria:

1. Atmospheric pressure: 15 mb
2. Gaseous composition: 67% carbon dioxide, 30% nitrogen, and 3% argon.
3. Incubation temperature: daily 16 hr at -65°C and 8-hr at 30°C.

III. RESULTS AND DISCUSSION

A. Rotating Chamber Experiments

It has been suggested by Horowitz et al (Science 155, 1501, 1967) that the ultraviolet radiation reaching the surface of Mars is of sufficient intensity to prevent the survival of terrestrial life forms. On the other hand, an opposing view was expressed by Sagan et al (Science 159, 1191, 1968), who suggested that limonite readily absorbs ultraviolet radiation and thus may protect life forms from the harmful effects of irradiation.

Ultraviolet irradiation, particularly in the 2400 to 2800 A region, has a profound effect on biological systems. The amount of ultraviolet irradiation required to reduce a population to about 37%, is approximately 1000-fold less (2 to 4 x 10^4 ergs/cm^2) than the daily exposure used in our studies (Demerec and Latarjet,

Cold Spring Harbor Symposia Quant. Biol. 11, 38, 1946; Herich, J. Gen. Physiol. 20, 589, 1936). Unshielded microorganisms, for example, are rapidly killed when exposed to ultraviolet radiation of the order of 10^5 to 10^6 ergs/cm^2. However, because of the low penetration of ultraviolet rays thin films of proteinaceous material of thicknesses approaching dimensions of bacterial cells greatly reduce ultraviolet intensity (Dunham, in Disinfection, Sterilization, and Preservation, Lea and Febiger, Phil. 1968, p. 480).

Our studies were designed to investigate the protective effect of soil particles in shielding bacteria from ultraviolet radiation. The survival in three types of environments was investigated:

a. Bacteria airborne in soil clouds maintained at constant 25°C without ultraviolet irradiation.

b. Bacteria airborne in soil clouds exposed daily to 16 hr at -65°C and 8 hr at 30°C with 2 to 9 x 10^7 ergs/cm^2 ultraviolet irradiation in the 2400 to 2800 A region.

c. Conditions similar to (b) but in a simulated Martian atmosphere consisting of 67% carbon dioxide, 30% nitrogen, and 3% argon at 15 mb pressure. The moisture of the environments ranged from 1 to 2% which was below the normal *in situ* moisture of 5%.

E. coli and S. marcescens did not survive two days in any of the environments. The rapid die-off was probably caused by desiccation and perhaps soil abrasion. The effect of the experimental environments (b) and (c) on cell death could not be determined since the viability decreased as rapidly in the control soil clouds (a) maintained at constant 25°C as in the other environments.

Daily exposures to ultraviolet irradiation ranging from 2 to 9 x 10^7 ergs/cm^2, freeze-thaw temperature cycle, or the simulated Martian atmosphere did not affect the survival of B. cereus (Figure 1) or B. subtilis (Figure 2). In fact at all sampling days the percent survival of these organisms was higher in the experimental environments (b) and (c), than in the control environment (a).

The survival of S. aureus (Figure 3) was essentially the same in all environments suggesting that the experimental environments (b) and (c) did not have any significant effect on the viability of this organism.

The decline in death of microorganisms, which was apprent from the flattening of the survivor curves between 14 and 21 days, indicated that long term survival of organisms in soil clouds is possible. This is an important observation when considered together with our other studies which showed that B. cereus and particularly S. aureus could grow in a simulated Martian environment if sufficient moisture was present

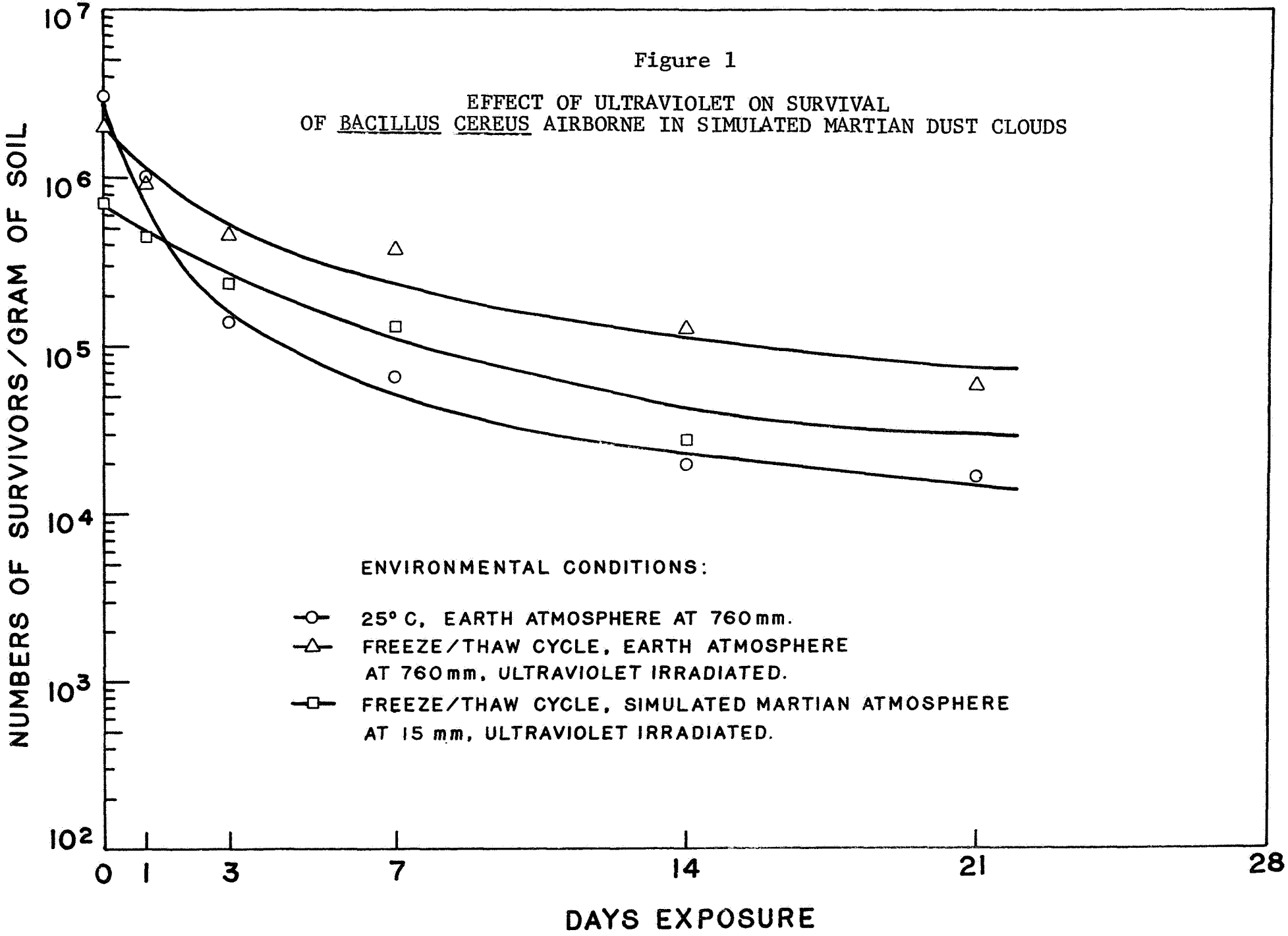


Figure 1
EFFECT OF ULTRAVIOLET ON SURVIVAL
OF BACILLUS CEREUS AIRBORNE IN SIMULATED MARTIAN DUST CLOUDS
NUMBERS OF SURVIVORS/GRAM OF SOIL
10^7
10^6
10^5
10^4
10^3
10^2
0
1
3
7
14
21
28
DAYS EXPOSURE
ENVIRONMENTAL CONDITIONS:
25° C, EARTH ATMOSPHERE AT 760mm.
FREEZE/THAW CYCLE, EARTH ATMOSPHERE AT 760mm, ULTRAVIOLET IRRADIATED.
FREEZE/THAW CYCLE, SIMULATED MARTIAN ATMOSPHERE AT 15 mm, ULTRAVIOLET IRRADIATED.

Figure 2

EFFECT OF ULTRAVIOLET ON SURVIVAL
OF BACILLUS SUBTILIS VAR. NIGER AIRBORNE IN SIMULATED MARTIAN DUST CLOUDS

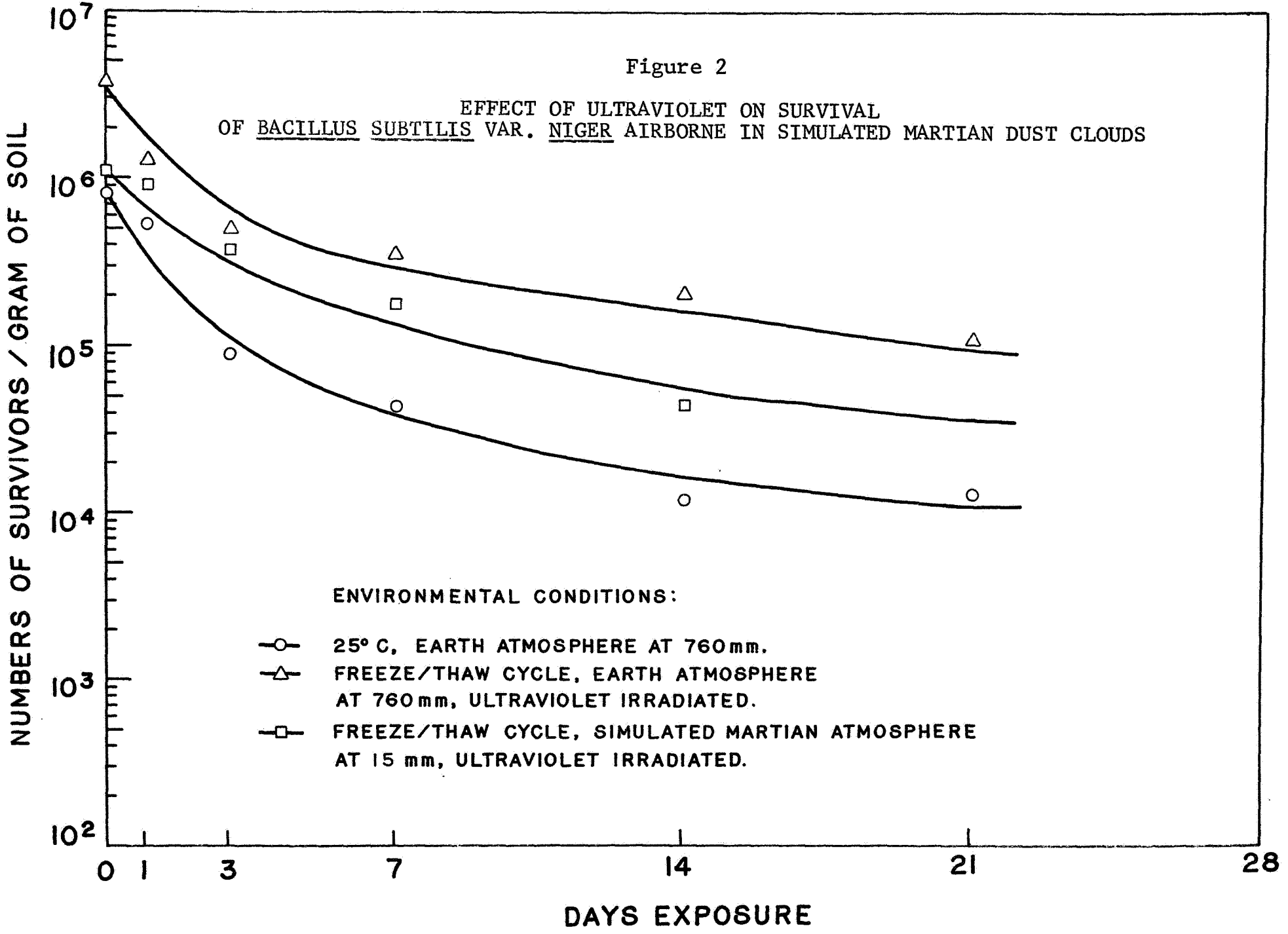
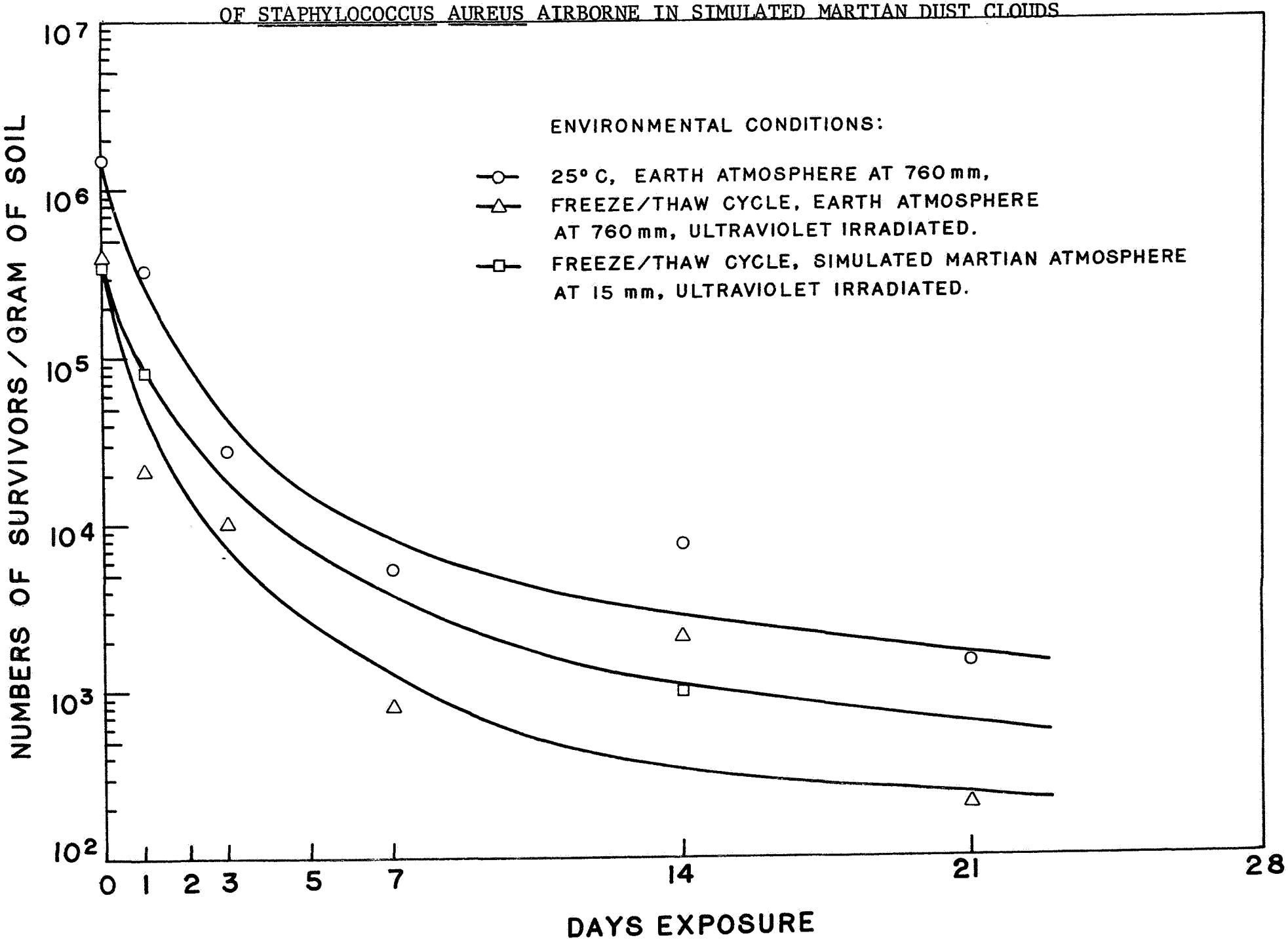


Figure 3

EFFECT OF ULTRAVIOLET ON SURVIVAL
OF STAPHYLOCOCCUS AUREUS AIRBORNE IN SIMULATED MARTIAN DUST CLOUDS



(Hawrylewicz et al., Life Sciences and Space Research. VI. North-Holland, Amsterdam, 1968, p. 146).

B. Salt Tolerance Experiments

The salt tolerant isolates were grouped according to their cellular morphology and minimum a_w requirement (Table 1).

Table 1

NUMBER OF SALT TOLERANT ORGANISMS ISOLATED FROM SOILS

Cell Morphology	Minimum a_w Requirements			Total Number of Isolates
	0.90	0.86	0.84	
Bacillus	26	19	9	54
Coccus	4	15	5	24

The term "salt tolerant" was given to those organisms that grew on trypticase soy agar with 7.5% sodium chloride (w/v) added.

All of the organisms examined were facultative anaerobes. Twelve of the bacilli isolated were lecithinase-positive, or cereus-type. Six of the cocci isolates were classified as Micrococcus based on their ability to utilize ammonium phosphate as the sole nitrogen source. Based on the nitrate reduction and anaerobic utilization of glucose and mannitol tests the remaining cocci were either species of Micrococcus or Sarcina.

The isolates that grew at low a_w's were not characteristic of any particular soil type. Organisms with low a_w requirements were isolated from the temperate climate soils in addition to the desert and tundra soils.

All 78 isolates were tested for their ability to survive and grow in a simulated Martian environment consisting of 67% carbon dioxide, 30% nitrogen, 3% argon at 15 mb pressure; daily 16 hr at -65°C and 8 hr at 30°C, felsite-limonite soil with 1% Ac (Difco), and an a_w of 0.99. Viable cell counts were determined before and after 7 days in the test environment. Those cultures that had a final viable cell count one log or greater than the initial count were scored as growing in the simulated Martian environment (Table 2).

Table 2

GROWTH OF SALT TOLERANT ORGANISMS IN A SIMULATED MARTIAN ENVIRONMENT

Cell Morphology	Minimum a_w Requirement		
	0.90	0.86	0.84
Bacillus	7/26*	0/19	3/9
Coccus	3/4	11/15	3/5

*Number of isolates that grew/number of isolates tested.

All of the isolates tested survived the simulated Martian environment with a greater proportion of the coccus than bacillus isolates growing in the simulated Martian environment. In general the organisms that grew in the Martian environment were isolated from soils from severe terrestrial environments: White Mountains, Mecca Hills, White Sands, and Libyan Desert soils. There was no apparent relationship between low a_w requirement and ability to grow in the simulated Martian environment.

IV. SUMMARY

A chamber was constructed to simulate Martian dust storms and to study the survival of airborne microorganisms exposed to a simulated Martian environment that included ultraviolet irradiation and daily freeze-thaw temperatures. The percent survivors of organisms exposed to ultraviolet radiation were equal to or greater than the non-irradiated organisms suggesting that some factors, probably the soil particles, protected the organisms from ultraviolet irradiation.

Salt tolerant, facultative anaerobes were isolated from desert, tundra, and temperate climate soils. All of the salt tolerant isolates, which included bacillus and coccus species, survived in a simulated Martian environment. The organisms that grew in the simulated Martian environment were chiefly isolates from desert and tundra soils which implied that growth was more

related to the native environment of the organism rather than to the organism's low a_w requirement.

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