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January 1, 1975 - June 30, 1975

RESPONSE OF SELECTED MICROORGANISMS TO EXPERIMENTAL PLANETARY ENVIRONMENTS

Submitted by

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RESPONSE OF SELECTED MICROORGANISMS TO

EXPERIMENTAL PLANETARY ENVIRONMENTS

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FOREWORD

This sixth semiannual progress report summarizes work performed for the National Aeronautics and Space Administration by the Science Research Center at Hardin-Simmons University supported by NASA Grant NGR 44-095-001, and covers the period January 1, 1975 - June 30, 1975.

This report includes a microbial population profile of mixed Cape Canaveral soil samples. During this investigation a few organisms were isolated which exhibit the ability to grow at 3°C, 32°C, and 55°C. Growth curves are shown for three of these isolates, one of which grows extremely well at all three temperatures.

Also included in this report are studies dealing with growth of soil populations at zero and subzero temperatures. Results indicate growth at 0°C and -5°C, but not at -15°C or -65°C. The effect of storage temperature on dry soil is also presented herein and results show that psychrophilic populations decrease when soil is stored at room temperature, but do not decrease when soil is stored at -65°C.

Results of another experiment with the simulated Martian environment are also presented and indicate that non-sporeforming rods, sporeforming rods, and cocci can reproduce in the simulated environment when nutrients and moisture are supplied. The sporeforming rods are the predominant survivors when dry soil is subjected to this environment.

The NASA Technical Officer for this grant is Lawrence B. Hall, NASA Planetary Programs, Code SL, Washington, D.C

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POPULATION PROFILE OF SOIL ISOLATES FROM CAPE CANAVERAL

Previous progress reports from Hardin-Simmons University dealing with psychrophilic populations from Cape Canaveral soil samples have shown that these soils do contain psychrophilic microorganisms in significant populations. These previous investigations have dealt only with populations which were originally isolated at 7°C; therefore the results indicate only a partial characterization of the Cape Canaveral soils. The objective of this present investigation was to perform a more complete population chacterization with initial isolations being performed at different temperatures, aerobically and anaerobically.

Fresh soil samples were obtained from Cape Canaveral and included samples from the VAB, SAEF (1 and 2), launch complex 41, and the fuel storage area. Equal portions of these were mixed to yield the mixed Cape Canaveral soil used in this analysis. Duplicate 10-gram soil samples were diluted and processed as shown in Figure 1. Immediately following the first dilution, the samples were divided, with half being non heat-shocked and the other half being heat-shocked. These were then diluted using a 10X dilution scheme, with all dilutions being replicate plated so that duplicate plates were incubated aerobically and anaerobically at 3°C, 32°C, and 55°C. Counts were performed on the 32°C and 55°C after 48 hours and the 3°C after three weeks. The results of these counts are shown in Table 1. These results are consistent with our previous work on Cape Canaveral soil samples, although this was our first attempt to selectively assay

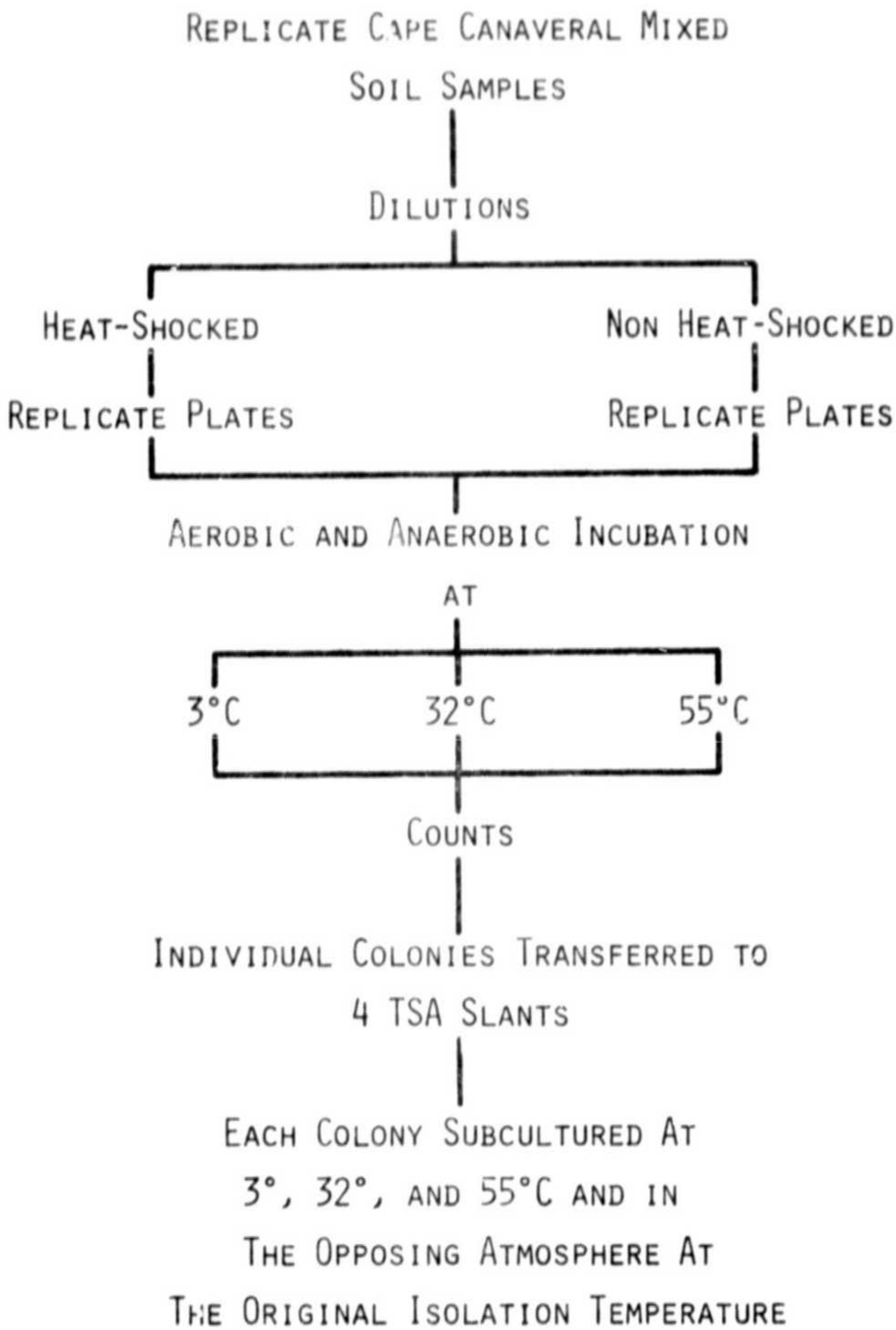

FIGURE 1. Schematic demonstrating procedures for population profile study of mixed Cape Canaveral soil samples.

Treatment	Atmosphere	Temperature	Count (Average of 4 plates)
Non Heat-Shocked	Aerobic	3°C	2.7×10^5
		32°C	6.6×10^6
		55°C	4.0×10^5
	Anaerobic	3°C	3.9×10^3
		32°C	5.1×10^5
		55°C	6.8×10^2
Heat-Shocked	Aerobic	3°C	2.1×10^3
		32°C	8.9×10^5
		55°C	2.2×10^5
	Anaerobic	3°C	$<1.0 \times 10^2$
		32°C	1.8×10^5
		55°C	2.4×10^2

TABLE 1. Counts of mixed Cape Canaveral soils incubated under various conditions (per gram of soil).

the thermophiles and anaerobes.

After counts were recorded individual colonies were transferred from plates having 30-300 colonies to four TSA slants. These slants were incubated at all three temperatures and in the opposite atmosphere at the original isolation temperature. After the prescribed incubation time the slants were observed for growth or no growth and the results recorded. By performing such a study it was possible to calculate the percentage population distribution of various types of microorganisms, and these results are shown in Table 2. The column showing percent of the total population gives the general distribution of the major types of organisms in Cape soils. Total population in this study is defined by the conditions of the investigation; that is, recovery in TSA at 3°, 32°, and 55°C aerobically and anaerobically. Psychrophiles, mesophiles, and thermophiles are defined as those that grow only at their respective temperature (3°C, 32°C, and 55°C respectively). Facultative organisms grow at more than one of the temperatures. For example, facultative psychrophiles grow at 3°C and 32°C. A significant fact seen in this table is that more than 95% of the isolates do grow at 32°C.

The remaining columns in Table 2 deal with the population distribution based upon oxygen requirements. The results show that the majority of the isolates grow aerobically with only 7.6% of the non heat-shocked population being obligate anaerobes and 2.2% of the heat-shocked population being obligate anaerobes. It is significant to note that this study revealed no obligately anaerobic psychrophiles capable of surviving heat-shock.

NON HEAT-SHOCKED

ORGANISM TYPE	% OF TOTAL POPULATION	% AEROBES	% FACULTATIVE	% ANAEROBES
PSYCHROPHILES	1.4	0.6	0.3	0.6
FACULTATIVE PSYCHROPHILES	32.7	2.4	26.7	3.5
MESOPHILES	41.3	12.5	26.0	2.8
FACULTATIVE THERMOPHILES	8.2	7.2	0.7	0.3
THERMOPHILES	1.1	0.6	0.4	0.1
OMNIPHILES	3.4	0.6	2.5	0.3
NO GROWTH ON SUBCULTURE	11.9	-	-	-

HEAT-SHOCKED

ORGANISM TYPE	% OF TOTAL POPULATION	% AEROBES	% FACULTATIVE	% ANAEROBES
PSYCHROPHILES	1.0	0.6	0.4	0.0
FACULTATIVE PSYCHROPHILES	8.4	6.2	2.2	0.0
MESOPHILES	43.7	20.0	23.2	0.6
FACULTATIVE THERMOPHILES	10.8	6.9	3.3	0.6
THERMOPHILES	3.7	2.4	0.4	0.8
OMNIPHILES	2.0	0.6	1.2	0.2
NO GROWTH ON SUBCULTURE	30.4	-	-	-

TABLE 2. POPULATION DISTRIBUTION OF VARIOUS TYPES OF ORGANISMS ISOLATED FROM HEAT-SHOCKED AND NON HEAT-SHOCKED CAPE CANAVERAL SOIL SAMPLES (GIVEN IN PERCENT).

By knowing the total population of a sample (calculated from Table 1) and the percentage distribution of the various types of organisms (Table 2), it is possible to calculate the load of each type of organism in the sample, and these results are presented in Table 3. For presentation in this table, all organisms capable of growth at 32°C are grouped together, and this makes up the majority of the population. In a percentage basis then, the psychrophiles, thermophiles, and anaerobes make up a small portion of the total population; but when this is calculated to give cells (or colony-forming units) per gram of soil, these populations appear to be much more significant. This is especially true when consideration is being given to probable contamination of planets. It appears worthwhile to consider performing a similar population distribution of air samples from inside spacecraft assembly areas to determine if the distribution is similar to that found in the soil.

<u>Preliminary results on organisms which grow at 3°, 32°, and 55°C</u>

In performing the investigation described above, it was observed that some isolates appeared to grow at all three of the designated temperatures. On the original population survey a total of 34 such isolates were found. Because of lack of a group designation and because of their wide temperature range we have tentatively called these "omniphiles" until a better term is suggested. Because these were only recently isolated we have had very little time to work with them at the time of writing of this report, but preliminary results will be presented here. All 34 isolates were streaked onto plates to

Non Heat-Shocked

Organism Type	Total	Aerobic	Facultative	Anaerobic
OBLIGATE PSYCHROPHILES	3.8×10^3	1.6×10^3	0.8×10^3	1.6×10^3
MESOPHILES + FACULTATIVES	5.6×10^6	1.5×10^6	3.7×10^6	4.6×10^5
OBLIGATE THERMOPHILES	4.4×10^3	2.4×10^3	1.6×10^3	4.0×10^2

Heat-Shocked

Organism Type	Total	Aerobic	Facultative	Anaerobic
OBLIGATE PSYCHROPHILES	2.1×10^1	1.3×10^1	0.8×10^1	0
MESOPHILES + FACULTATIVES	5.8×10^5	3.0×10^5	2.7×10^5	1.2×10^4
OBLIGATE THERMOPHILES	8.1×10^3	5.3×10^3	8.8×10^2	1.8×10^3

TABLE 3. Counts of various types of organisms isolated from mixed Cape Canaveral soil samples (per gram of soil).

determine purity of the culture, and these were again placed at all three temperatures when purity was confirmed. At this time only 10 of the original 34 have repeated the ability to grow at all three temperatures, but work is still in progress with the others. During this investigation, it has been observed that some of the isolates do not grow in TSB at high and/or low temperatures. Turbidity studies have been performed on five of these isolates: one of them failed to grow at 3°C, three grew at 3°C only sparsely, and one grew very well at 3°C. All five grew well at 32° and 55°C. The results of three of these isolates are shown in Figures 2, 3, and 4. Figures 2 and 3 indicate slight turbidity at 3°C, and visual observation of the flasks shows very fine particles of growth with little gross turbidity. Figure 4 shows good growth at 3°C after three weeks with turbidity being greater than 40 hours at 55° or 32°C. All three isolates show 32°C to be the optimum growth temperature. Isolate G-38B is a small spore-forming rod, exhibiting similar morphology at all three temperatures. The other two are small rods, but spores have not yet been detected.

Detailed studies of these unusual isolates has recently been initiated. They will be studied in detail and results will be presented in our next report.

B. Population Changes of Mixed Cape Canaveral Soil Samples Stored in TSB at Various Low Temperatures over an Extended Time Period

An experiment was performed to determine if soil populations could increase at 0°C or subzero temperatures when supplied with

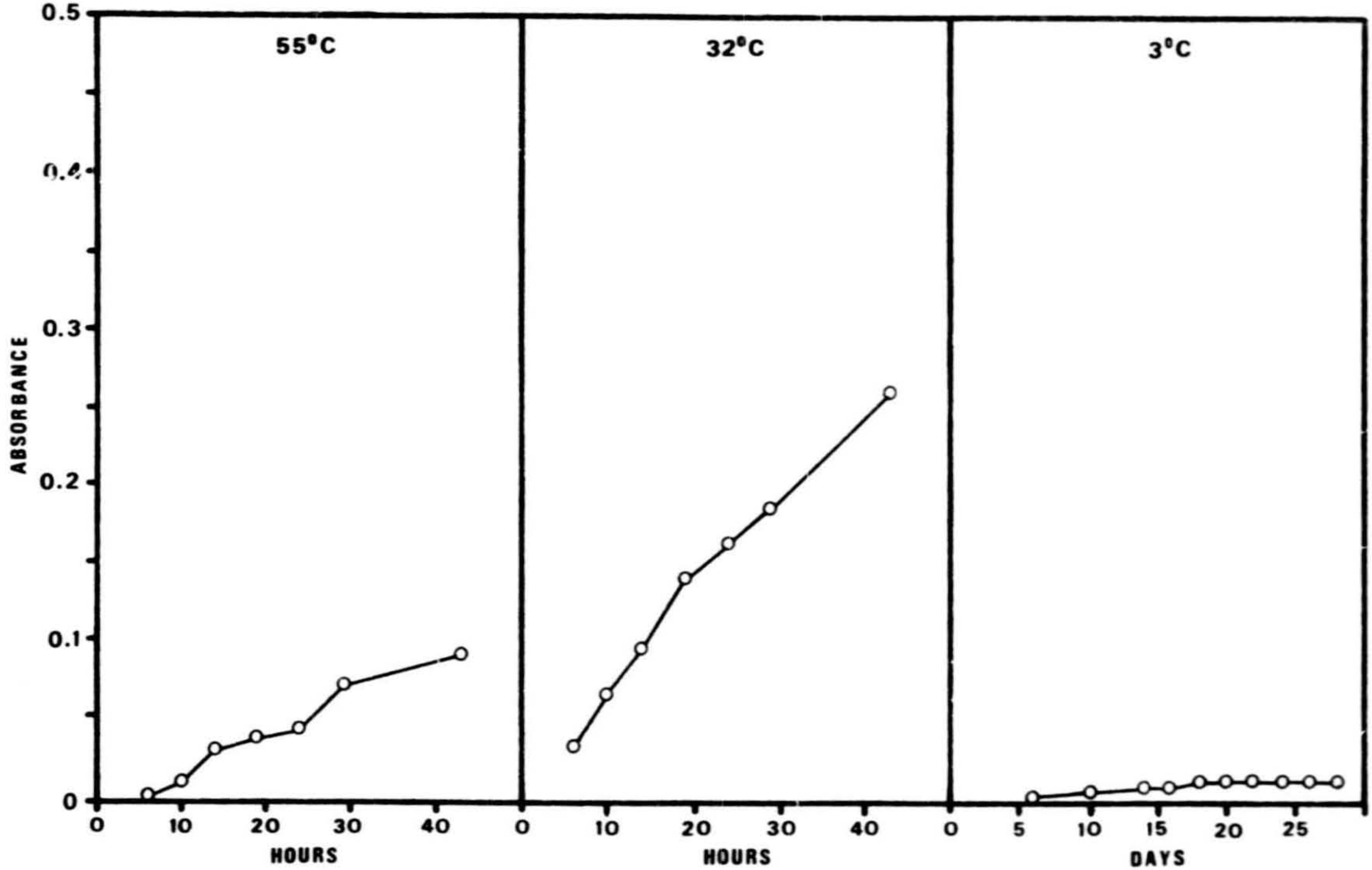

FIGURE 2. RESPONSE OF KSC SOIL ISOLATE A-28 AT THREE DIFFERENT TEMPERATURES.

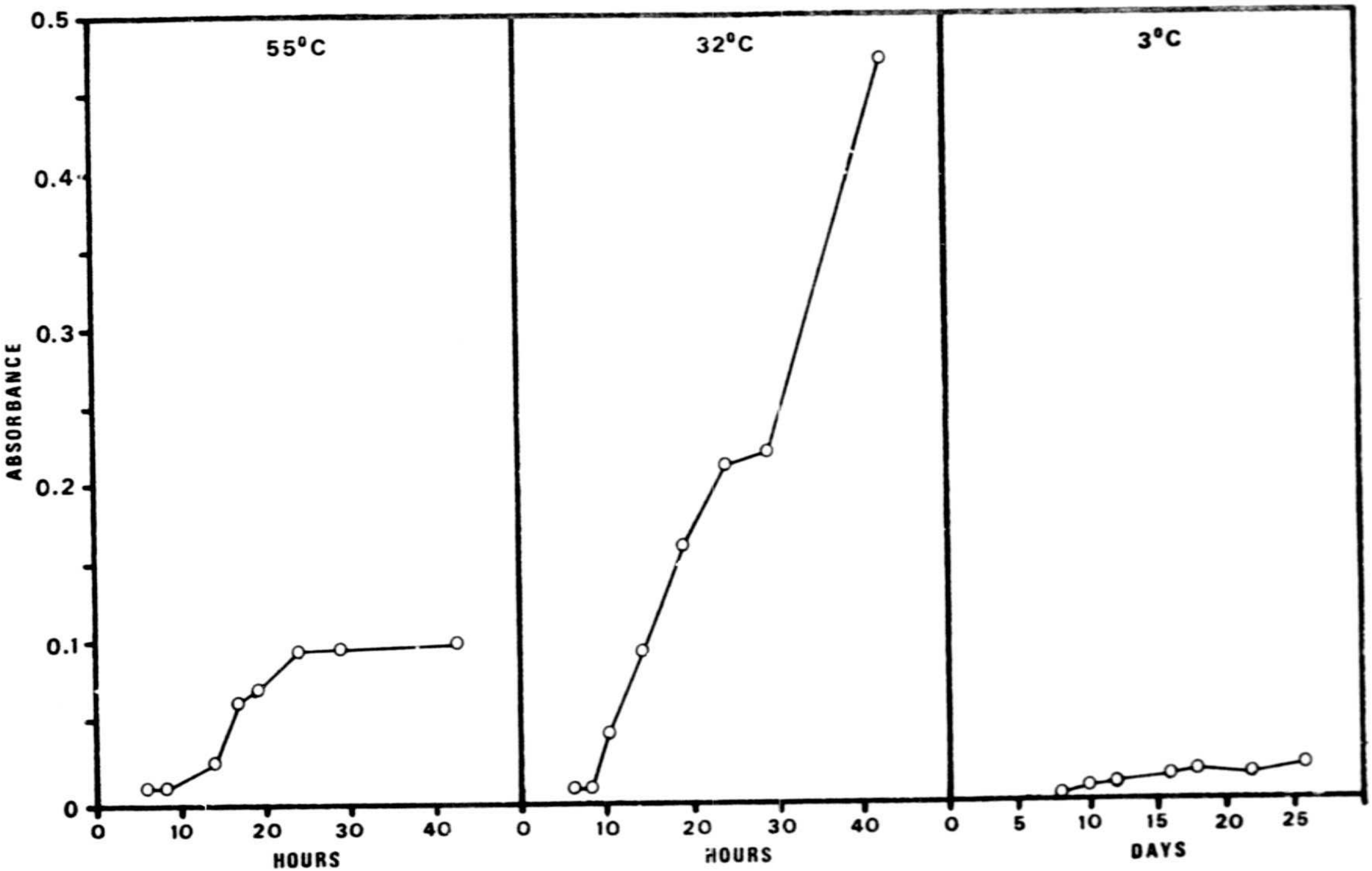

FIGURE 3. RESPONSE OF KSC SOIL ISOLATE C-16 AT THREE DIFFERENT TEMPERATURES.

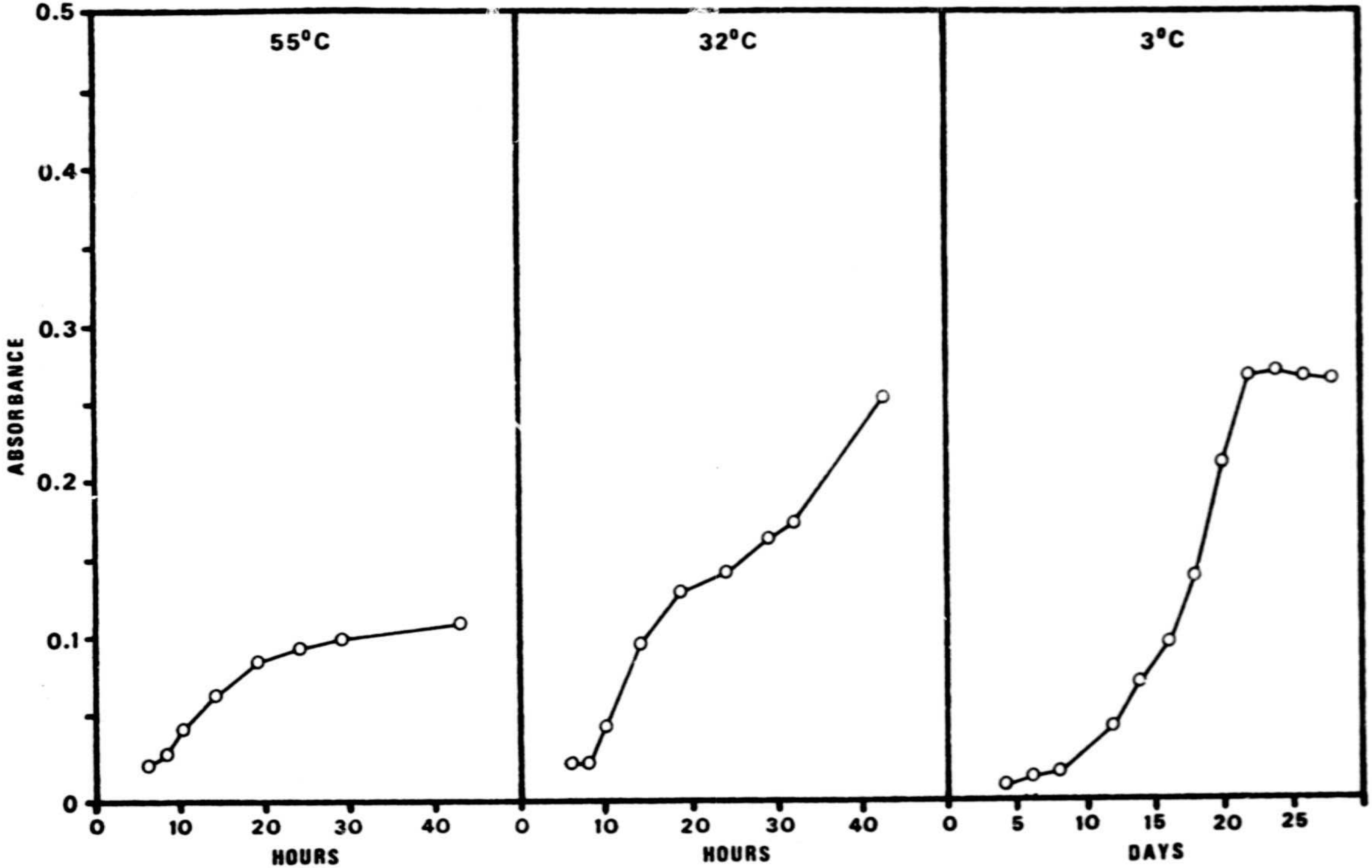

FIGURE 4. RESPONSE OF KSC SOIL ISOLATE G-38B AT THREE DIFFERENT TEMPERATURES.

sufficient nutrients. In this procedure, replicate 0.25-gram soil samples were weighed into vials, 0.25 ml TSB was added, and the vials were stored at 0°C, -5°C, -15°C, and -65°C. Duplicate vials were removed approximately every two weeks, diluted, and plated in duplicate. Plates were incubated at 7°C and read after 14 days. At the time of this writing, this investigation had covered 112 days and is still in progress. The results of this investigation are presented in Table 4. At 0°C the population increases rapidly and at this time has maintained approximately its maximum population. At -5°C there appears to be significant growth after 77 days, which is followed by a decline. At -15°C the counts are variable, but the population never increases one log, and the changes are not considered significant. At -65°C the population increases at about 44 days, then remains constant. It was at this point that the -65°C vials had to be moved to another freezer to facilitate defrosting of the -65°C incubator, and it is felt that the slight increase may be attributed to this. Again, however, the increase is never as much as one log and is considered insignificant. In summary, it appears that the populations do increase at 0°C and -5°C, but not at -15°C or -65°C.

C. Effect of Storage Temperature on Various Populations in Mixed Cape Canaveral Soil Samples

Because many of our studies and the studies of others in this field deal with various population analyses of soil samples, we are investigating the effect of storage temperature on populations in soil samples from Cape Canaveral. Replicate samples of mixed

Storage Temperature	Days Incubation 4	30	44	77	94	112
0°C	3.7×10^{7}	3.0×10^{8}	2.5×10^{8}	6.9×10^{7}	2.5×10^{8}	8.5×10^{7}
- 5°C	8.6×10^{4}	-	-	9.7×10^{5}	4.6×10^{4}	7.8×10^{4}
-15°C	7.9×10^{4}	2.0×10^{5}	1.3×10^{5}	-	6.3×10^{4}	1.3×10^{5}
-65°C	3.8×10^{4}	8.4×10^{4}	2.6×10^{5}[a]	3.2×10^{5}	1.6×10^{5}	2.1×10^{5}

NOTE: Original count was 4.5×10^{4}

[a]-65°C freezer was defrosted during this time.

TABLE 4. Population changes of mixed Cape Canaveral soil samples stored in TSB at various low temperatures over an extended period of time.

Cape Canaveral soils were placed into sterile plastic containers with airtight lids. These were then stored at room temperature and -65°C with duplicate samples being removed and counted every month. Counts were performed at 3°C, 32°C, and 55°C. The current results of this study are shown in Figures 5 and 6. Figure 5 is a mesophilic count and demonstrates that storage at 24°C causes a slight initial increase during the first 30 days, but shows no change during the next four months. Storage at -65°C causes a slight initial decrease which then becomes stationary.

Figure 6 shows the psychrophilic and thermophilic counts of soils stored at 24°C and -65°C. There is no significant difference in the thermophilic counts at the two storage temperatures, but the psychrophiles show a declining population in the sample stored at 24°C. The psychrophilic count of soil stored at -65°C is unchanged after five months of storage.

These are only partial results as this investigation is still in progress. Further results well be shown in our next report.

D. Recovery of Survivors from Mixed Cape Canaveral Soil Samples after Exposure to the Viking Dry Heat Cycle

In some of our previous work (Report 5), we had been concerned with the recovery of hardy organisms from the Teflon ribbon experiments in which the heated ribbons were subjected to spacecraft conditions. These consisted of N_2 gas (5 inches water pressure) followed by 10^{-6} Torr vacuum followed by storage in the simulated Martian environment at 10°C. Recovery medium was then added to the ribbons after exposure to these spacecraft conditions. That

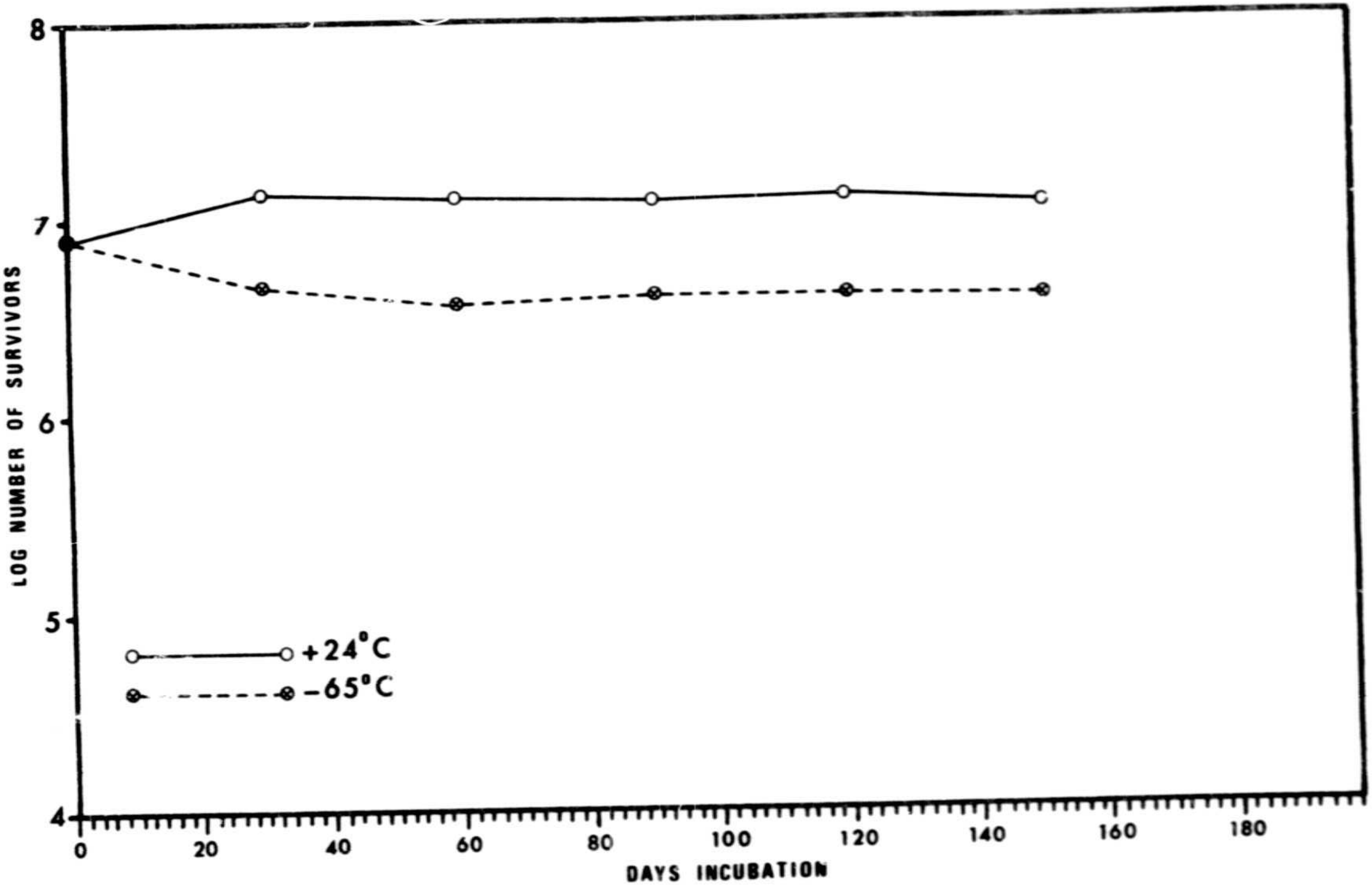

FIGURE 5. SURVIVAL OF SOIL POPULATIONS WHILE STORED IN A CLOSED CONTAINER AT +24°C OR -65°C.

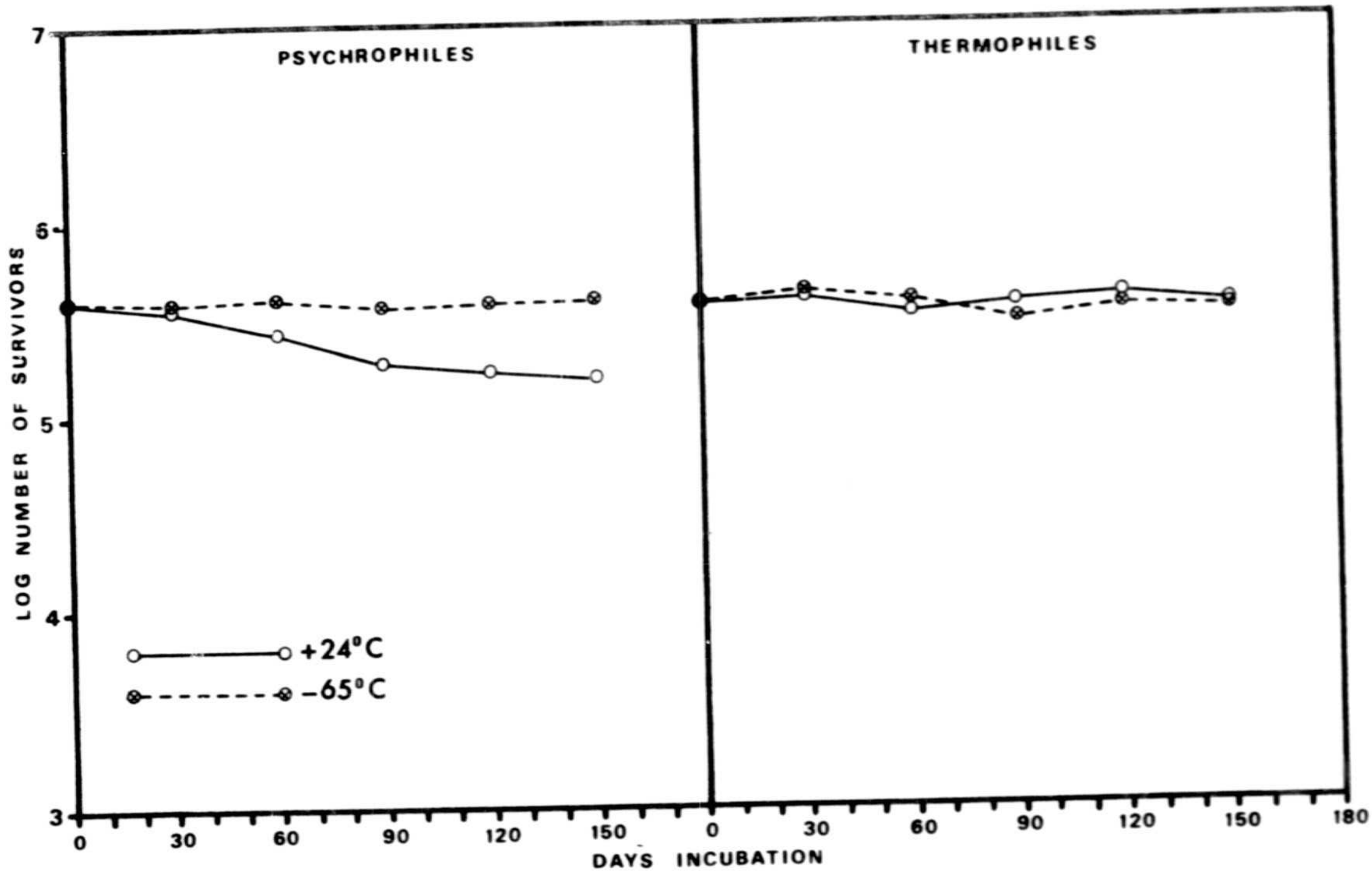

FIGURE 6. SURVIVAL OF PSYCHROPHILIC AND THERMOPHILIC KSC SOIL POPULATIONS WHILE STORED IN A CLOSED CONTAINER AT +24°C AND -65°C.

investigation indicated that spacecraft conditions might lower the recovery rate of hardy organisms, but the number of samples and the N_o were too small to place a great deal of confidence in the results. For this reason we performed a similar study in which we subjected 0.1 gram replicate Cape Canaveral soil samples to the Viking dry heat cycle. In doing this study, we used the ovens at the JPL-Planetary Quarantine Lab at Cape Canaveral. Recovery of hardy organisms was performed by adding buffer to the vials, sonicating, and plating the total volume of buffer. Replicate samples were plated immediately after heating, aerobically and anaerobically. Other samples were subjected to spacecraft conditions prior to recovery. These results are presented in Table 5. A comparison of aerobic recovery to recovery after exposure to spacecraft conditions shows that there is a decrease in the recovery of hardy organisms after exposure to spacecraft conditions, and this decrease is significant at the 0.05 probability level.

The JPL-PQ lab has recently installed a second dry-heat oven, and during this investigation we ran replicate samples in oven A and oven B to determine if there were differences in their heating characteristics. Replicate samples were assayed aerobically and anaerobically. The results shown in Table 5 indicate that there is no significant difference in the thermal characteristics of the two ovens. This is in agreement with results found by other investigators.

The anaerobic assays in this investigation were performed to determine if any hardy survivors were obligate anaerobes. Of these

Recovery Condition	Oven A	Oven B	Statistical Test $T_{0.05}$ Theoretical	Calculated	Significant
Aerobically	5.6×10^{3}[a]	4.1×10^{3}	2.10	1.79	No
Anaerobically[b]	7.2×10^{2}	8.4×10^{2}	2.10	0.87	No
Aerobically after exposure to spacecraft conditions[c]	2.4×10^{3}	-	2.10	3.93	Yes

[a] All counts are an average of 10 soil samples.

[b] All of these proved to be facultative anaerobes.

[c] N_2 (5 in. water pressure for 2 weeks) followed by 10^{-4} Torr vacuum for 2 weeks followed by simulated Martian environment at 10°C for 2 weeks.

TABLE 5. Recovery of survivors from mixed Cape Canaveral soil samples after exposure to the Viking dry heat cycle (per gram of soil).

survivors examined, all were facultative anaerobes.

E. Response of Mixed Cape Canaveral Soil Samples to the Simulated Martian Environment

In previous reports we have presented results of various experiments dealing with growth of organisms in the simulated Martian environment. We were asked to report this with fresh, mixed Cape Canaveral soil samples, and study the general population changes throughout the experiment. These results using various variables are presented in Figures 7-14. The growth patterns themselves follow the typical results which we have presented previously, i.e. given moisture and nutrients the populations will increase in the simulated Martian atmosphere; in a dry system the population is unchanged as far as total count.

In studying the changes of individual populations, the organisms were grouped into one of three groups, the non-sporeforming rods, the Bacillus spp., and the Gram + cocci. Figure 8 shows that when the soil population increases, the increase appears to be attributable to all three groups, with the non-sporeforming rods yielding the greatest increase in the last few days. The simulated environment with incubation at constant 24°C (Figures 9 and 10) also shows an increase in population, but it drops faster than the freeze-thaw incubation (Figure 7). Again all three populations appear to respond in similar fashion. Figure 11 shows that the total population is unchanged in the dry atmosphere, but Figure 12 shows that both the non-sporeforming rods and cocci decline whereas the Bacillus group remains stationary or increases slightly. Figures 13 and 14 also indicate that the

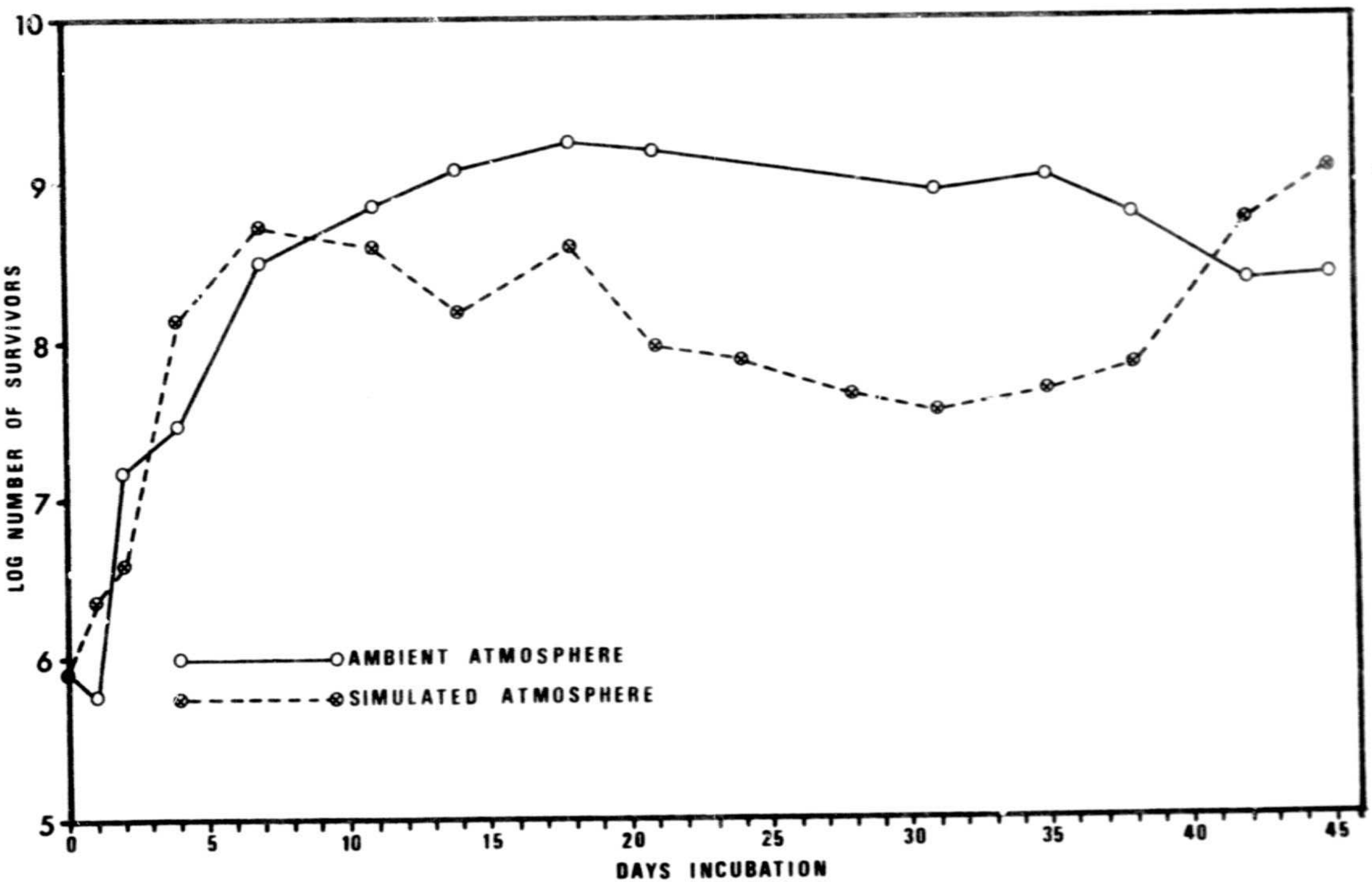

FIGURE 7. RESPONSE OF SOIL POPULATIONS TO AN ENVIRONMENT CONTAINING EXCESS MOISTURE AND ADDED NUTRIENTS WHILE INCUBATED USING AN ALTERNATING FREEZE-THAW CYCLE OF -65°C FOR 16 HOURS AND +24°C FOR 8 HOURS. SIMULATED ATMOSPHERE BEING 99.9% CO_2 AND 0.1% O_2 AT 7 MB PRESSURE.

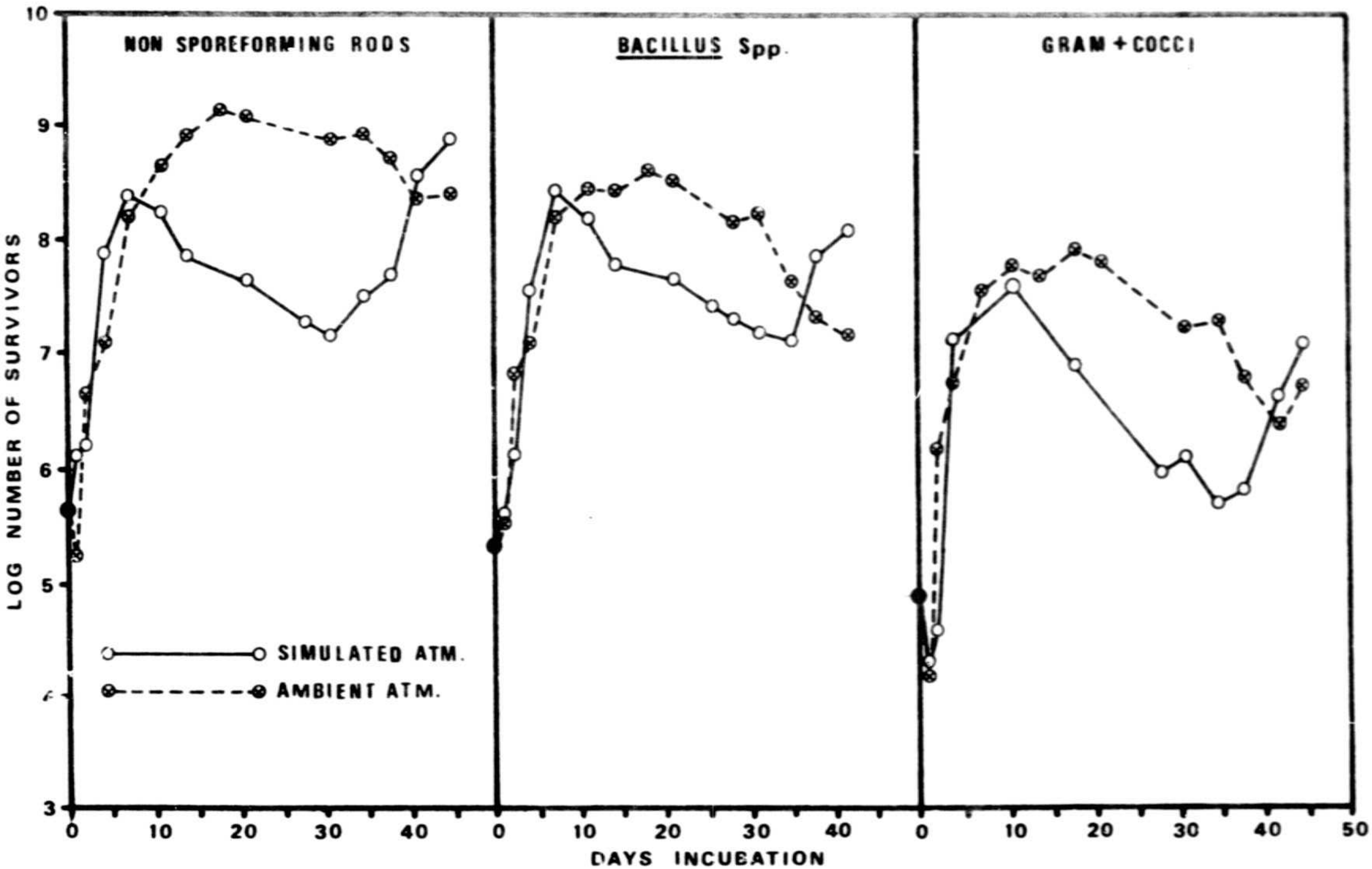

FIGURE 8. POPULATION CHANGES OF VARIOUS BACTERIAL GROUPS WITH ADDED MOISTURE AND NUTRIENTS USING A FREEZE-THAW INCUBATION CYCLE.

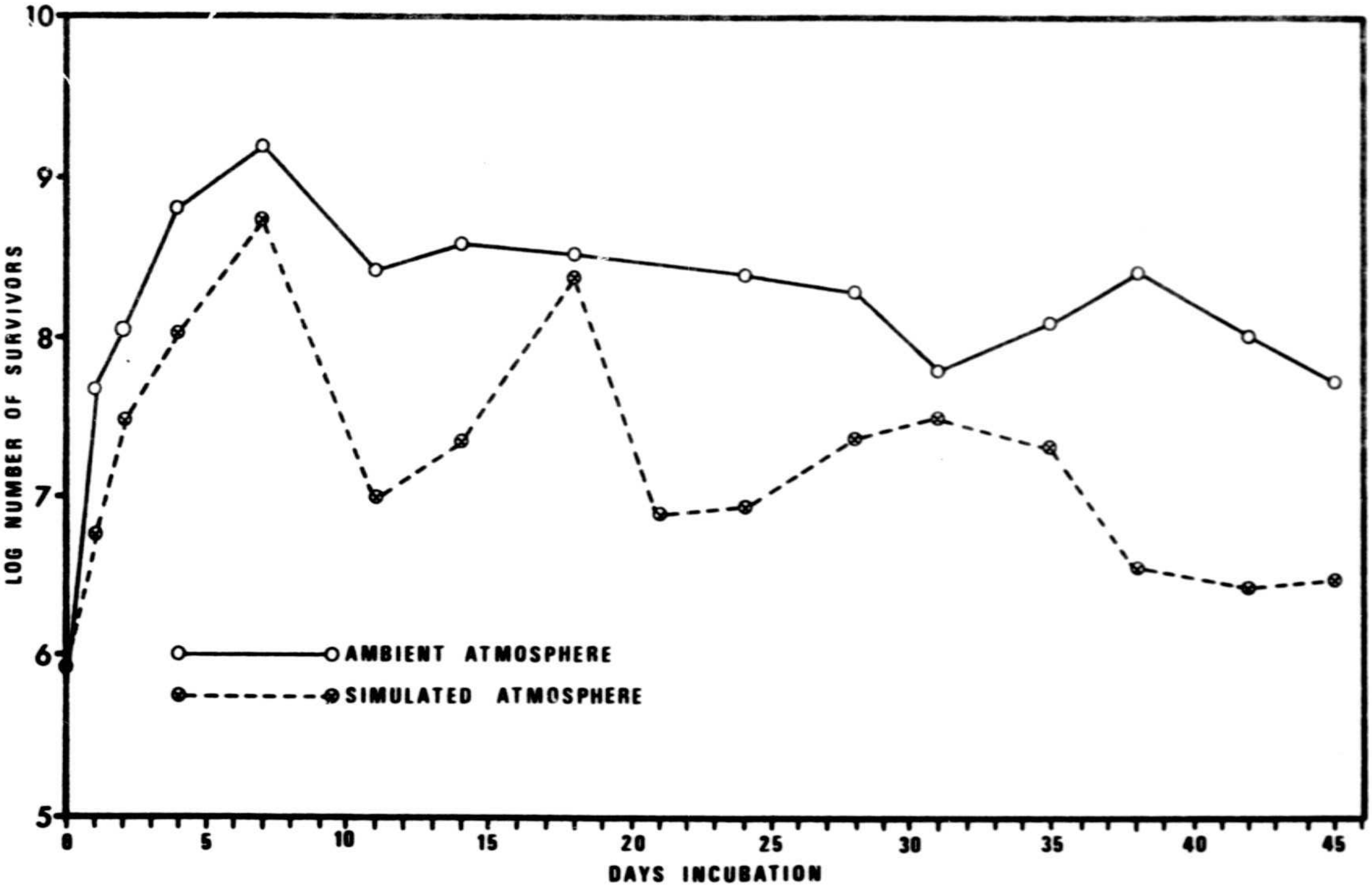

FIGURE 9. RESPONSE OF SOIL POPULATIONS TO AN ENVIRONMENT CONTAINING EXCESS MOISTURE AND ADDED NUTRIENTS WHEN INCUBATED AT A CONSTANT 24°C. SIMULATED ATMOSPHERE BEING 99.9% CO_2 AND 0.1% O_2 AT 7 MB PRESSURE.

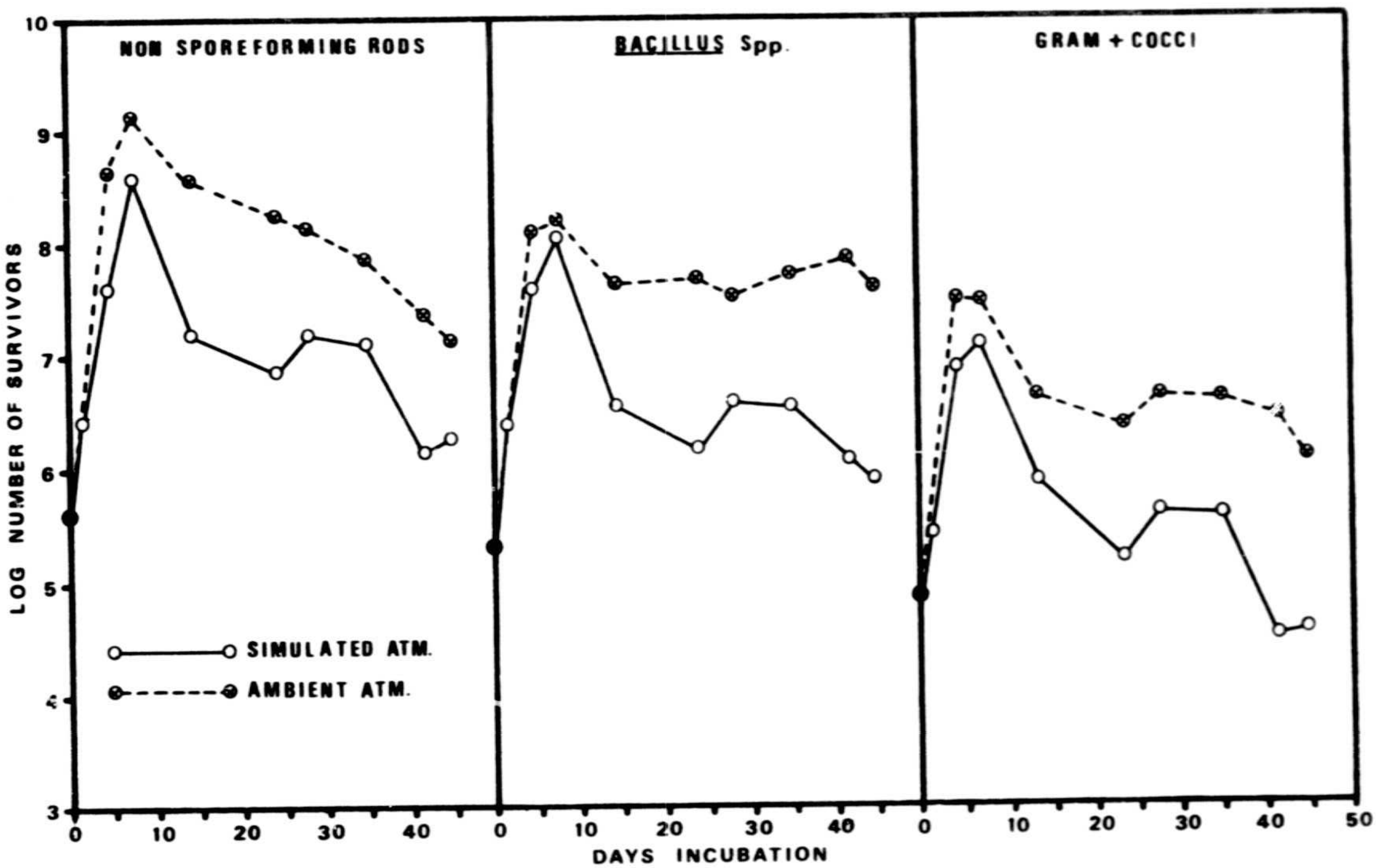

FIGURE 10. POPULATION CHANGES OF VARIOUS BACTERIAL GROUPS WITH ADDED MOISTURE AND NUTRIENTS USING A 24°C INCUBATION TEMPERATURE.

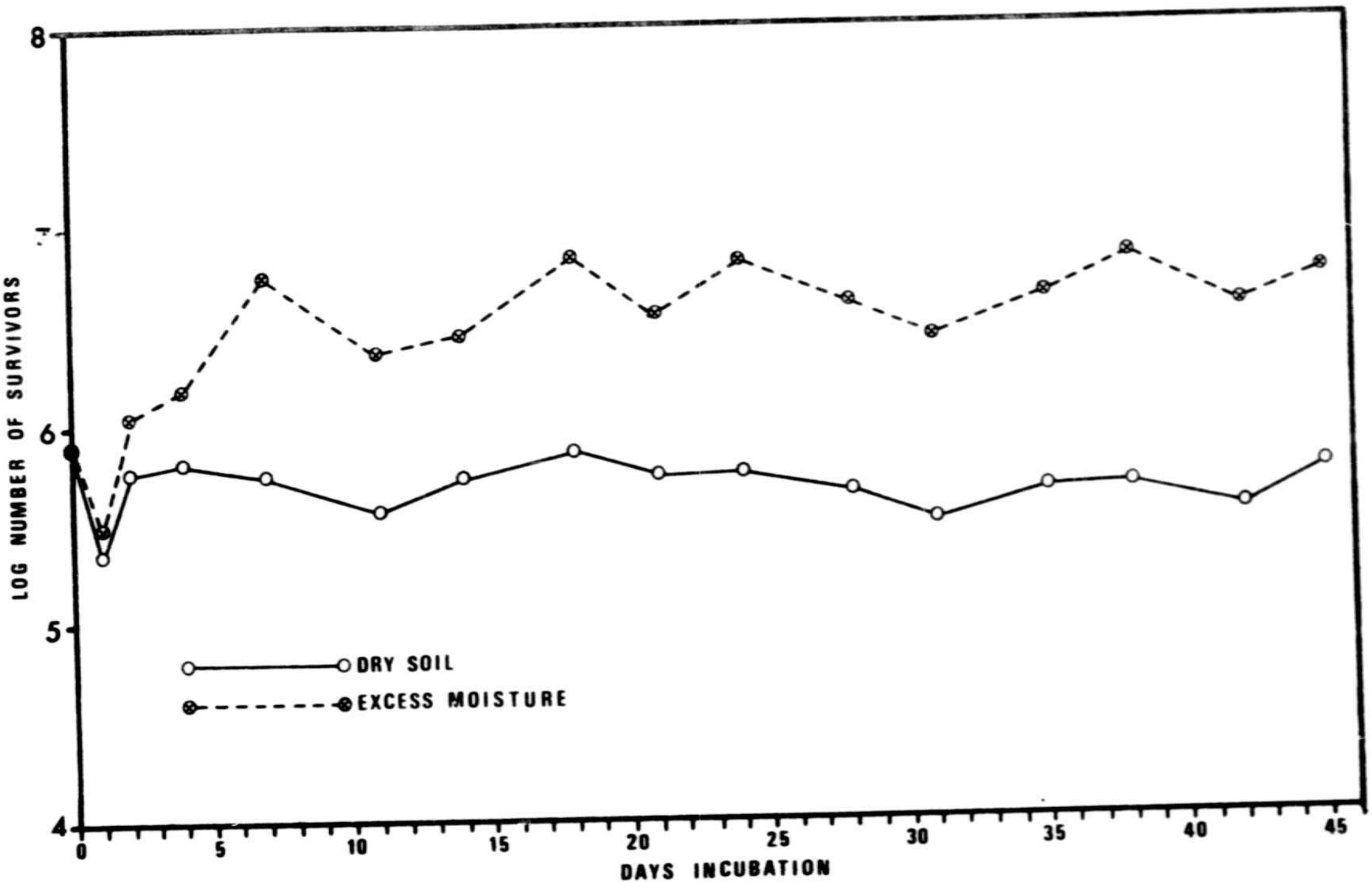

FIGURE 11. RESPONSE OF CAPE CANAVERAL SOIL POPULATIONS TO A SIMULATED ENVIRONMENT OF 99.9% CO_2 AND 0.1% O_2 AT 7 MB PRESSURE DURING AN ALTERNATING FREEZE-THAW CYCLE OF -65°C FOR 16 HOURS AND +24°C FOR 8 HOURS.

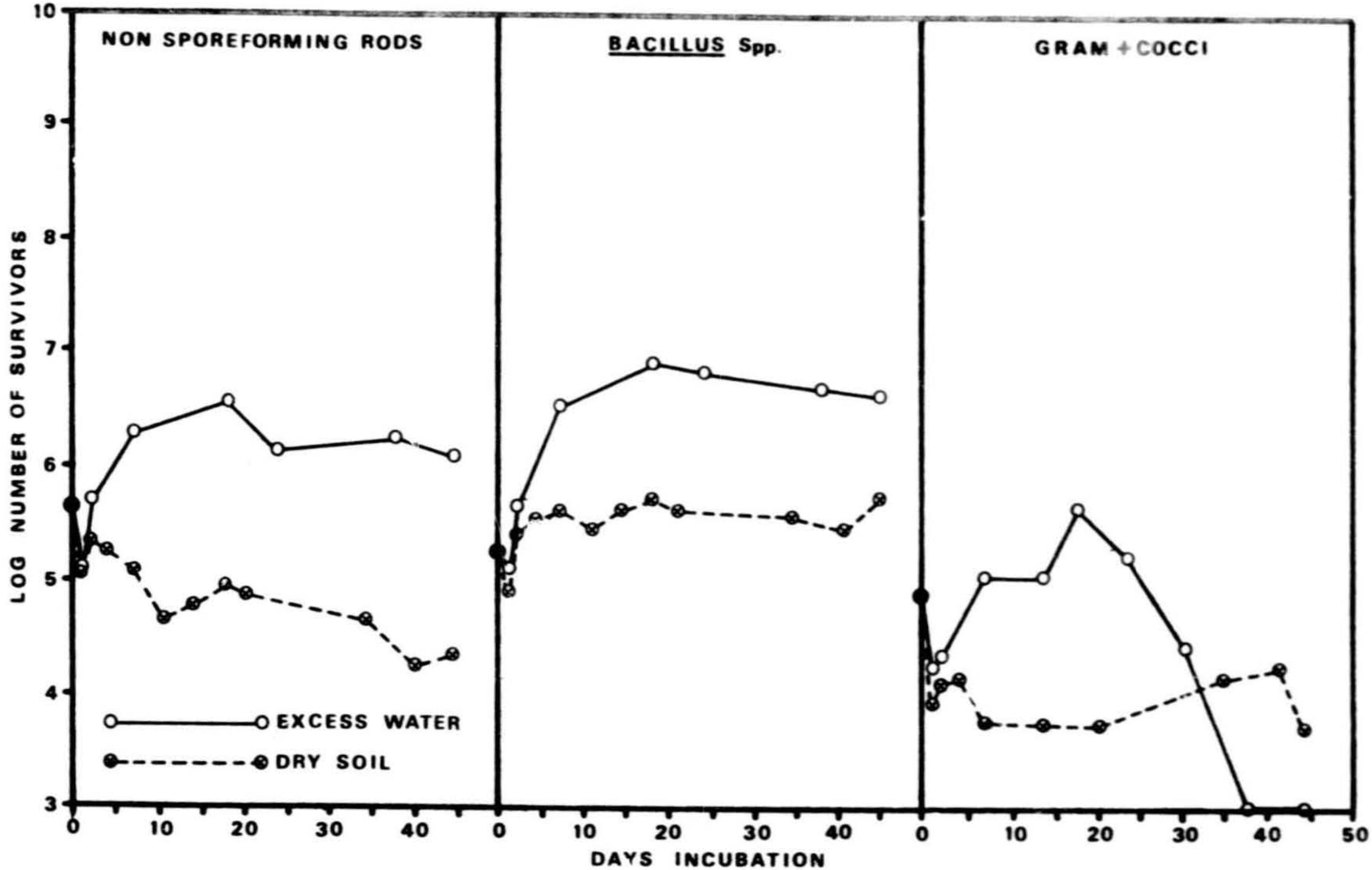

FIGURE 12. POPULATION CHANGES OF VARIOUS BACTERIAL GROUPS IN A SIMULATED MARTIAN ENVIRONMENT.

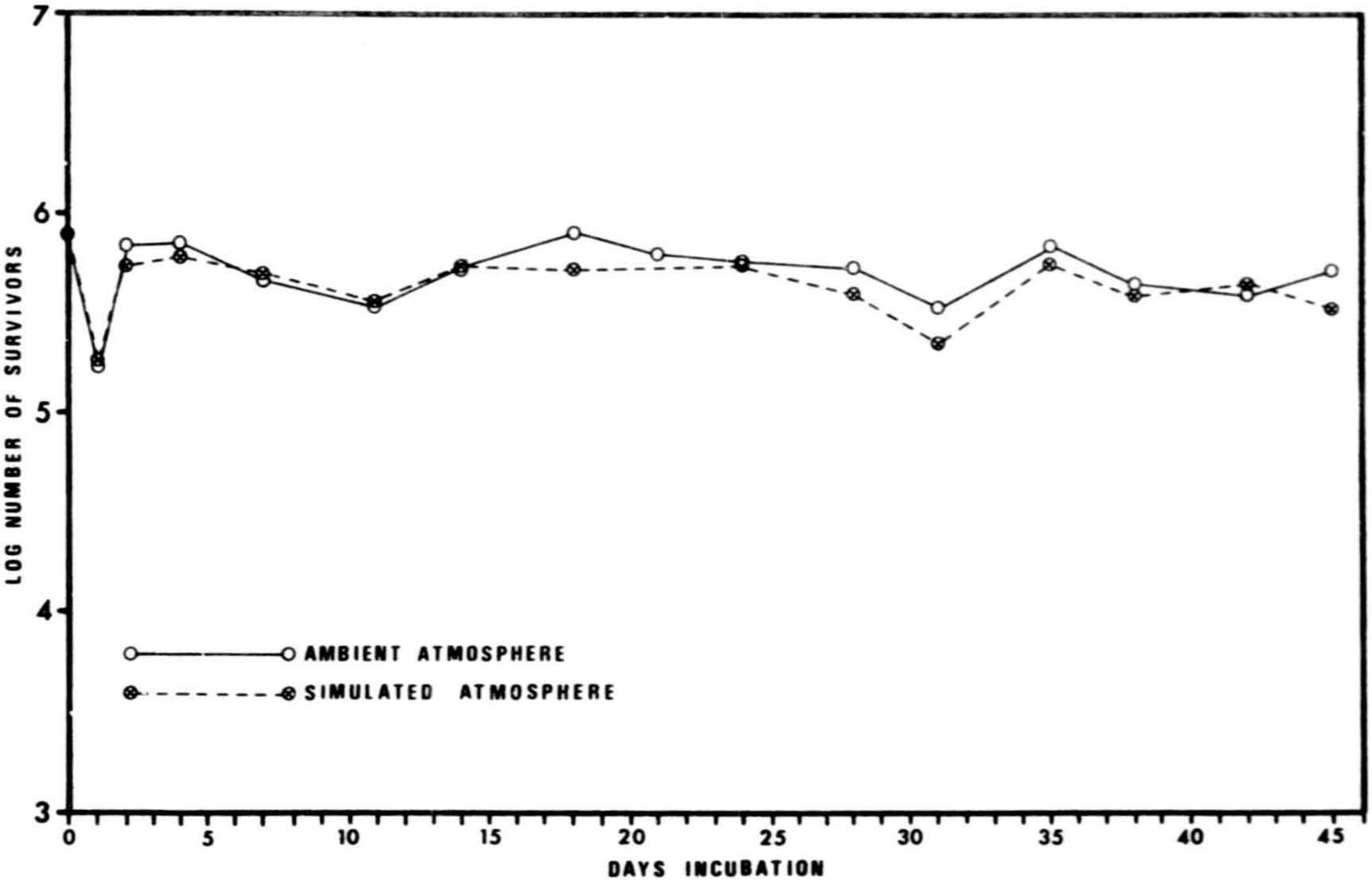

FIGURE 13. RESPONSE OF CAPE CANAVERAL SOIL POPULATIONS TO A DRY ENVIRONMENT WHEN INCUBATED AT A CONSTANT 24°C FOR 45 DAYS. SIMULATED ATMOSPHERE BEING 99.9% CO_2 AND 0.1% O_2 AT 7 MB PRESSURE.

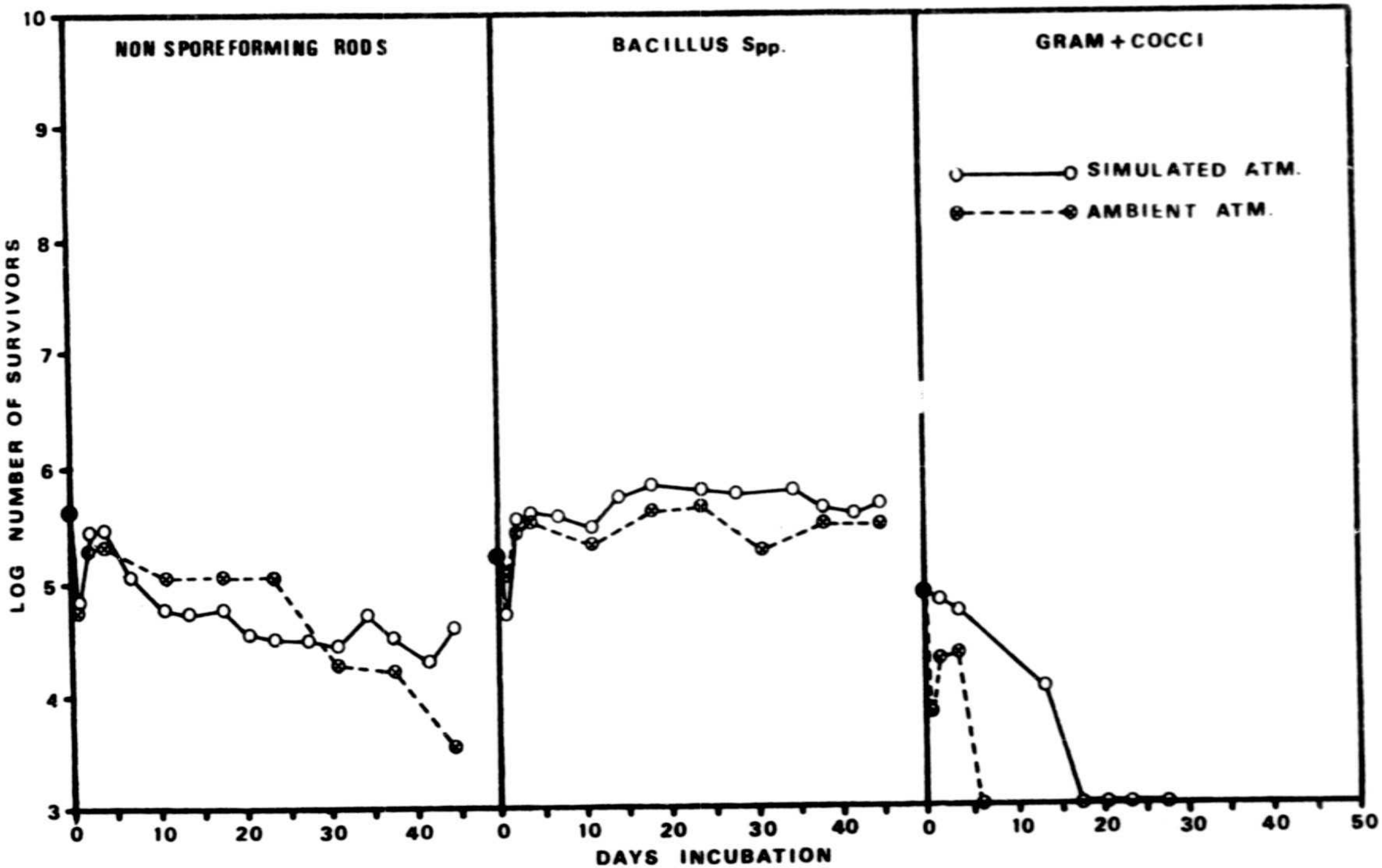

FIGURE 14. POPULATION CHANGES OF VARIOUS BACTERIAL GROUPS IN A DRY ENVIRONMENT AND INCUBATED AT 24°C.

Bacillus group is the surviving population in a dry environment.

In comparing all of these graphs it appears that the populations in the simulated environment are lower than those in the ambient environment, but they follow the same basic pattern of growth. It also appears that the freeze-thaw cycle allows the population to increase over a longer period of time than does incubation at constant 24°C.

F. Effect of Pressure on Changes in Soil Populations

In working with the simulated Martian environment, we were uncertain of the need for reducing the pressure to 7 mb. An experiment was performed to determine the effects of pressure and the results are shown in Figure 15. At constant 24°C there appears to be no difference in reduced vs. ambient pressure; but when using the freeze-thaw cycle there is a difference which is significant at the 0.05 probability level. In future experiments then, we will continue to reduce the pressure in the simulated Martian environment to 7 mb.

G. Plans for Future Work

This report has demonstrated the presence of a group of organisms which have not previously been investigated in detail. The omniphiles, or those organisms capable of growth over broad temperature ranges (3°C - 55°C), may have special significance to planetary space flights because of their adaptability to various temperatures. For this reason, we will investigate these in detail in an attempt to better characterize them. Planned experiments include a detail temperature profile to determine their maximum, minimum, and optimum temperatures; biochemical characteristics at different temperatures; heat resistance;

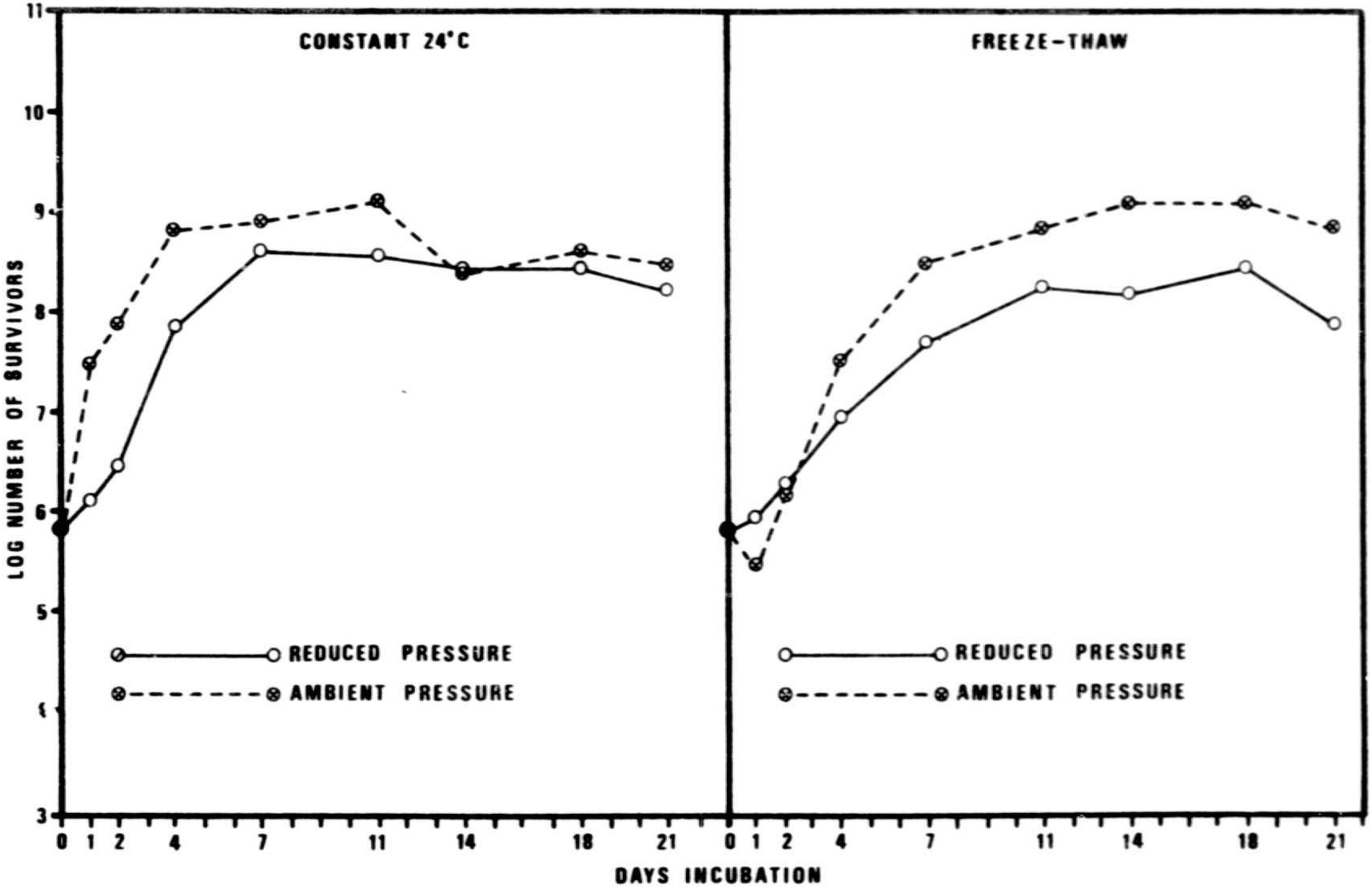

FIGURE 15. RESPONSE OF SOIL POPULATIONS TO EFFECTS OF REDUCED PRESSURE ON GROWTH. AMBIENT PRESSURE IS ATMOSPHERIC PRESSURE AND REDUCED PRESSURE IS 7 MB.

and other investigations.

Another investigation recently begun in our lab includes the anaerobic utilization of phosphite and/or phosphine. This has special significance in relation to exploration of Jupiter. Preliminary results indicate an isolate capable of utilizing phosphite anaerobically. This work has only recently begun and we plan to perform detailed investigations to demonstrate that this isolate is indeed utilizing only phosphite. Similar studies will also be performed for utilization of phosphine.

Other future activities include continued study of psychrophiles and anaerobes from planetary spacecraft environments.