NATIONAL AERONAUTICS AND SPACE ADMINISTRATION

Technical Report 32-1454

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Preface

The work described in this report was performed by the Space Sciences Division of the Jet Propulsion Laboratory.

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Abstract

Survivability of indigenous aerobic, anaerobic, and microaerophilic bacteria, fungi, algae, and protozoa occurring as a microbial community in a Sonoran Desert algal soil crust was determined after 4 to 5 years of exposure to continuous very high vacuum of 10^{-6} to 10^{-8} torr. Aerobic and microaerophilic bacteria showed some decrease in survivability (approximately 1 log unit) after 4 and 5 years. In the sieved and powdered samples, there were no survivable thermophiles after 4 years and the numbers of algae were reduced from 10^6 to 10^2 per g of soil. No protozoa survived after 5 years in vacuum, but they did survive after 4 years. In the sieved sample, viable microaerophilic bacteria had decreased from 10^6 to 10^5 per g of soil, and there were no survivable anaerobes or molds. Results of survivability of microorganisms in powdered samples were more variable than in sieved samples. Survivors in both sieved and powdered samples included several *Bacillus* spp., and diphtheroids, an imperfect mold, *Alternaria* sp., the coccoid green alga, *Chlorococcum humicola,* and filamentous and coccoid blue-green algae, including various species of *Schizothrix, Anacystis* and *Coccohloris, Scytonema hofmannii,* and *Nostoc muscorum.* The reduction in abundance of various groups of microorganisms exposed to vacuum shows some similarity to survivable microbial groups occurring in the naturally harsh, cold Antarctic desert.

Survival of Microorganisms in Desert Soil Exposed to Five Years of Continuous Very High Vacuum

I. Introduction

In preparation for the microbiological examination of returned samples of lunar and Martian soil, the investigators selected a hot terrestrial desert soil for a long-term period of testing in very high vacuum to determine survival rates of microorganisms. Then, for the first time, a terrestrial desert soil was subjected to continuous very high vacuum to determine the survival of mixed indigenous populations of microflora and microfauna after 4- and 5-year periods.

This study was undertaken subsequent to two earlier experiments with microorganisms in vacuum (Refs. 1 and 2). In the first experiment (see Ref. 1), it was found that bacterial spores could survive in very high vacuum (10^{-8} torr) after 35 days at room temperature. In the second experiment (see Ref. 2), it was demonstrated that a significant number of bacteria and molds could survive after being maintained in a vacuum of 5×10^{-7} to 1×10^{-10} torr for 140 days. This report presents the results of one phase of a soil storage study undertaken at the request of the NASA Bioscience Subcommittee.

For the present study, an algal crust sample was collected in November 1963, from an extremely dry, barren area of the Colorado Desert of the greater Sonoran Desert. Aseptic collecting and handling procedures were followed in obtaining the sample from the surface to a depth of 2 to 4 mm (Ref. 3). Descriptions and photographs of the general area, topography, soil site, and algal crust are presented in Refs. 4–6.

II. Soil Properties

The soil used for this experiment consisted of a light-gray Coachella sand and an entisol derived from ancient, granitic beach materials. It had a high quartz content, microcline was evident, and weathered sea shells were present. Physically, this soil had a particle size range of 91.7% medium sand, 5.4% silt, and 2.9% clay. In the field, bulk density was 1.6 g/cm^3 and porosity was 44.2%. Air-dry moisture content was 0.5% (determined at 105 $\pm$5°C to constant weight); this was also the moisture content of the samples at the beginning of the vacuum experiment.

Chemical properties included total C (0.4%), total N (0.04%), and a relatively narrow organic C–organic N ratio of 9.6, which was indicative of very little exogenous or undecomposed organic matter. Saturated soil

paste values showed a *p*H of 7.9, an E*h* of +375 mV, and an electrical conductivity value of 3×10^{-6} mhos/cm^2 at 25°C. There was a relatively high buffer capacity, 15 meq/100 g of soil and a cation exchange capacity of 6.0 meq/100 g of soil, which was occupied primarily by Ca^{++}, Na^+, and K^+ in association with HCO_3^-, $SO_4^=$, and Cl^-.

An aliquot of sieved soil ($\lesssim$ 2 mm) was powdered with a sterilized automatic mortar and pestle for this experiment. The textural classification subsequently was altered from medium sand to a finer textural class, silty clay loam. Mechanical analysis of the powdered soil showed a new size range of separates of 20% very fine sand, 51.8% silt, and 28.2% "clay" ($\lesssim 2\,\mu$).

III. Equipment and Methods

A. Vacuum System

An apparatus, which enabled its 24 chambers to be exposed to vacuum simultaneously, was constructed of Pyrex glass.* Each chamber contained 2-g samples, 12 chambers with sieved soil and 12 with powdered soil (Fig. 1). The detailed design of sample chambers, which were detached from the vacuum system for culturing, is shown in Fig. 2 (included in this figure is one of the 4-oz bottles used for storage of air-dry sieved and powdered control samples).

An ionization gauge (Bayard–Alpert type) was mounted at the lower end of the appartus to provide pressure readings at the point of poorest vacuum. Vacuum was obtained with a Vacion pump (Varian V-11411) and high-voltage power supply (Varian V-7411), which were kept in continuous operation since March 16, 1964. The initial pressure (6×10^{-4} torr) of the pump was lowered to 3×10^{-5} torr after 1 mo, and then reduced to 1×10^{-6} torr after 3 mo. Finally, after 6 mo, pressures were maintained in the range of 10^{-7} to 10^{-8} torr. This pressure is equivalent to an altitude of 1.2×10^6 to 1.7×10^6 ft above sea level and a molecular density of 5×10^9 to 5×10^8 mol/cm^3 at 25°C. Temperature inside the system was measured with a copper–constantan thermocouple, and room temperature was checked with a mercury thermometer suspended parallel to the glass apparatus. Room temperature was monitored on a long-term basis with a recording thermometer (Tempscribe). Temperatures varied between 18 and 27°C, but were usually from 21 to 23°C. Temperatures within the vacuum system usually varied no more than 1 to 2°C from room temperature.

*Apparatus constructed by Hermann Heyn and Robert Condon.

B. Cultural Methods

Two powdered and two sieved samples were sealed off and removed from the lowest sample tier of the apparatus on April 9, 1968, when the pressure was 1.5×10^{-8} torr. On April 17, 1969, two additional sets of sieved and powdered samples were sealed off at a pressure of 1.2×10^{-7} torr. All cultural analyses have been performed since May 1, 1969. A washing technique was used to remove the samples from their chambers into appropriate dilution bottles for subsequent microbiological determinations.

Microbiological analyses were performed on samples diluted serially from 10^{-1} through 10^{-7} for microaerophiles and algae. Dilutions for agar pour plates were from 10^{-1} through 10^{-5}, or else soil was sprinkled on the agar surface when low numbers of microflora were expected. Trypticase soy agar was used to cultivate the aerobic bacteria (including streptomycetes); fluid thioglycollate was used for microaerophiles; and the anaerobes were cultivated on trypticase soy agar in a CO_2 atmosphere. Rose bengal agar, Cook's or Martin's formula (without antibiotics), was used in an attempt to culture fungi. Algae were grown in inorganic salt media (either Pochon's or Thornton's standard medium). The formulas for the latter two media consist of the salts per l of distilled H_2O indicated in Table 1.

Table 1. Formulas for Pochon's and Thornton's media

Pochon's solution		Thornton's medium	
Formula	Quantity	Formula	Quantity
$Ca(NO_3)_2$	0.1 g	K_2HPO_4	1.0 g
K_2HPO_4	0.4 g	$MgSO_4 \cdot 7H_2O$	0.2 g
$MgSO_4$	0.3 g	$CaCl_2$	0.1 g
KNO_3	0.1 g	NaCl	0.1 g
$FeCl_3$	0.001 g	KNO_3	0.5 g
EDTA	Trace	$FeCl_3$	0.002 g
		EDTA	Trace

Incubations for the various groups of microorganisms were conducted at temperatures of 4 to 55°C, as indicated in Table 2, for approximately 30 days (except for thermophiles, which were incubated for 2 days). The algae were incubated for 9 wk under Sylvania "Gro-lux" fluorescent tubes at approximately 500 ft-cd light intensity. Cultures were checked macroscopically and microscopically for growth. Algal dilution tubes also were checked microscopically for protozoa.

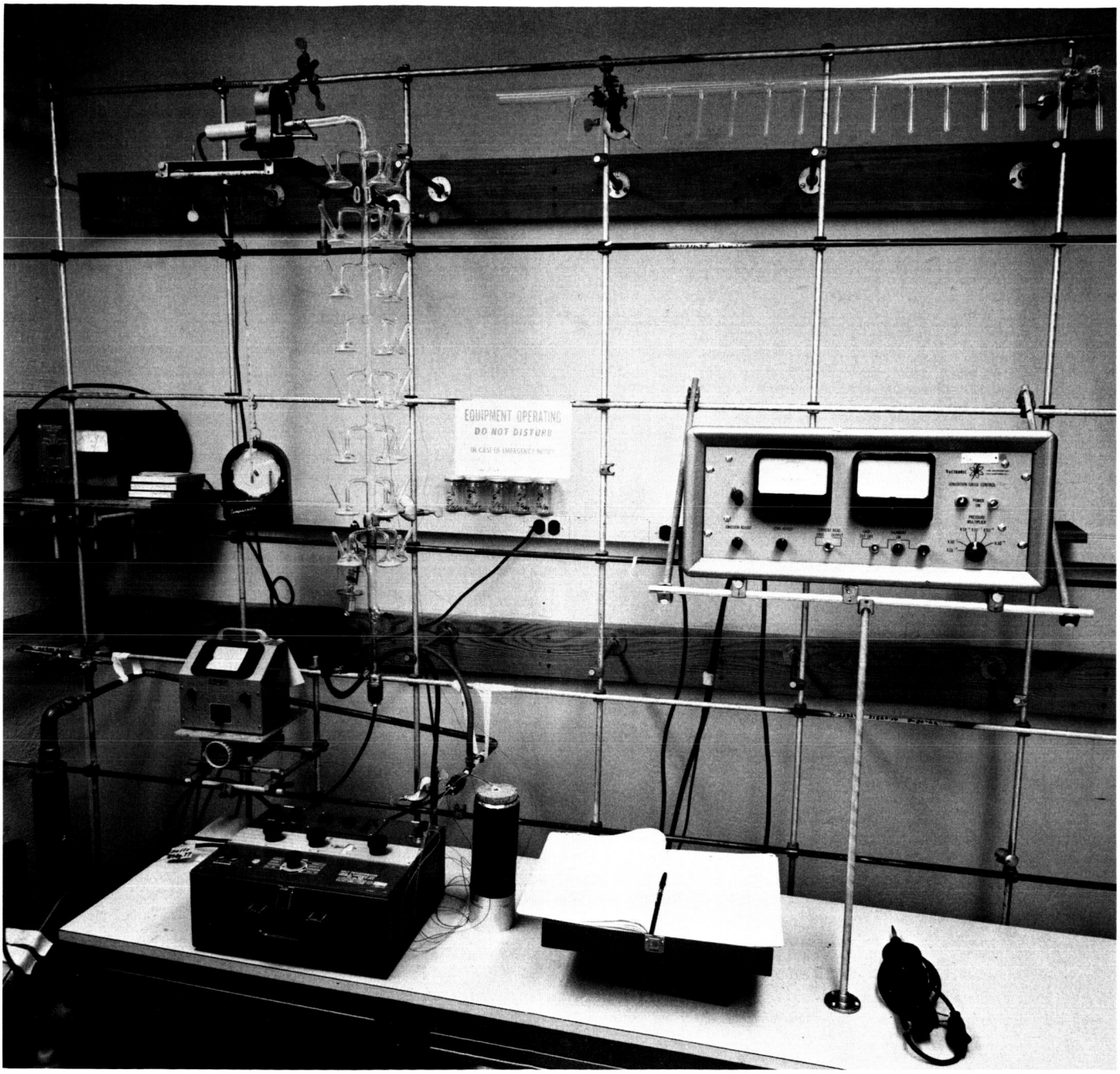

Fig. 1. Vacuum system arrangement

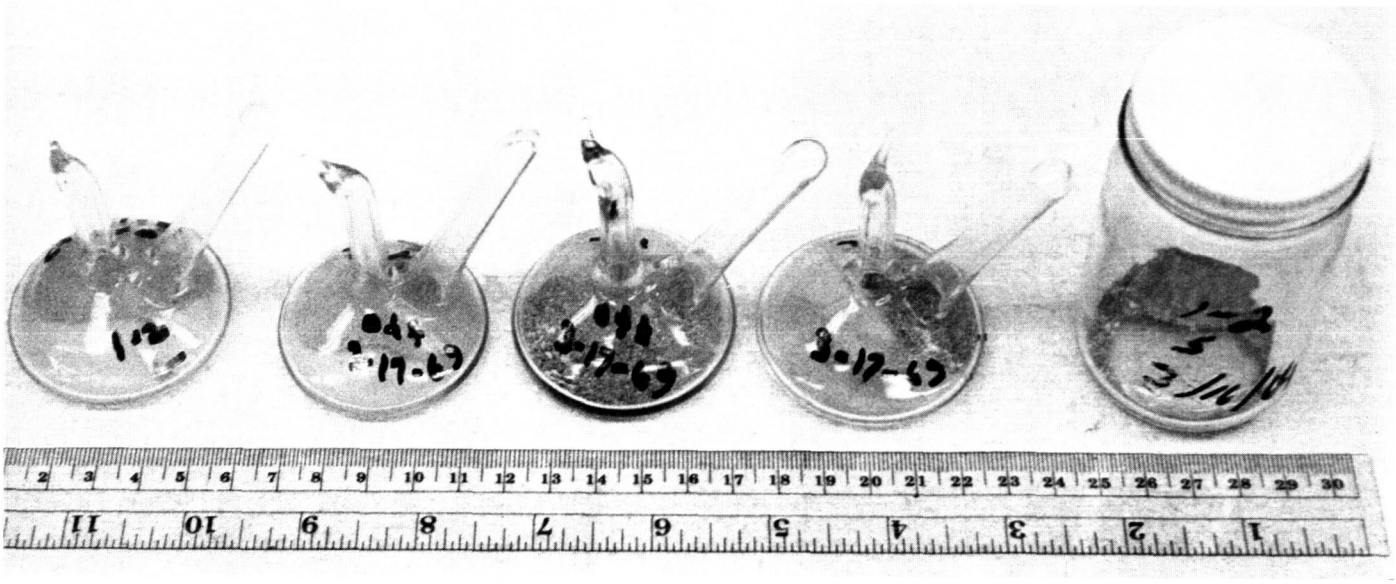

Fig. 2. Soil sample chambers and control sample bottle

IV. Results and Discussion

Average abundance of microorganisms in sieved and powdered soils is shown in Table 2. Abundances are given for sieved and powdered samples at the beginning of the experiment in 1964, and also for samples sealed off from the vacuum apparatus in 1968 and 1969.

For both sieved and powdered samples, there was a significant survival of aerobic bacteria cultured on trypticase soy agar at +20°C, but with a drop from 10^5 to 10^4 bacteria per g of soil in the sieved sample exposed to vacuum. The 1969 powdered control also showed a decrease in aerobic bacteria compared with values obtained for the control in 1964, as well as for powdered samples of similar age that were exposed to vacuum. The decrease in aerobic bacteria cultured from all stored, powdered samples may have been the result of injury to cells or subsequent death caused by grinding by mortar and pestle.

Original numbers of aerobic bacteria with psychrophilic and thermophilic capabilities were not determined. Because the sample was procured from the hot, dry, subtropical Coloradan subdesert of the greater Sonoran Desert, it is doubtful if psychrophiliclike bacteria were originally present. Thermophilic bacteria were present in both sieved and powdered air-dry control soils after 5 yr, but they did not survive in vacuum.

A large number of microaerophilic bacteria survived in both sieved and powdered samples, although there was a decrease of approximately 1 log unit after 4- to 5-yr exposure in vacuum. Aerobic bacteria, which were nonpigmented forms, also grew in this medium; they were usually 1 log unit lower in abundance than the microaerophiles, and comparable in number to those obtained by culturing in trypticase soy agar pour plates.

The anaerobic count was considerably lower than the aerobic or microaerophilic count. In the sieved control samples, there was a slight decrease in anaerobes after 4 yr, and none was recovered after 5 yr. Original counts of anaerobes were not made for the powdered control samples, and variable counts were obtained for both 1969 control samples and 1968 and 1969 *in vacuo* samples. There were still some anaerobes after 4- and 5-yr exposure in vacuum.

Molds did not survive following the 5-yr exposure of sieved samples to vacuum. Regarding anaerobes, the the results obtained for powdered samples were variable, and could again indicate the effect of grinding and the destruction of mold spores or fragmentation of mycelia. Surprisingly, a few molds were culturable at 4°C from powdered samples and a few yeasts were also recovered from one sample exposed to vacuum. The molds were apparently of the same species as was the one cultured at 20°C.

Algae gave the most consistent response in both sieved and powdered samples exposed to vacuum, with a large decrease in abundance after 5 yr, from 10^6 to 100 per g of

Table 2. Abundance of microorganisms per gram of desert soil

Soil treatment	Aerobic bacteria (including actinomycetes)			Microaerophiles (positive at highest dilution)	Anaerobes	Fungi				Positives at highest dilution	
						Molds		Yeasts		Algae	Protozoa
	+4°C	+20°C	+55°C	Room temperature	Room temperature	+4°C	+20°C	+4°C	+20°C	Room temperature	Room temperature
Sieved (control), 1964	—	5.5×10^5	—	10^6	2.6×10^3	—	150	—	—	10^6	—
Sieved (control), 1969	0	4.7×10^5	100	10^6	2.5×10^3	0	20	0	0	10^5	10^3
Sieved (vacuum), 1968	0	3.3×10^4	0	10^5	1.1×10^3	0	23	0	0	10^5	10^3
Sieved (vacuum), 1969	0	4.1×10^4	0	10^5	0	0	0	0	0	10^2	0
Powdered (control), 1964	—	3.7×10^5	—	10^5	—	—	300	—	—	10^6	—
Powdered (control), 1969	0	4.6×10^4	700	10^5	15	10	90	0	0	10^5	10
Powdered (vacuum), 1968	0	2.5×10^4	0	10^5	2.5×10^3	400	1.5×10^3	0	0	10^4	100
Powdered (vacuum), 1969	0	5.7×10^4	0	10^5	25	0	710	25	0	10^2	0
Media	Trypticase soy agar; Baltimore Biological Laboratory (BBL)			Fluid thioglycollate; BBL	TSA in CO_2	Cook's rose bengal agar or Martin's rose bengal agar (less antibiotics)				Pochon's solution or Thornton's medium (less organics)	

soil. However, there also was a decrease in both sieved and powdered control samples from 10^6 to 10^5 per g of soil after 5-yr, air-dry storage.

Also, the algae samples were observed for protozoa. As has been found for other desert soils collected from favorable microenvironments, protozoa were present, but were less numerous than the algae. Although the original abundance of protozoa was not determined, a decrease in algal numbers would undoubtedly result in a subsequent decrease in dependent protozoa; in both sieved and powdered samples, no protozoa were recovered after 5 yr in vacuum. The low abundance of protozoa in both the powdered control and 4-yr vacuum samples, compared to the sieved samples, can probably be attributed to the injury and death of these microorganisms during and following the grinding procedure.

Variable degrees of soil "stickiness," "hardening," or cohesion have been observed in the behavior of soil at similar very low pressures (Ref. 7). There was no discernible increase in cohesion of single-grain particles or aggregates in either powdered or sieved samples subjected to vacuum. No surface crust formation occurred in the powdered sample; however, a greater degree of variability in powdered samples, as compared to results for sieved samples, may be indicative of some protective microenvironments formed by very small aggregates of fine particles that were undiscernible macroscopically.

Although the primary objective of this study was to determine survivable numbers of the various groups of microorganisms, some attention was given to determining the identity of the most abundant surviving populations of each of the groups of microorganisms. These groups are indicated in Table 2.

Aerobic bacterial survivors included several *Bacillus* spp. and some diphtheroids. The diphtheroids most closely resembled *Nocardia* sp. (commonly included with the actinomycetes), *Arthrobacter* sp., *Micrococcus* sp., *Mycobacterium* sp., and *Corynebacterium* sp. This complex group of bacteria in desert soils is described in Refs. 8–10. Except for a few orange-colored colonies of one diphtheroid cultured from samples exposed to vacuum, none of the survivors was prominently pigmented.

In the air-dry control samples, approximately one third of the colonies were pigmented *Micrococcus* spp. Bacterial survivors were primarily nonpigmented or pale, translucent, cream-colored *Bacillus* spp. or diphtheroids (Fig. 3). The bacterial survivors also were isolated on TSA from algae dilution tubes. Microaerophilic bacteria in fluid thioglycollate, or those that survived cultivation in CO_2 atmosphere, also appear to be the same types of bacteria. An unusual desert-soil bacterium resembling large-polar flagellated, pleomorphic, spindle-shaped sulfur organisms, but without reddish pigmentation, *Rhabdochromatium* sp. (Ref. 11), was recovered from the sieved control sample cultured in fluid thioglycollate (Fig. 4). However, similar (but less morphologically distinct) bacteria were recovered from aliquots taken from algal dilution tubes and subcultured on trypticase soy agar.

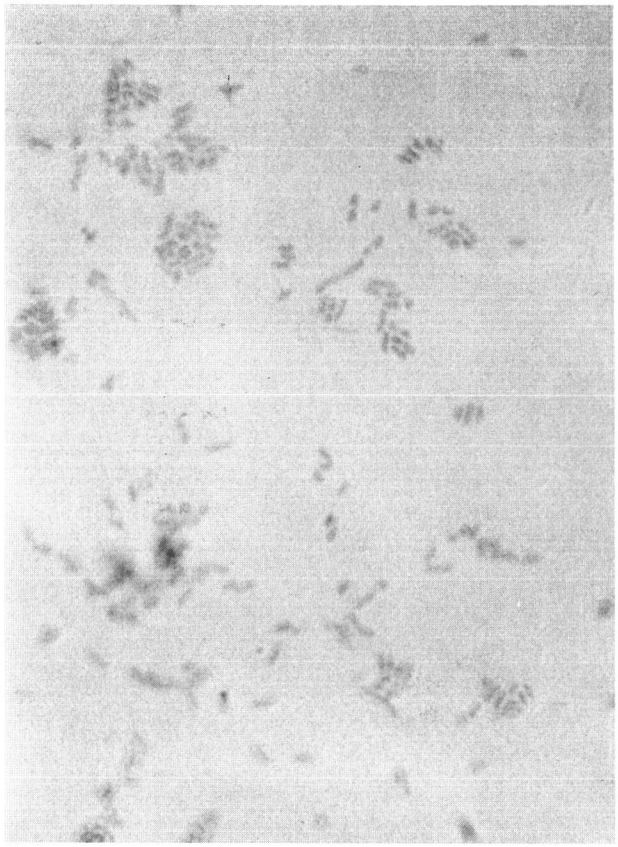

Fig 3. Photomicrograph (1250 ×) of stained diphtheroid

It has been observed that relatively few pigmented bacteria are found in the cold, barren Antarctic dry valley deserts (Refs. 12–14). A decrease in pigmented bacteria was observed from hot desert samples stored for 4 and 5 yr at −195 and −80°C. These samples included number 1-2 from Thermal, Calif., and numbers 9, 14-1, and 17, which were collected from the White Mountain Range in California at elevations of 12,000, 13,000, and 14,250 ft. Of the initial 58 bacteria isolated from California soils

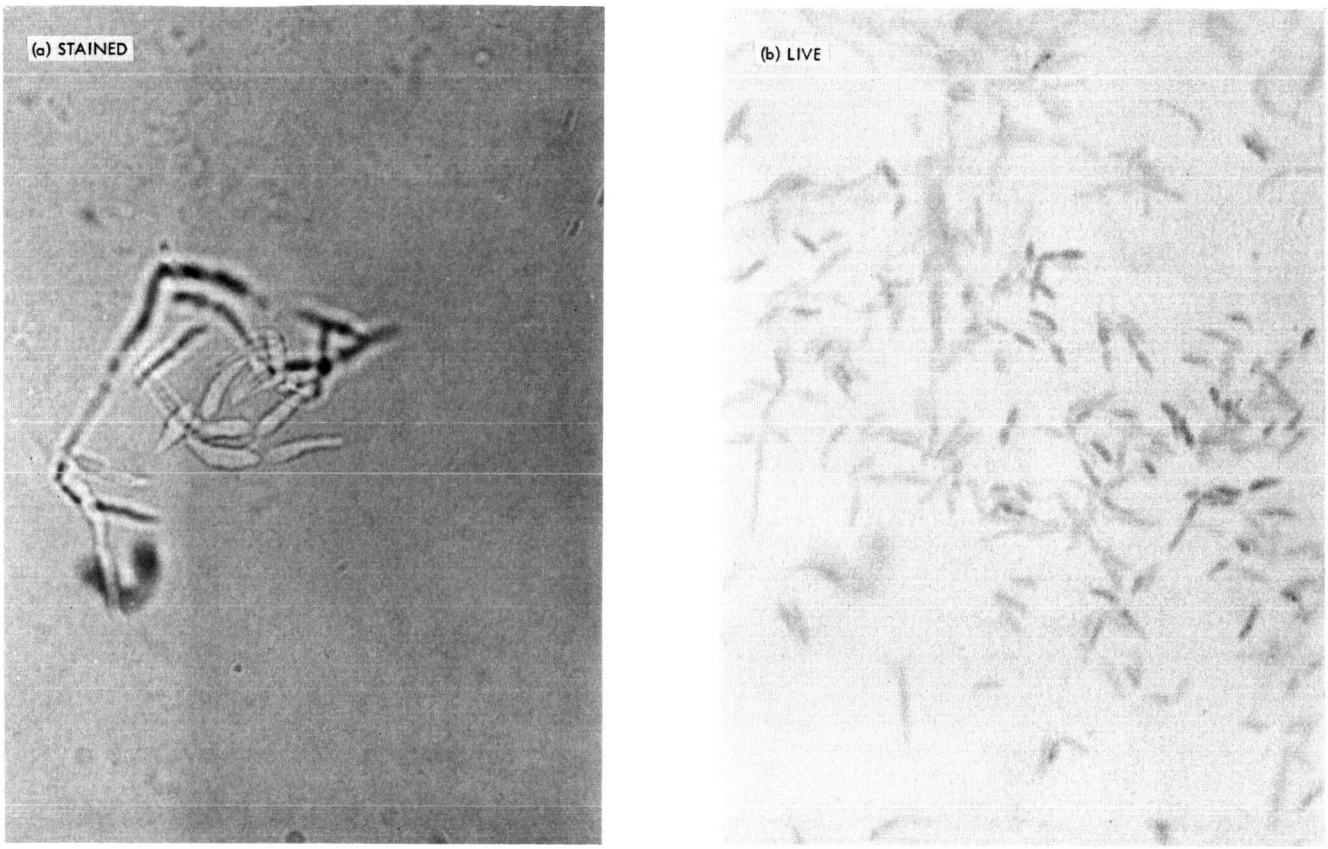

Fig. 4. Photomicrograph (1250 ×) of bacteria resembling *Rhabdochromatium*

stored air-dry for 65–85 yr, 75% were of *Bacillus* spp., 15% were of diphtheroids, and only 2% of micrococci (Ref. 15).*

The *Alternaria* sp. was the only prominent fungus in both control and evacuated samples. A few penicillia colonies observed in control samples were not recovered in the experimental samples. It has been reported that fungal mycelia survived better than spores after short exposures to a vacuum of 10^{-8} to 10^{-9} torr for 72 h at 23°C (Ref. 16). The fungus in the samples of this study was primarily in the form of mycelia intertwined in the algal soil crust. However, other experiments have indicated that spores of *Aspergillus niger* have a relatively high survival rate after exposure to ultrahigh vacuum (see Refs. 2, 17, and 18).

The algae survived in both the air-dry control and evacuated samples, but the survivability of the coccoid green alga, *Protococcus grevillei* (more commonly identified as *Chlorococcum humicola*), was reduced in abundance in the samples exposed to vacuum. The green alga grew well in one of the powdered evacuated samples, but in others it was generally unhealthy and showed degenerate forms readily attacked by bacteria. Only one dilution culture of a powdered vacuum sample showed any viable cells of the diatom, *Navicula* sp.

As far as could be determined from microscopic observations, all of the blue-green algal species survived, but the survival rate was irregular and not consistent with dilution of a given sample. However, this is not an uncommon phenomenon in the cultivation of mixed indigenous algal populations in soils. Some species, as has been observed in other desert soils, grow rapidly, predominate, and persist throughout all dilutions and throughout the tenure of the cultivation period, but other species are ephemeral or tenuous in their ability to survive, grow, and reproduce in mixed populations or communities of algae.

The blue-green algae included both filamentous and coccoid forms, and xeric as well as mesic species (Ref. 19), which depend on soil microenvironments (Ref. 20). The

*JPL Desert Microflora Program, unreported data.

following blue-green species were recovered in both control and experimental samples:

A. Filamentous species:

1. *Schizothrix arenaria.*
2. *Schizothrix calcicola.*
3. *Schizothrix rubella.*
4. *Scytonema hofmannii.*

B. Coccoid species:

1. *Anacystis marina.*
2. *Anacystis montana.*
3. *Coccochloris peniocystis.*

Although the spore-forming, nitrogen-fixing alga, *Nostoc muscorum,* forms protective akinetes (Fig. 5), it was recovered only from the sieved air-dry control samples. When present in soils, *Nostoc muscorum* is commonly found in association with *Schizothrix calcicola* (Fig. 6), which has many ecophenes, and is the most widely distributed and hardy of all species of the *Oscillatoriaceae* (Ref. 21). It was the most abundant alga in these samples, both in the control and in the experimental samples.

The flagellated protozoan, *Cercobodo angustus,* was found in many of the algal dilution cultures, in air-dry controls as well as in experimental samples subjected to 4 yr in vacuum. This microorganism was not recorded as present unless the active flagellated stage was observed. Cysts were also observed, and could have been viable resting stages of this same species. In one powdered sample exposed to vacuum, the large algal-containing protozoan, *Peranema trichophorum,* was observed, but it was not found in any other cultures. Flagellated as well as amoeboid protozoans have been observed in cultures of other hot deserts and Antarctic cold desert soil samples.*

*JPL Desert Microflora Program, unreported data.

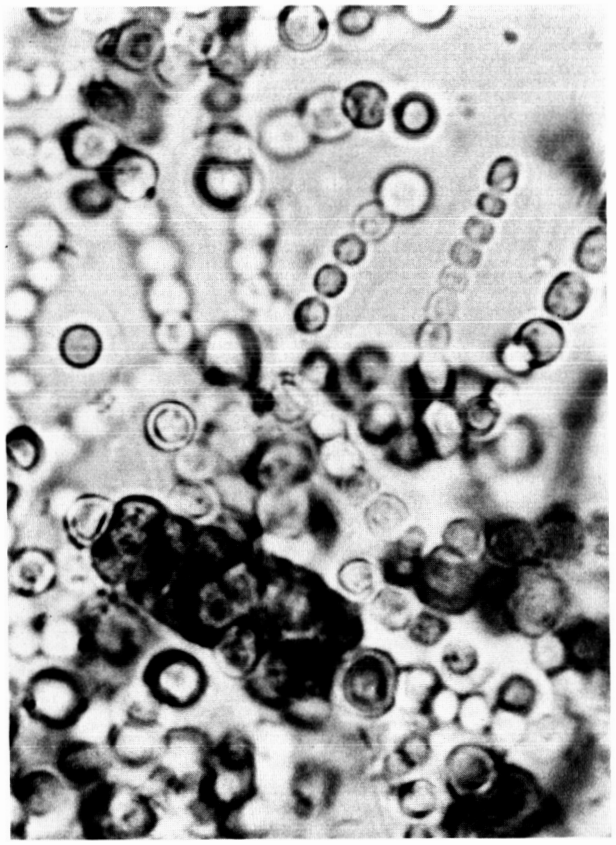

Fig. 5. Photomicrograph (1250 ×) of akinetes and filaments of *Nostoc muscorum*

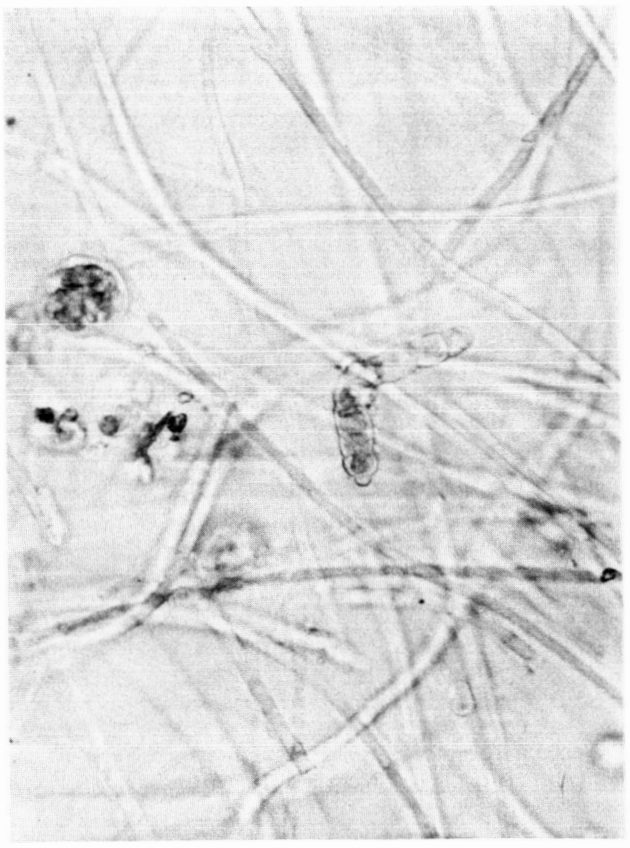

Fig. 6. Photomicrograph (500 ×) of filaments of *Schizothrix calcicola*

V. Conclusion

Exposure of an indigenous desert soil microbial community in sieved and powdered algal soil crust for 4 and 5 yr of continuous very high vacuum of 10^{-7} to 10^{-8} torr resulted in a decrease of most of the prominent groups of microorganisms after 5 yr. The most prominent survivors were nonpigmented, mesophilic, heterotrophic, aerobic, and microaerophilic bacteria. These were *Bacillus* spp. and various members of the diphtheroid group. There was a progressive decrease in the algae in both sieved and powdered samples. After 5 yr, no bacteria were recovered in the sieved samples following culturing in a CO_2 atmosphere, and no molds survived. No protozoa survived after 5 yr in vacuum in either powdered or sieved samples.

Results obtained with powdered samples exposed to vacuum were less conclusive than for sieved samples. Although reasonable care was taken to provide for homogenous samples, it is quite likely that injury or death of cells caused by grinding in mortar and pestle should be considered in an interpretation of results on powdered samples. It is also to be considered that the fine particles may have provided protective microenvironments for some microorganisms.

The decrease in microbial groups or elimination of some groups following exposure to vacuum is, in some ways, analogous to the survivable groups of microorganisms found in nature in the cold Antarctic desert. In the harshest areas within the Antarctic dry valleys, none or only a few nonpigmented aerobic or microaerophilic heterotrophic bacteria have been found (see Refs. 12–14). Coccoid green algae, filamentous blue-green algae, flagellated and amoeboid protozoa, and molds and lichens do not generally appear in these areas until there is an increase in favorable ecological factors—especially an increase in the duration of "available" water, which must be of a suitable quality. Thermophilic bacteria may sometimes survive (Ref. 22), but bacteria with anaerobic capabilities were not found unless other groups of microorganisms were also present, as well as moisture and organic matter.

Vacuum samples were incubated for longer periods than were the control samples before growth was observed. For example, the first appearance of bacterial growth at 20°C in trypticase soy agar usually took approximately 2 days in air-dry control samples, but more than twice as long for the vacuum experimental samples. This, too, is a general similarity for first appearance of bacteria cultured on agar from the Antarctic cold desert.

In conclusion, the testing of indigenous microorganisms in a desert soil exposed to long-term, very high vacuum has provided additional information on survivability of microorganisms in harsh environments. Long-term testing with other environmental parameters and soil microorganisms should provide further insight into understanding of harsh terrestrial environments and soil microbial ecology, as well as the possibility of life existing in extraterrestrial environments.

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