DRY HEAT EFFECTS ON SURVIVAL OF INDIGENOUS SOIL PARTICLE MICROFLORA AND PARTICLE VIABILITY STUDIES OF KENNEDY SPACE CENTER SOIL

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INTRODUCTION

The NASA Planetary Quarantine Program has been concerned with various facets of microbial contamination and sterilization problems relevant to space exploration research. One area of interest is in the small soil particles and their associated natural microorganisms as a source of contamination for space probes. In a recent publication, Reynolds et al. (1974)* have reported that a certain fraction of the soil microbial spore population may be quite resistant to heat treatment. In earlier reports other workers (Koesterer, Favero and Pflug) have also discussed the heat resistance of soil microflora and suggested that this phenomenon should be recognized in the design of sterilization cycles. Thus, current information about dry heat effects on soil particulate fractions and the viability of their natural organisms appear desirable.

During the period covered by this report, we have been investigating the effects of dry heat on the natural *in situ* microbial population of soil, especially the effect of heat treatment on soil particle viability profiles. This work has involved studies of both single soil particles as well as heat treatment of multiple particle samples. It is anticipated that data from these experiments will be helpful in establishing effective heat treatment cycles required to inactivate *in situ* soil particle microflora and to better understand the soil contamination problem.

Experimental data included in the present report were obtained from our most recent studies of microscopic sized particles of Kennedy Space Center soil fractions. This research is one phase of our continuing laboratory investigations concerned with the dry heat effects on micro-

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* Reynolds, M.C. et al. (1974) Thermoradiation Inactivation of natural occuring bacterial spores in soil. Appl. Microbiol. 28 406-410.

bial spores and related phenomena relevant to the spacecraft sterilization programs. The project is part of a more extensive program on heat resistance of microorganisms conducted by the Environmental Sterilization Laboratory, Space Science Center, University of Minnesota.

OBJECTIVES

In this investigation, research efforts were concentrated on attempts to obtain data concerning the dry heat resistance of particle microflora in Kennedy Space Center soil samples. Specific objectives for the project were: (1) to determine the *in situ* dry heat resistance profiles at selected temperatures for the aggregate microflora on soil particles of certain size ranges. (2) to compare viability profiles of older with more recently stored soil samples and (3) to investigate the effect of increased particle numbers on viability profiles after dry heat treatment. These soil particle viability data for various temperatures and times provide information on the soil microflora response to heat treatment and may be useful in making selections for spacecraft sterilization cycles.

MATERIALS AND METHODS

All aspects of this investigation have been concerned with the natural, mixed microbial populations of soil particles. Spore crops cultured under laboratory conditions were not studied in this research phase. The experimental work was done directly with soil particles and their naturally associated microflora. This approach seemed to be desirable because it provided a system which appeared to be reasonably representative of natural soil particle contamination.

Soil Particles

In our studies of heat effects on survival of soil particle microflora, two samples of Kennedy Space Center soil were used. One of these was an older soil sample (Identification Code WAJJ Series) that had been stored in our laboratory following completion of earlier work. This sample had been collected in June 1970 by the Spacecraft Bioassay Laboratory. The

dried soil was received by our laboratories in September 1971 and initially stored at 4°C. In December 1971, this sample was separated into particle size ranges and stored at room temperature since that time. A second soil sample was collected more recently at the Space Center and was sent to our laboratory in June 1973. The sample was designated by code as WAKM series and was also stored at room temperature in the dried state. This "new" soil has been used extensively in some of the recent particle viability profile analyses.

When the soil samples were received by our laboratory, they were processed in a Ro-Tap soil separator to fractionate each sample into a series of particle size ranges using ASMT standard sieves (See University of Minnesota, School of Public Health NASA Report for December 1972 - May 1973). Each soil fraction collected from the selving process was stored at ambient laboratory conditions in a clean, covered glass jar until analyzed. Among the soil fractions stored, the smallest particle range separated was 44-53 µm and the largest fraction size range was 105-125 µm. Most of the viability profile studies were done with the intermediate sized 74-88 µm soil particles.

The soil particle viability analyses were run with either a series of randomly selected particles or a series of dark particles. In the random series, particles were transferred to test cups without any conscious selection for structure, color, texture, etc. However, for the dark particle series, a special effort was made to select the more dark, organic or clay type soil particles while quartz-like particles were especially rejected.

Microbiological Media

In the experiments to determine the survival of microflora on soil particles following heat treatment, Trypticase Soy Agar (TSA) modified by the addition of triphenyltetrazolium chloride (TTC) was used. The TSA-TTC modified medium had been found to aid in the detection of microbial growth and determination of particle viability (University of Minnesota, School of Public Health, NASA Progress Report June-November 1973). Use of the redox indicator dye was particularly helpful in facilitating recognition of microbial colony development emanating from the

microscopic sized soil particles. The red precipitate formed by microbial growth in the medium provided a sharp contrast to the particles and normal background.

Soil Particle Viability Profiles

The soil particle viability experiments were done using dry heat treatment at 110°C or 125°C for selected times. Individual soil particles were placed in separate, sterile, stainless steel TDT cups and heated on temperature controlled hot plates in our clean room facility. The TDT Cup-Aluminum Boat-TTC procedure was used in all experiments. For all these studies ranging from analyses of one particle to 25 particles per cup, the soil particles were selected and transferred to TDT cups by micromanipulation with the aid of a stereoscopic microscope. A detailed description of the methodology has been reported previously (University of Minnesota, School of Public Health, NASA Progress Report June - November 1973).

After appropriate heating periods and cooling of test units, TTC media was added directly to the particles in the TDT cups. All cups were then incubated at 32°C for two weeks. Following incubation, the cups were carefully checked under a stereoscopic microscope for any evidence of growth and the number of particles or cups demonstrating growth was recorded. From these data the proportion of cups with viable microorganisms after each heating time was calculated for all test units. The resulting values were used to plot the viability profiles for each series of soil fractions investigated.

Because of the meticulous and tedious nature of the microscopic work as well as personnel limitations only certain particle size fractions of soil have been analyzed. In most experiments, only randomly selected particles were studied. However, for certain particle investigations, series of random or series of dark soil particles were also selected for analysis and comparison of data.

Additional information concerning the procedures have been outlined in the University of Minnesota, School of Public Health, NASA Progress Report June - November 1973.

RESULTS AND DISCUSSION

The determinations of viability profiles for soil particles subjected to dry heat has depended extensively on microscopy for particle selection, transfer and microbial growth detection. Microscopy was an essential part of these studies because of the small particle sizes involved (44 to 125 μm). The fact that micromanipulation techniques are required has to some extent limited the number of experiments that could be completed in this reporting period. However, it is noteworthy that viability data on microbial survival presented in this report has required manipulation of large numbers of particles. The data presented here are based on results obtained from the processing and analysis of more than 40,000 individual Kennedy Space Center soil particles of microscopic size. These analyses have provided an extensive series of viability profiles for particle types at selected temperatures, including comparison of stored soil samples, effect of the size and number of particles on the time required for heat inactivation of the microflora, reproducibility of viability profiles, etc.

Particle Size Effect on Viability Profile

The earlier studies dealing with particle viability analyses using TSA-TTC media were confined to preliminary experiments with the 74-88 μm fraction of WAKM series soil. More recently, we have completed a viability analysis for the WAKMG (88-105 μm) particles as well. Random single soil particles were used in this experiment and the particles were heated at 125°C for time intervals from 15 to 120 minutes. Experimental data obtained in this study are listed in Table I and the particle viability profile is shown in Figure 1.

Among the unheated WAKMG particles tested, 67 of 74 demonstrated microbial growth. Apparently, some particles did not retain microorganisms which were capable of growth under the test conditions or these particles did not have viable forms associated with them. The viability response of the unheated particles was slightly greater than 90 per cent. After dry heat treatment for 100 minutes at 125°C, only one particle out of 74

Table 1

Soil Particle** Viability After Dry Heat Treatment

On Clean Room Hotplate at 125°C

WAKMG Soil (88-105 μm) Proportion Positive Data*

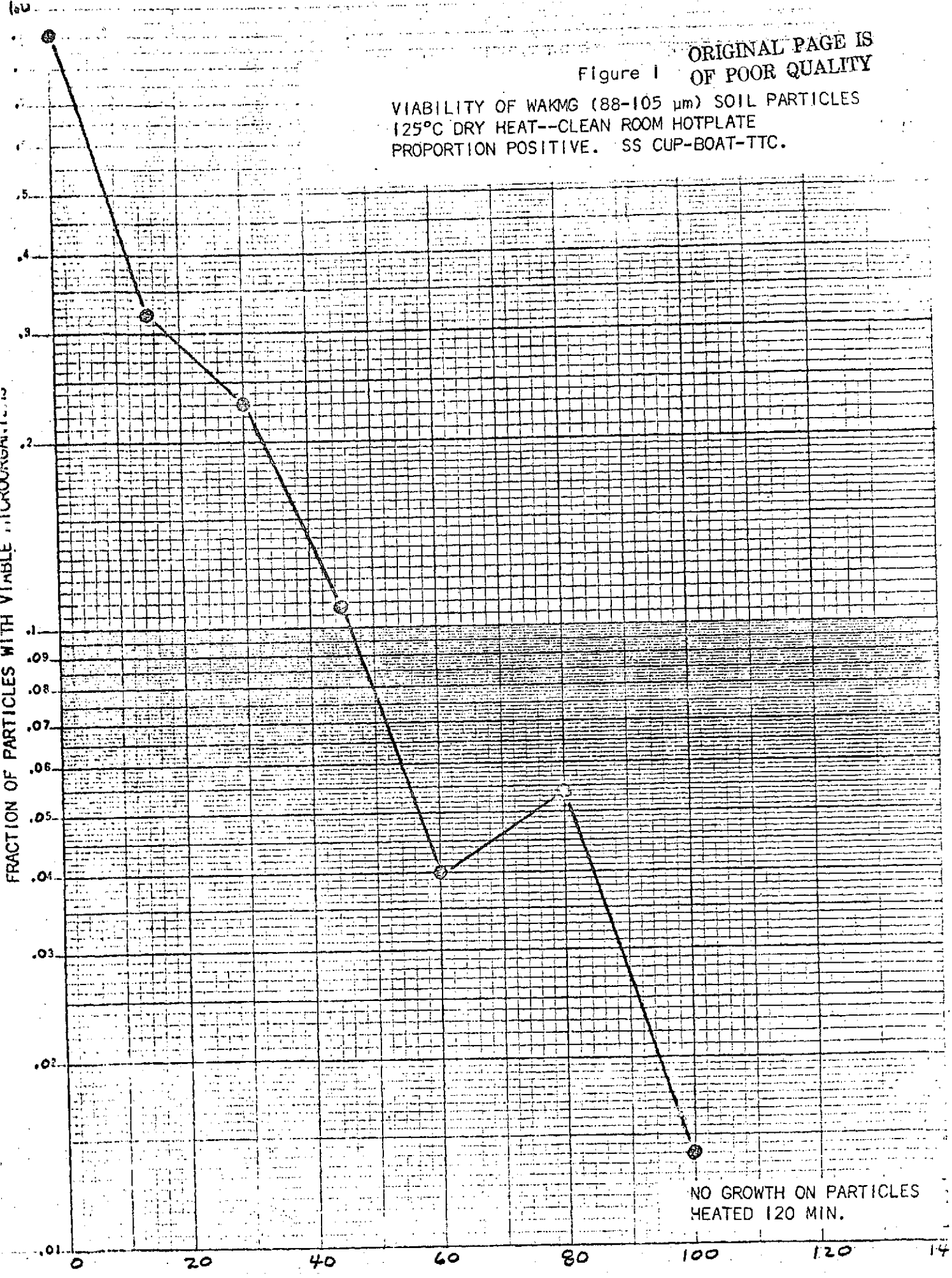
Experiment Number	Heating Time (Minutes)	Proportion Positive	
		Fraction	Decimal
OR4003A	0	67/74	0.905
OR4003B	15	24/74	0.324
OR3354A	30	17/74	0.230
OR4004A	45	8/74	0.108
OR3354B	60	3/74	0.040
OR4004B	80	4/74	0.054
OR4023A	100	1/74	0.014
OR4023B	120	0/74	0.000

* Refers to fraction of particles with viable microorganisms

** Particles selected randomly

Figure 1

VIABILITY OF WAKMG (88-105 μm) SOIL PARTICLES
125°C DRY HEAT--CLEAN ROOM HOTPLATE
PROPORTION POSITIVE. SS CUP-BOAT-TTC.



tested showed evidence of viability. No growth was observed on particles tested after 120 minutes heating time.

For purposes of comparison, the viability profile for random particles of the WAKMG (88-105 µm) soil fraction has been graphed together with similar data for WAKMF (74-88 µm) soil particles (Figure 2). These data suggest that the larger sized particles retain a viable microflora for a somewhat longer period of time when heated at the same temperature. This effect of particle size is similar to that observed earlier for WAJJ series soil studied by Moore et al. (University of Minnesota, School of Public Health NASA Progress Report, December 1972 - May 1973).

Random vs. Dark Particle Profiles

As part of the experimental soil work, it was also of interest to run a comparative viability profile series to note any differences in the profiles when only single dark particles were used as opposed to single randomly selected ones. For this experiment a series of 74-88 µm particles of both WAKMF and WAJJF soil samples were tested. Particles were treated with dry heat at 110°C for various time intervals up to 24 hours for some cups. The proportion of particles retaining viable microorganisms after each heating interval was determined and recorded for viability profile plots.

Experimental data from the dark particle study with WAKMF particles are listed in Table II. The corresponding graph illustrating the fraction of particles with surviving microflora at the various heating time intervals has been plotted in Figure 3. These data show that of the 74 unheated particles tested, 67 particles had viable, detectable microorganisms associated with them. This proportion constitutes about 90 per cent of the tested dark particles. After eight hours of heat treatment at 110°C, only one particle of the 74 tested retained viable microflora. No growth was observed on WAKMF dark particles heated for time intervals longer than eight hours.

The graph in Figure 4 shows the viability profile of the WAKMF dark particles compared with a profile for WAKMF randomly selected particles determined in an earlier experiment. In this case the two

Figure 2

COMPARATIVE VIABILITY DATA FOR RANDOM
CAPE KENNEDY SOIL PARTICLES
125°C DRY HEAT--CLEAN ROOM HOTPLATE
PROPORTION POSITIVE. SS CUP-BOAT-TTC

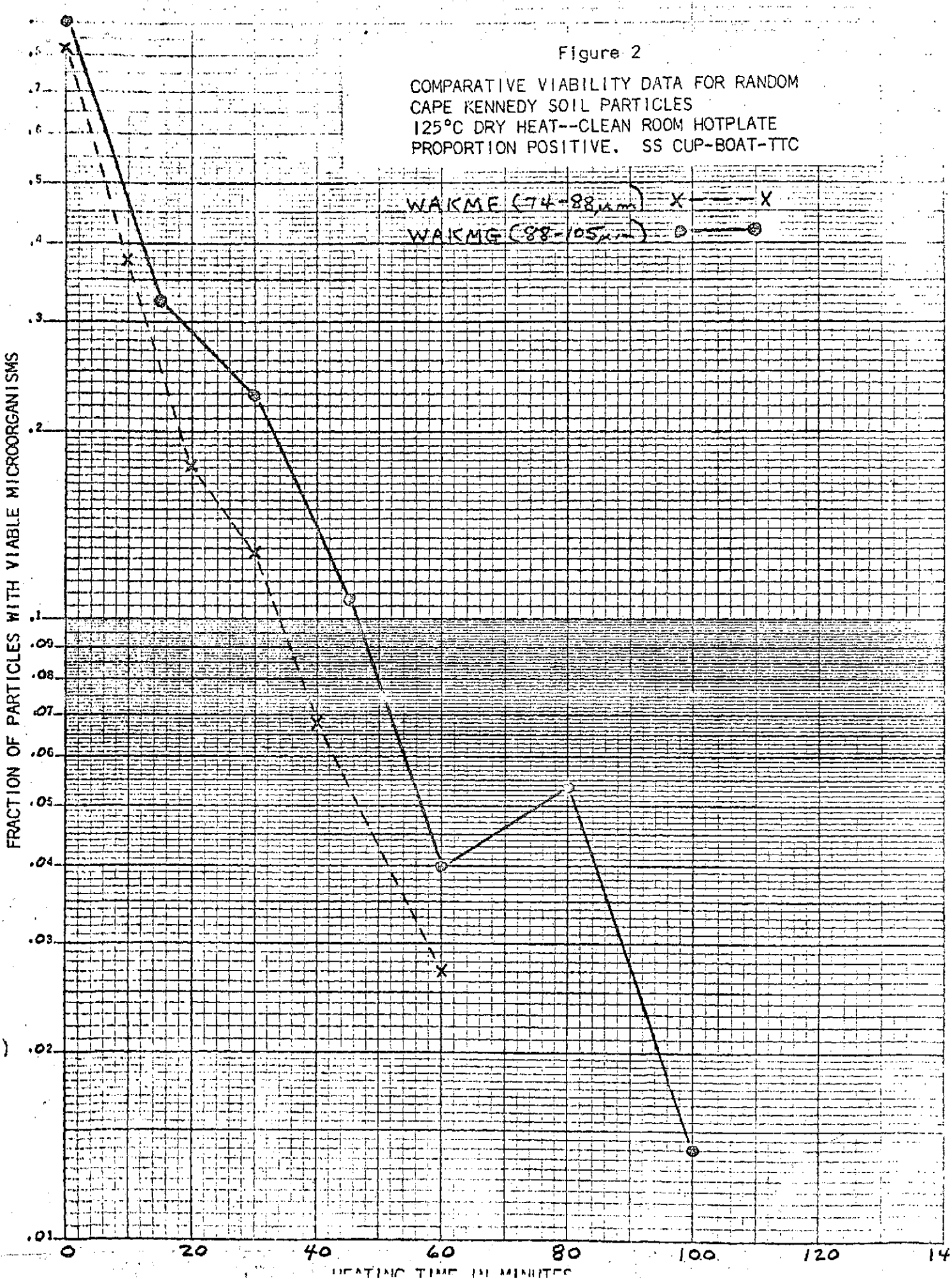


Table II

Soil Particle Viability After Dry Heat Treatment on Clean Room Hotplate at 110°C. WAKMF (74-88 μm) Dark Particles. Proportion Positive Data*

Experiment Number	Heating Time (Hours)	Proportion Positive	
		Fraction	Decimal
OR4039A	0	67/74	0.905
OR4039B	1	37/74	0.500
OR4039C	2	17/74	0.230
OR4045A	4	7/74	0.095
OR4045B	6	5/74	0.068
OR4045C	8	1/74	0.014
OR4052A	10	0/74	0.000
OR4052B	12	0/74	0.000
OR4050A	16	0/74	0.000
OR4050B	24	0/74	0.000

* Refers to fraction of particles with viable microorganisms

Figure 3

VIABILITY OF WAKMF (74-88 μm) DARK SOIL PARTICLES
110°C DRY HEAT--CLEAN ROOM HOTPLATE
PROPORTION POSITIVE. SS CUP-ALUM BOAT-TTC
(Expts. OR4039-4052)

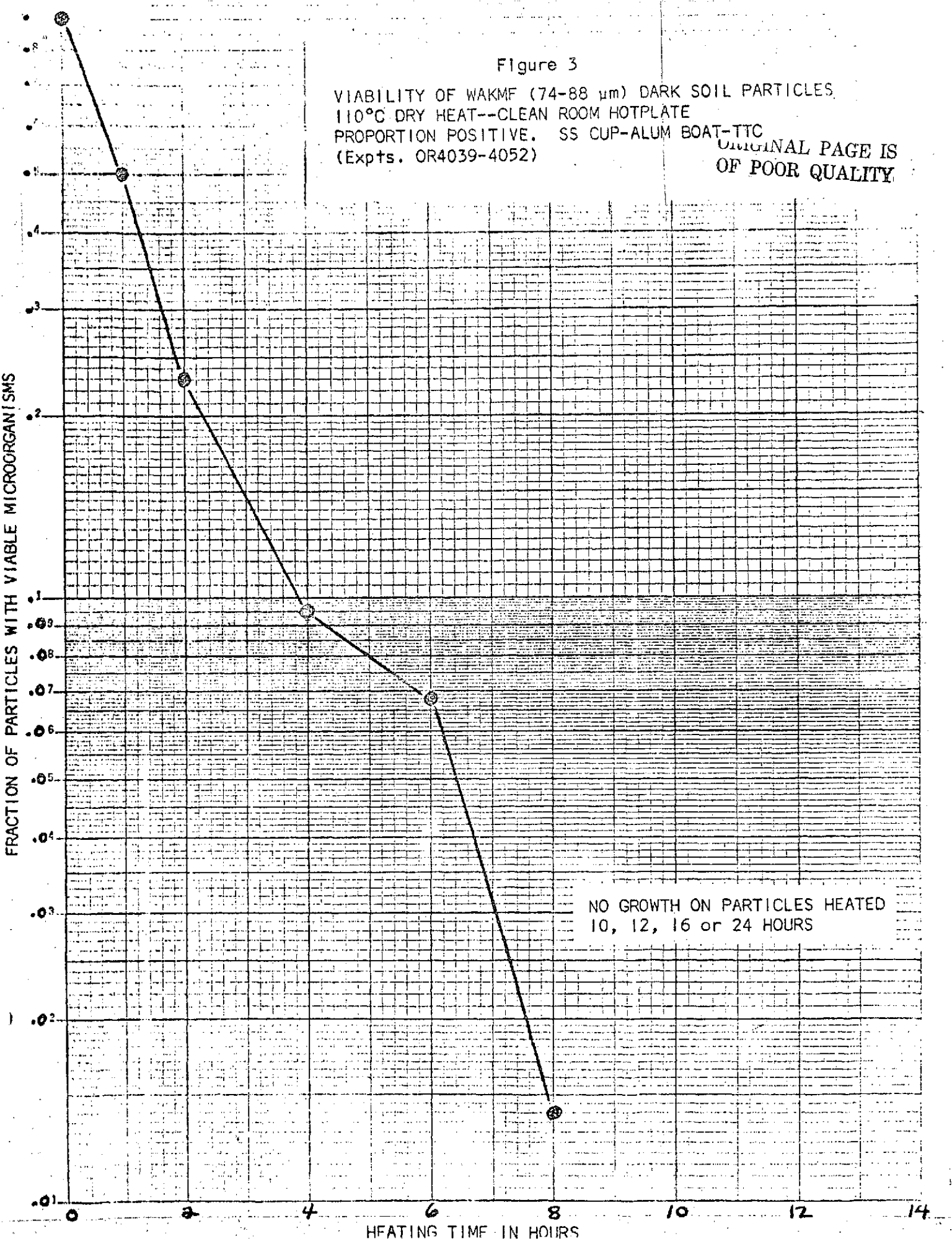
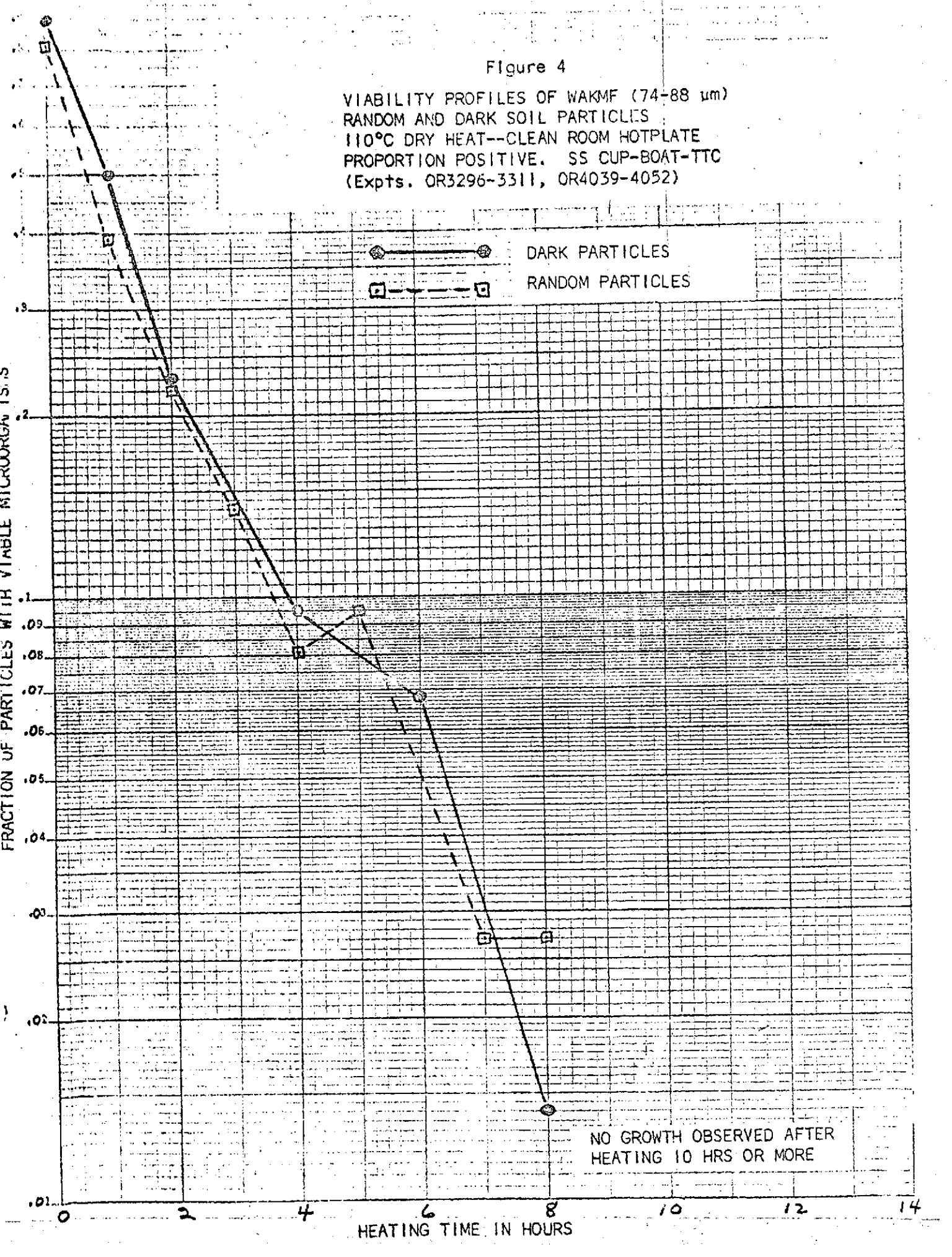


Figure 4

VIABILITY PROFILES OF WAKMF (74-88 µm)
RANDOM AND DARK SOIL PARTICLES
110°C DRY HEAT--CLEAN ROOM HOTPLATE
PROPORTION POSITIVE. SS CUP-BOAT-TTC
(Expts. OR3296-3311, OR4039-4052)



profiles are quite similar in slope and no growth was detected upon the analysis of either set of particles after 10 hours heating time. Although no differences in viability profiles are evident in the results of this experiment, it is recognized that these data are limited in that they represent only one experiment for each series of WAKMF particles. Additional work may reveal differences not detected in this experiment.

On the basis of the limited data available for WAKMF particles it appears that dark particles and randomly selected ones yield a similar inactivation response to dry heat effects. Because light colored particles, with generally fewer organisms per particle, constitute part of the random particle series, one might have expected some effect of selection on the viability profile. It is also conceivable that, due to special coincidence, the random particle set for the current experiment may have contained a higher proportion of darker particles than generally occur in such selections.

A second experiment with single random versus dark particles was carried out with the 74-88 μm WAJJF "old" soil fractions. These particles were also heated at 110°C for various time intervals. The proportion of random particles with associated microflora capable of surviving heat treatment at each time interval are listed in Table III. A viability profile plot for these random WAJJF particles is shown in Figure 5. For this particular experiment, approximately 73 per cent of the unheated random particles showed evidence of growth. It was also observed that about four per cent of the particles heated for four hours still retained viable organisms. However, after six hours or more of heat treatment, none of the random particles from this series demonstrated viability in the test medium.

A viability profile for WAJJF dark particles, together with the random particle profile is shown in the graph of Figure 6. The experimental data from which the profile of the WAJJF dark particle response was drawn are listed in Table VII, page 24. In this experiment, comparison of data from dark and random particle analyses indicates that for all heating time intervals the dark series had a higher proportion of particles that retained viable microflora through eight hours of heating time.

Table III

Soil Particle Viability After Dry Heat Treatment At 110°C On Clean Room Hotplate WAJJF (74-88 μm) Single Random Particles Proportion Positive Data*

Experiment Number	Heating Time (Hours)	Proportion Positive	
		Fraction	Decimal
OR4247A	0	54/74	0.730
OR4247B	1	14/74	0.189
OR4248A	2	7/74	0.095
OR4248B	4	3/74	0.040
OR4249A	6	0/74	0.000
OR4249B	8	0/74	0.000
OR4249C	10	0/74	0.000

* Refers to fraction of particles with viable microorganisms

Figure 5

VIABILITY OF WAJJF (74-88 μm) SINGLE RANDOM SOIL PARTICLES. 110°C DRY HEAT--CLEAN ROOM HOTPLATE PROPORTION POSITIVE. SS CUP-BOAT-TTC (Expts. OR4247A-49C)

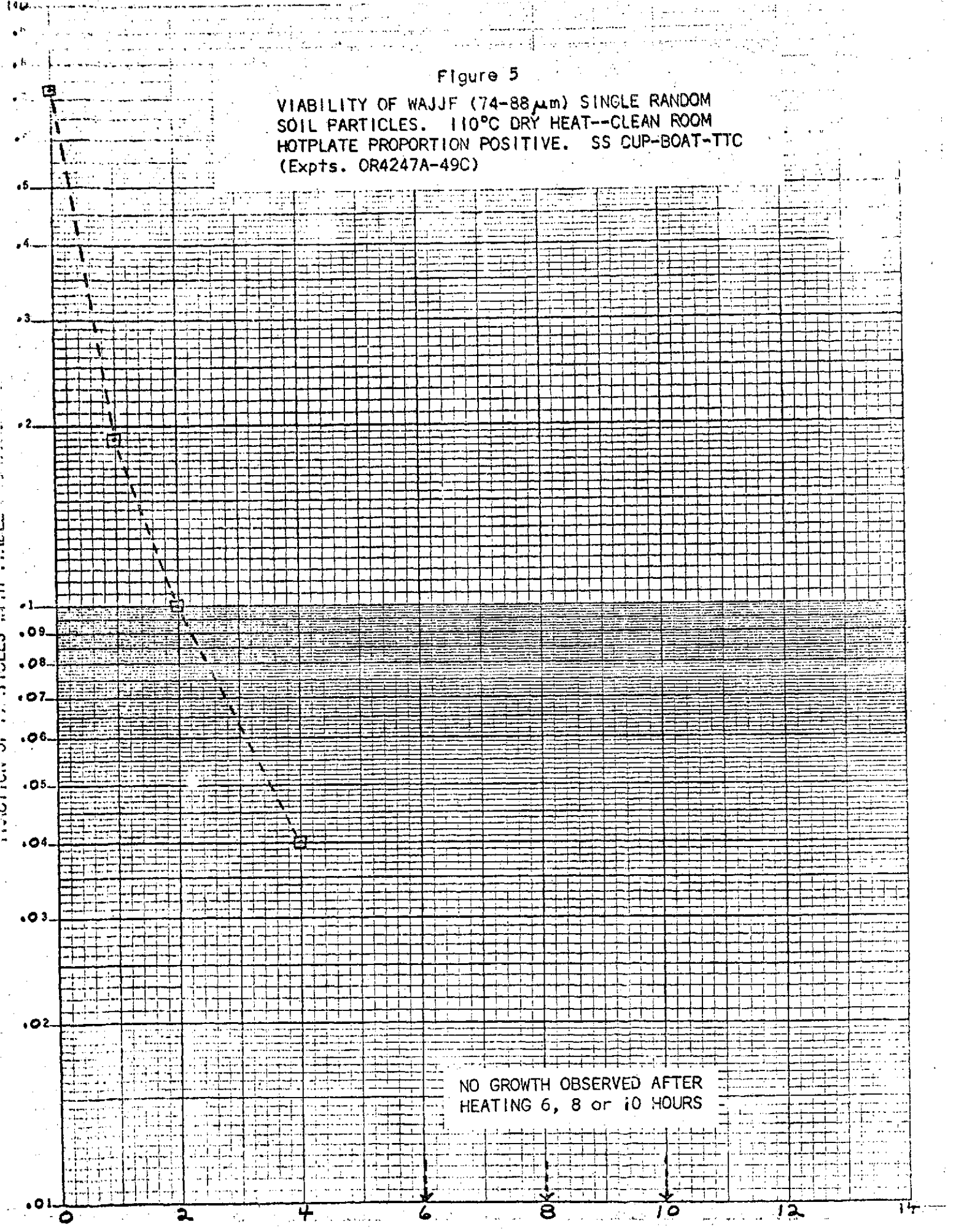
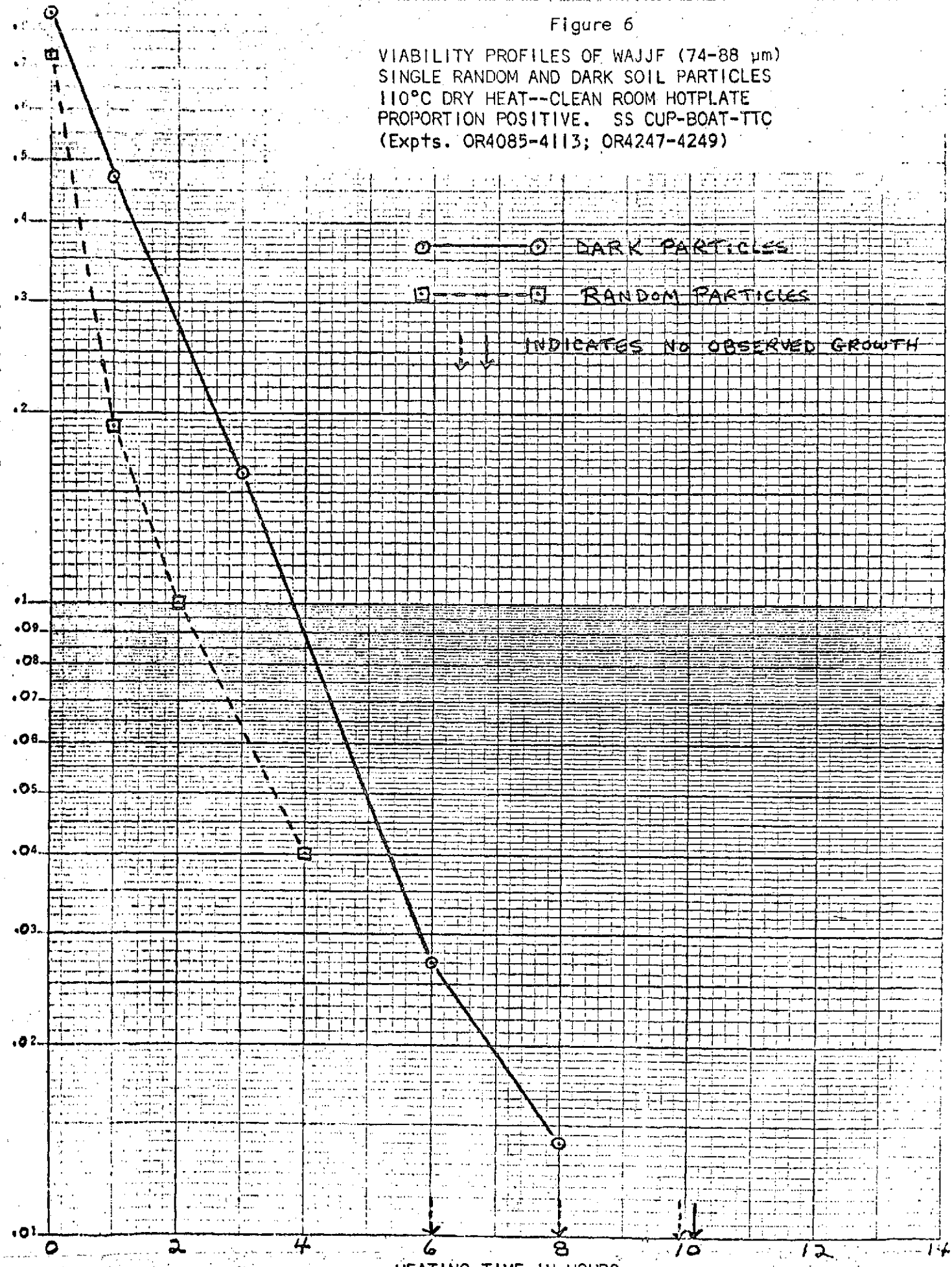


Figure 6

VIABILITY PROFILES OF WAJJF (74-88 µm)
SINGLE RANDOM AND DARK SOIL PARTICLES
110°C DRY HEAT--CLEAN ROOM HOTPLATE
PROPORTION POSITIVE. SS CUP-BOAT-TTC
(Expts. OR4085-4113; OR4247-4249)



The results from studies of random versus dark particles are inconclusive. Although the data obtained from the WAJJF series suggest that a difference in viability profiles can be expected, this phenomenon was not demonstrated with the WAKMF soil fraction. Whether or not this is due to the inherent variability in particle selection is unknown at this present time.

Viability Profile Reproducibility

During the initial investigations of WAKMF soil particles and the response of associated microorganisms to dry heat treatment, the question of viability profile reproducibility occurred. Therefore, plans were made to run a replicate series of three experiments to provide information on how well the viability profiles for a soil fraction would be reproduced from one experimental series to another. Throughout these experiments, Kennedy Space Center WAKMF single dark particles were used in three similar dry heat treatment series at 110°C. In total this study alone involved the selection, heat treatment and analysis of 1,628 individual soil particles for the combined replicate series experiments.

Results obtained from the first replicate experiment analyses were presented earlier in Table II, p. 10. Data which were obtained from the two additional replicate series determinations with individual WAKMF particles are listed in Table IV and Table V. Figure 7 shows the viability profiles that have been plotted for the initial series of soil particles (Series I) and the replicate experiments (Series II and Series III).

When one considers the problems of selection, manipulation and treatment of these microscopic sized particles, it is surprising to find that the agreement between data from these replicate experiments appears to be quite good. The results from these studies indicate that reasonable reproducibility of the viability profiles for soil particle microflora from a sample can be achieved. It should be recognized that this experiment was limited to only one soil fraction because laboratory time requirements precluded a more extensive experimental series with additional soil fractions. This was especially the situation whenever micromanipulation was required for the particle studies.

Table IV

Soil Particle Viability After Dry Heat Treatment On Clean Room Hotplate at 110°C WAKMF (74-88 μm) Dark Particles. Replicate II Proportion Positive Data

Experiment Number	Heating Time (Hours)	Proportion Positive	
		Fraction	Decimal
OR4059A	0	66/74	0.892
OR4067A	2	21/74	0.284
OR4059B	4	8/74	0.108
OR4067B	6	3/74	0.041
OR4059C, 4071A	8	1/148	0.007

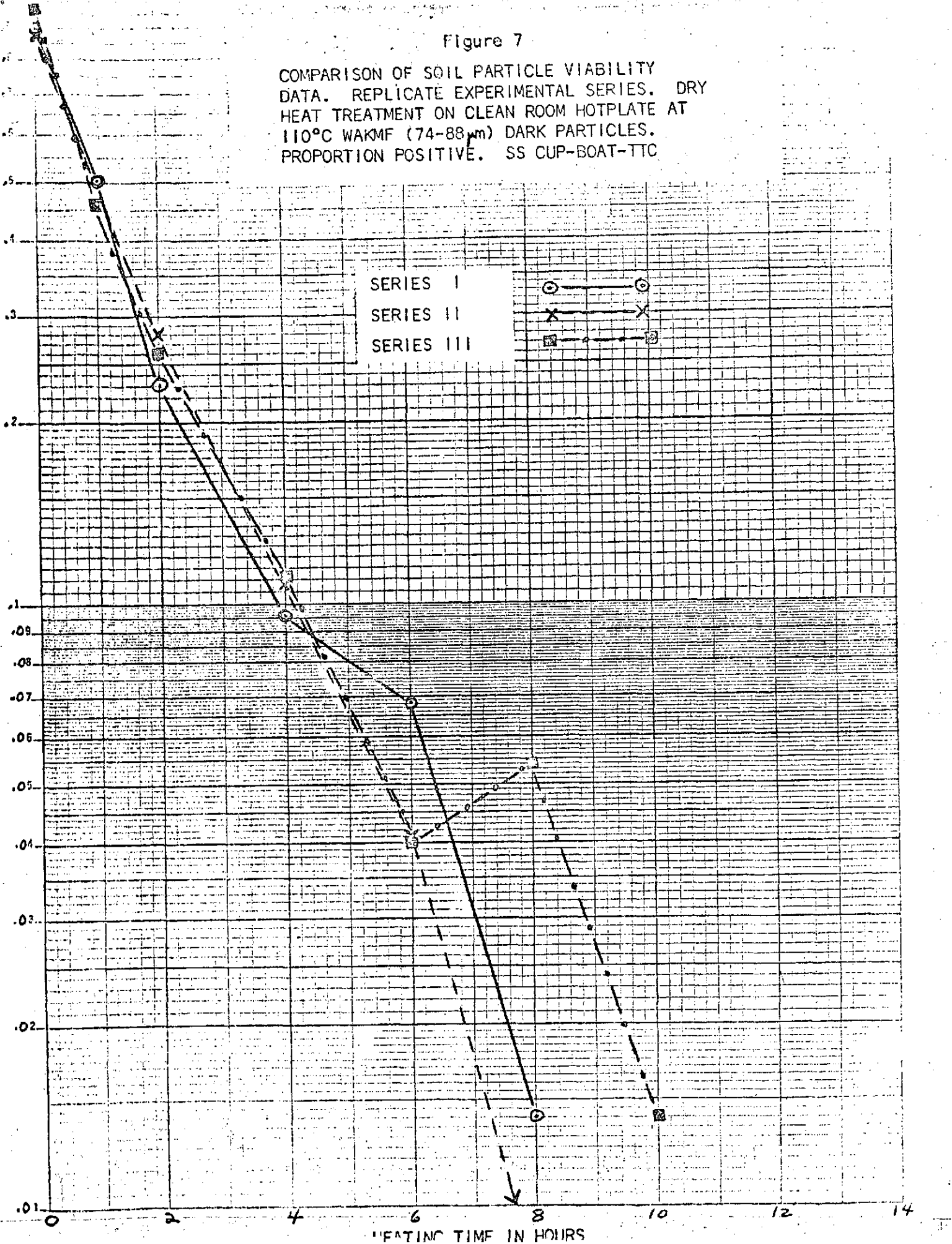
Table V

Soil Particle Viability After Dry Heat Treatment On Clean Room Hotplate at 110°C. WAKMF (74-88 µm) Dark Particles. Replicate III Proportion Positive Data*

Experiment Number	Heating Time (Hours)	Proportion Positive	
		Fraction	Decimal
OR4255A	0	72/74	0.973
OR4255B	1	33/74	0.446
OR4255C	2	19/74	0.257
OR4256A	4	8/74	0.108
OR4256B	6	3/74	0.040
OR4262A	8	4/74	0.054
OR4262B	10	1/74	0.014

Figure 7

COMPARISON OF SOIL PARTICLE VIABILITY DATA. REPLICATE EXPERIMENTAL SERIES. DRY HEAT TREATMENT ON CLEAN ROOM HOTPLATE AT 110°C WAKMF (74-88 μm) DARK PARTICLES. PROPORTION POSITIVE. SS CUP-BOAT-TTC



Viability Profiles of Stored Soil Particles

Another aspect of the soil particle microbiology problem has been the effect of long term storage on particle viability. For this reason some experimental studies were done to obtain data relevant to the viability of soil particles which had been stored in our laboratory over several years. In these experiments, individual WAJJF (74-88 μm) dark soil particles were subjected to heat treatment. These particles had been stored in covered glass jars at ambient laboratory conditions for approximately 2.5 years. Viability profiles at 125°C and 110°C have been determined for these soil particles.

For comparative purposes, a similar profile at 110°C for WAKMF (74-88 μm) dark soil was also determined during the same period. The WAKMF particles had been stored in our laboratories for approximately one year at the time of heat treatment and analysis.

Results from the analyses of WAJJF "old" particles heated at 125°C are listed in Table VI and plotted on the graph of Figure 8. These data show that, despite the 2.5 year storage period, more than 74 per cent of the soil particles still retained viable microorganisms as demonstrated by the response of the unheated series. After 60 minutes of heat treatment at 125°C, most of the microbial population on the particles was rendered non-viable. Only 1.4 per cent of the particles heated for one hour showed growth under test culture conditions. None of the particles tested demonstrated viability after 120 minutes heating at 125°C. The viability plot in Figure 8 shows the trend of the particle inactivation curve with time.

Particle viability data from the 110°C dry heat treatment of stored WAJJF dark particles are presented in Table VII. Analyses of the unheated particles indicated that approximately 85 per cent of the individually tested particles still retained viable microorganisms. Figure 9 shows the particle inactivation profile which was obtained from this experiment with the "older" soil. The time required to inactivate the particles at 110°C was at least eight times longer than the time observed for 125°C. After eight hours of heat treatment at 110°C, slightly over one per cent

Table VI

Soil Particle Viability After Dry Heat Treatment at 125°C on Clean Room Hotplate. Single WAJJF (74-88 µm) Dark Particles Proportion Positive Data*

Experiment Number	Heating Time (Hours)	Proportion Positive	
		Fraction	Decimal
OR4177A	0	56/74	0.757
OR4177B	15	22/74	0.297
OR4177C	30	12/74	0.162
OR4179A	45	3/74	0.040
OR4178B	60	1/74	0.014
OR4178B	120	0/74	0.000
OR4178C	180	0/74	0.000

* Refers to fraction of particles with viable microorganisms

Figure 8

VIABILITY OF WAJJF (74-88 μm) DARK SOIL PARTICLES 125° DRY HEAT--CLEAN ROOM HOT-PLATE PROPORTION POSITIVE. SS CUP-BOAT-TTC (Expts. OR4177-79)

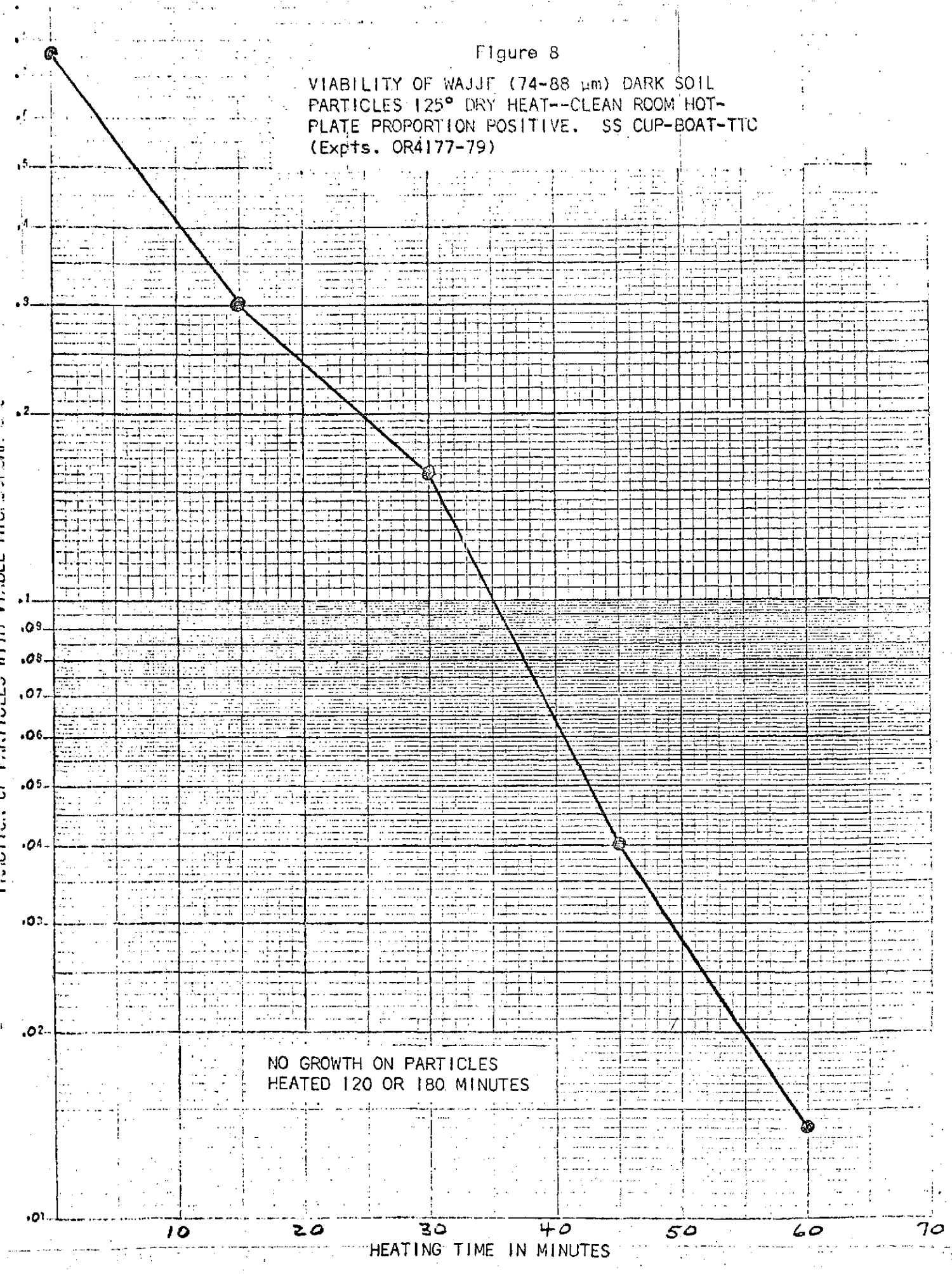


Table VII

Soil Particle Viability After Dry Heat Treatment at 110°C on Clean Room Hotplate. WAJJF (74-88 µm)

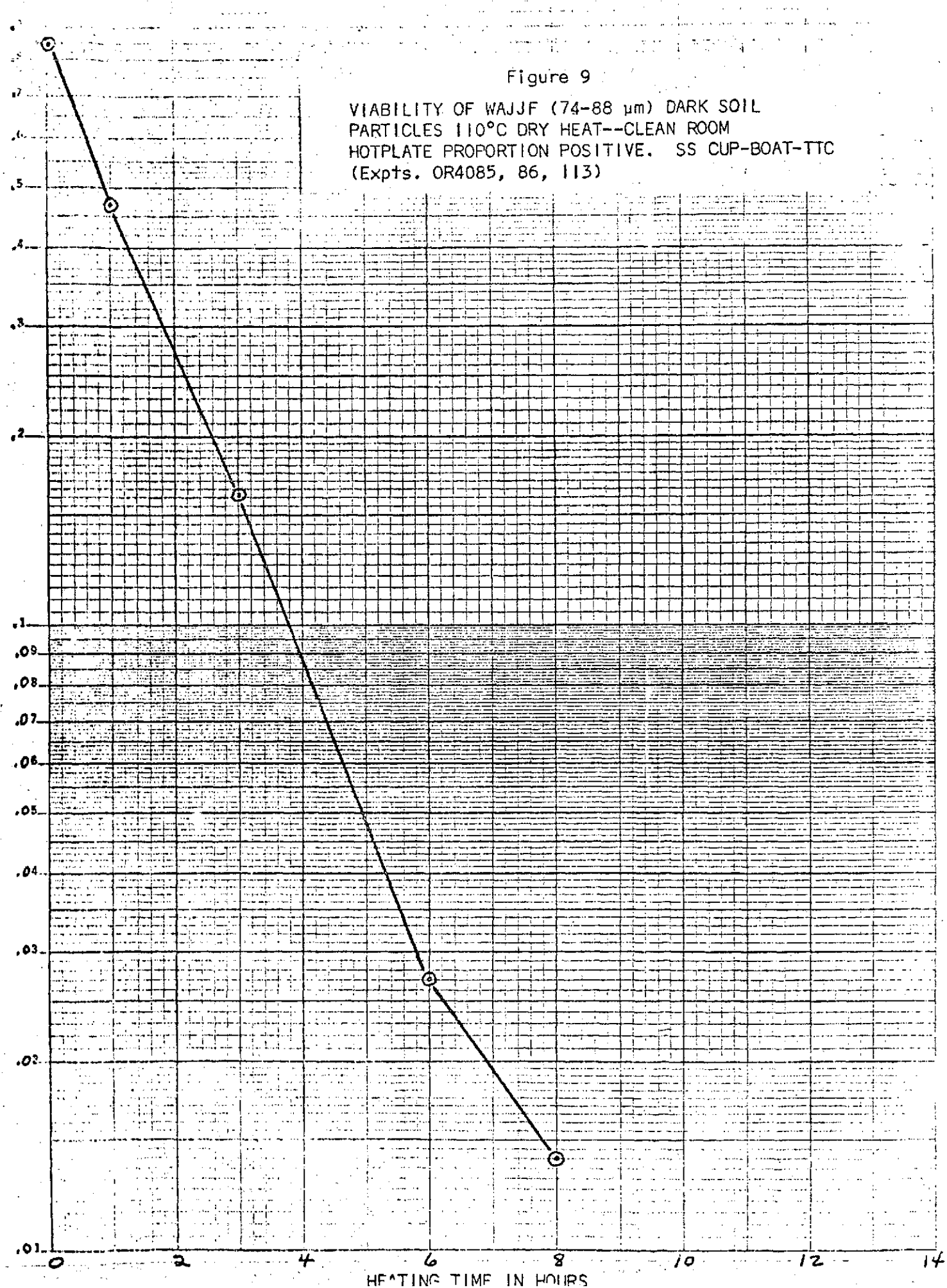
Dark Particles Proportion Positive Data*

Experiment Number	Heating Time (Hours)	Proportion Positive	
		Fraction	Decimal
OR4113A	0	63/74	0.851
OR4086A	1	35/74	0.473
OR4085	3	12/74	0.162
OR4086B	6	2/74	0.027
OR4113B	8	1/74	0.014

* Refers to fraction of particles with viable microorganisms

Figure 9

VIABILITY OF WAJJF (74-88 µm) DARK SOIL PARTICLES 110°C DRY HEAT--CLEAN ROOM HOTPLATE PROPORTION POSITIVE. SS CUP-BOAT-TTC (Expts. OR4085, 86, 113)



of the treated particles still demonstrated the presence of viable microorganisms.

Figure 10 provides a comparison of the particle inactivation profiles obtained when dark particles of WAJJF (old) and WAKMF (new) soil fractions were treated at 110°C. It is of interest to note that, despite the longer storage time of approximately 2.5 years for WAJJF soil particles, both particle viability profiles appear to be quite comparable. Thus, the results suggest that the dry heat survival response of these soil sample microflora are apparently similar regardless of the longer storage time for WAJJF soil. Furthermore, these data also suggest that there was no marked loss of heat resistant forms from the particles during the storage period. This is not an unusual phenomenon with spore forming bacteria; since some forms have been found to remain viable for many decades in dried soils. Anthrax spores are a classic example and these forms have been reported to survive for many years in certain soils of Europe and the United States.

Effect of Particle Number on Viability Profile

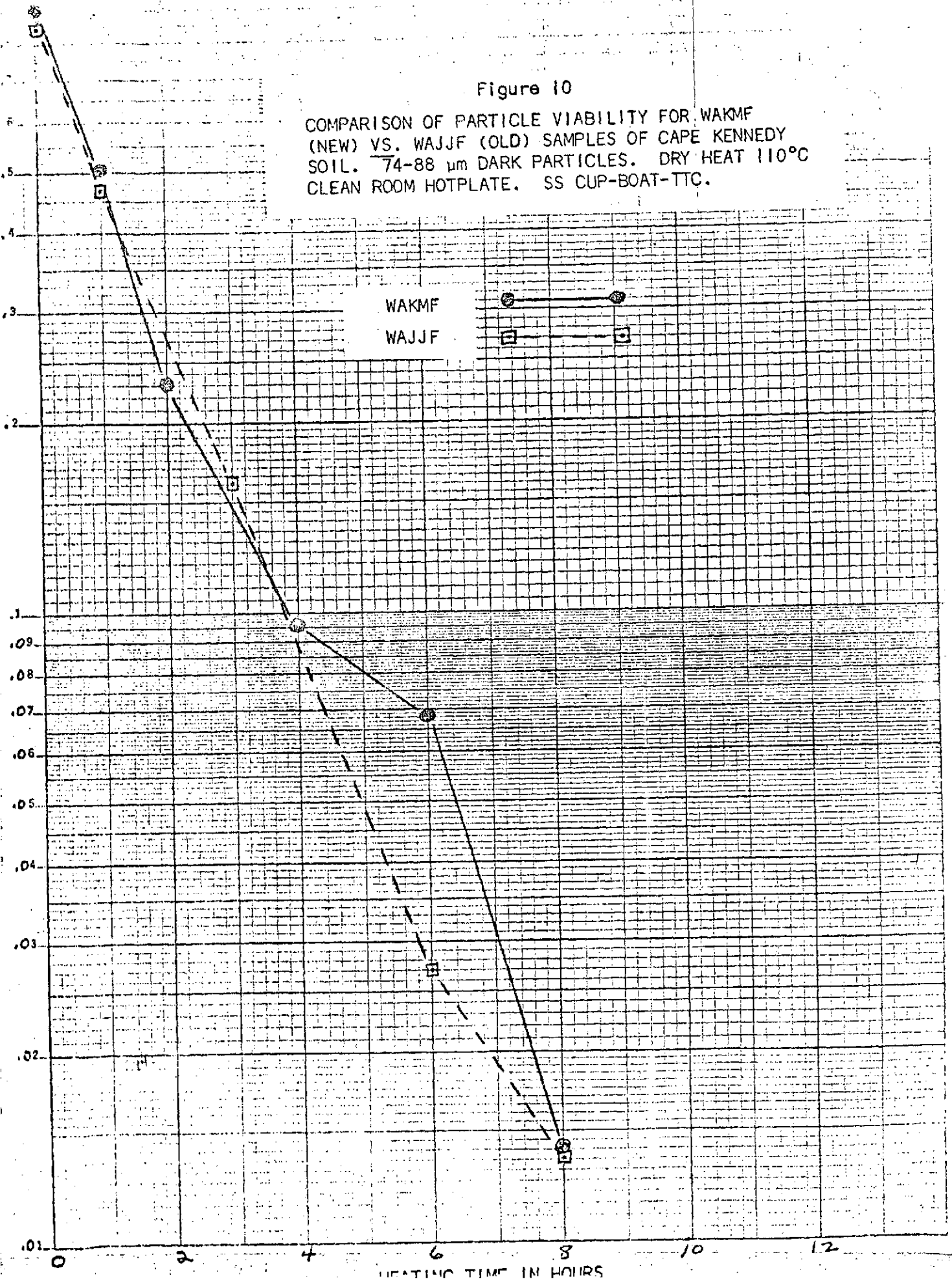
As the various investigations of soil particle microflora were carried on, an interest was also developed with regard to any effects on particle viability that might result from an increased particle density per unit area. Of particular concern was the question of how an increase in the number of soil particles per TDT cup would influence the configuration of the viability profile graph. An extensive series of experiments was completed in order to obtain the data relevant to the effect of particle load on the soil particle viability profiles.

In these experiments, numerous series of 74-88 μm sized random particles, drawn separately from the "new" WAKMF and "old" WAJJF soil samples, were subjected to dry heat treatment. For each of the soil samples studied, analyses were done to obtain viability profiles for concentrations of one, ten and 25 soil particles per TDT cup.

The randomly selected soil particles were placed in each TDT cup and series of 74 cups were heated at 110°C for each selected time interval. In aggregate, the test groups for both soils were comprised of a total of

Figure 10

COMPARISON OF PARTICLE VIABILITY FOR WAKMF (NEW) VS. WAJJF (OLD) SAMPLES OF CAPE KENNEDY SOIL. 74-88 μm DARK PARTICLES. DRY HEAT 110°C CLEAN ROOM HOTPLATE. SS CUP-BOAT-TTC.



more than 22,000 individual particles. The particles were generally separated into sequences of six to seven experimental heating time series. This number of heating times furnished a sufficient number of points to allow graphing of the viability response curves and note trends in time required for inactivation.

Results obtained in the series of experiments with WAKMF soil particles are presented in Tables VIII, IX and X. The tabulated data refer to the proportion of cups which retained viable soil particles after each interval of heating time. These data have also been plotted as viability profiles for one, ten and 25 particles per cup. Graphs of these data are shown in Figure II and provide an interesting comparison of viability profiles for the increasing loads of WAKMF soil particles per cup.

Inspection of the graphs in Figure II suggests that there was an influence on the particle viability curve as the number of particles per cup was increased. In the series using ten particles per cup, the inactivation of the associated microorganisms showed an initial lag up to about two hours of heating time. Following this period, the rate of particle inactivation apparently increased and, after ten hours of heat treatment, no growth was detected in any of the cups tested.

Results from using single particles of WAKMF soil per cup did not demonstrate any evidence of delayed inactivation of particles. The viability profile for the single particles was of approximately uniform slope and no growth was found in these cups at heating time intervals that were longer than eight hours. Although these single particles generally appeared to undergo more rapid inactivation, a few retained viable organisms for the same length of heating as was observed with the ten particle per cup series.

Of the three groups of experiments for WAKMF soil particles, the most noticeable effect was shown by the studies with 25 particles per cup. In this case no marked increase in the slope of the viability profile was observed until after four hours heating time. The lag in the inactivation rate was clearly more pronounced than that observed for ten particles per cup. Some particles of this group remained viable through ten hours of heat treatment. However, after 12 hours heating time none of the cups

Table VIII

Soil Particle Viability After Dry Heat Treatment On Clean Room Hotplate at 110°C. WAKMF (74-88 µm) Random Particles--1 Particle/Cup Proportion Positive Data*

Experiment Number	Heating Time (Hours)	Proportion Positive	
		Fraction	Decimal
OR3296A	0	60/74	0.811
OR3296B	1	29/74	0.392
OR3298A	2	16/74	0.216
OR3311A	3	10/74	0.134
OR3298B	4	6/74	0.081
OR3311B	5	7/74	0.095
OR3311C	7	2/74	0.027
OR3303A	8	2/74	0.027
OR3303B	16	0/74	0.000
OR3305A	24	0/74	0.000

* Refers to fraction of particles with viable microorganisms

Table IX

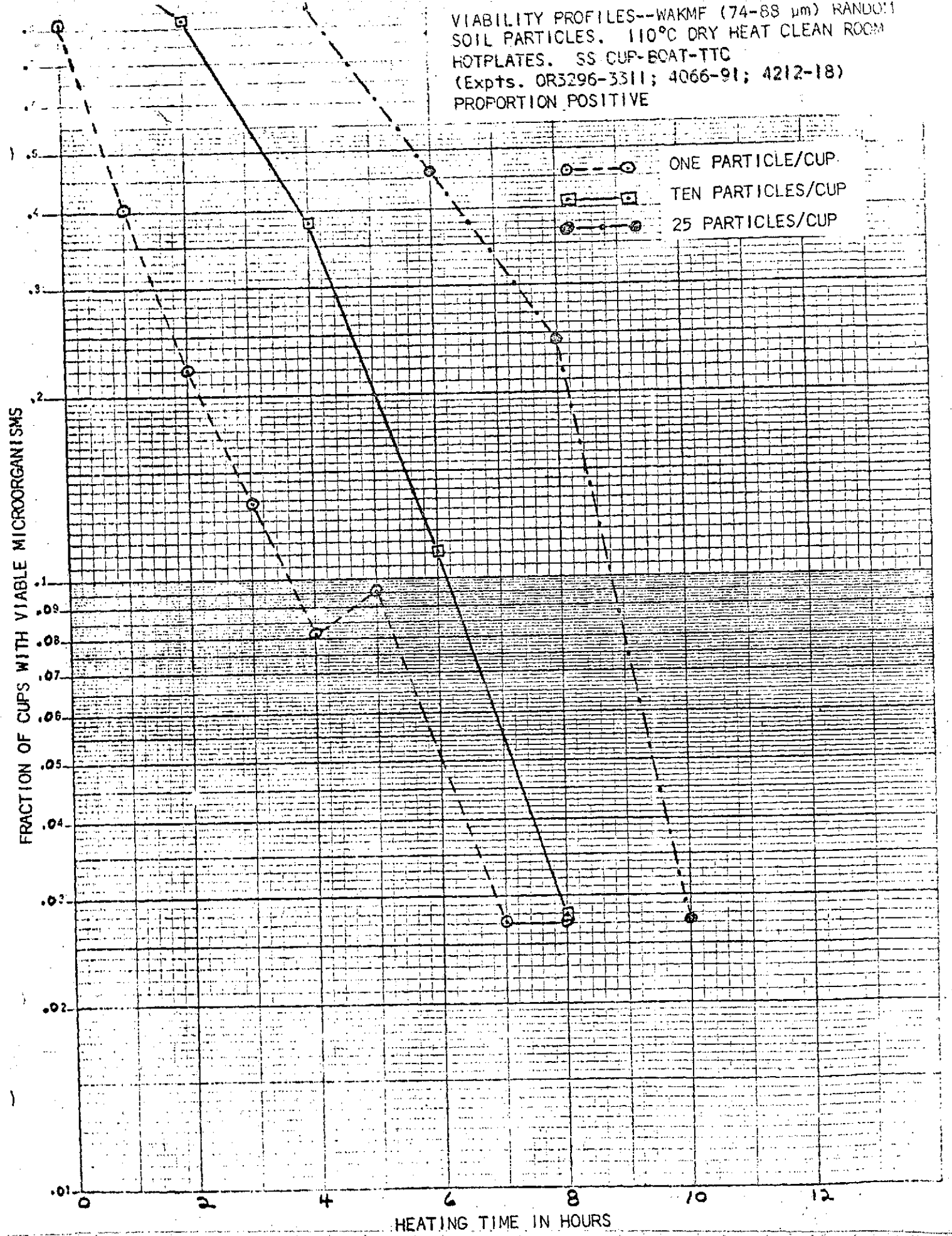
Soil Particle Viability After Dry Heat Treatment On Clean Room Hotplate at 110°C. WAKMF (74-88 µm) Random Particles--10 particles/Cup Proportion Positive Data

Experiment Number	Heating Time (Hours)	Proportion Positive	
		Fraction	Decimal
OR4081A	1	72/74	0.973
OR4066A, 91A	2	91/111	0.820
OR4066B	4	28/74	0.378
OR4081B	6	8/74	0.108
OR4074A	8	2/74	0.027
OR4074B	12	0/74	0.000

Table X

Soil Particle Viability After Dry Heat Treatment On Clean Room Hotplate at 110°C. WAKMF (74-88 µm) Random Particles--25 Particles/Cup Proportion Positive Data

Experiment Number	Heating Time (Hours)	Proportion Positive	
		Fraction	Decimal
OR4212A	1	74/74	1.000
OR4212B	2	73/74	0.986
OR4213A	4	64/74	0.865
OR4213B	6	34/74	0.459
OR4218A	8	18/74	0.243
OR4218B	10	2/74	0.027
OR4218C	12	0/74	0.000


VIABILITY PROFILES--WAKMF (74-88 µm) RANDOM SOIL PARTICLES. 110°C DRY HEAT CLEAN ROOM HOTPLATES. SS CUP-BOAT-TTC
(Expts. OR3296-3311; 4066-91; 4212-18)
PROPORTION POSITIVE
ONE PARTICLE/CUP
TEN PARTICLES/CUP
25 PARTICLES/CUP
FRACTION OF CUPS WITH VIABLE MICROORGANISMS
.5
.4
.3
.2
.1
.09
.08
.07
.06
.05
.04
.03
.02
.01
0
2
4
6
8
10
12
HEATING TIME IN HOURS

from this series showed any growth response on TSA-TTC media under test conditions.

Similar experimental work with the "old" WAJJF soil particle fraction revealed a somewhat similar trend to that found for the WAKMF soil sample. However, in the case with the WAJJF soil particles, the differences were not as marked and the graphed data was not as clear cut.

The data from the experimental series using ten and 25 random particles per cup of WAJJF soil are shown in Tables XI and XII. Results from the work with one random particle per cup have been listed in Table III, page 14 of this report. The three viability profiles obtained for the different particle loads of WAJJF soil per cup are drawn in the graphs of Figure 12.

These data reveal a trend which is similar to that found with the WAKMF particles. The viability profile for single, random particles of WAJJF soil per cup indicated that particle inactivation occurred without any noticable lag period. Data in Table III (page 14) shows that none of the particles produced microbial growth after four hours heating time. Tests run after heating times of six, eight and ten hours, and representing a total of 222 individual particles, showed no evidence of viability.

In contrast to the single particle data, the results with 25 particles of WAJJF soil per cup showed a readily detectable lag in the viability profile plot. Furthermore, some of the particles in these cups retained viable microorganisms through ten hours of heat treatment. Both of these results are similar to those found with WAKMF soil studies.

The ten particle per cup profile for WAJJF soil showed a somewhat intermediate response but was not as clearly defined as those for one particle or 25 particles. Some of the microflora associated with particles in these cups were also found to survive ten hours heating time.

Data from the two experimental series with WAKMF and WAJJF soil fractions indicate that as the particle load per cup is increased, a lag in the inactivation rate occurs in the early heating periods. Furthermore, as the particle load per cup was increased from one to 25 particles. the time required to achieve inactivation of all cups was increased. These data suggest that increasing the particle load per cup beyond the number

Table XI

Soil Particle Viability After Dry Heat Treatment On Clean Room Hotplate at 110°C. WAJJF (74-88 µm) Random Particles--10 Particles/Cup Proportion Positive Data

Experiment Number	Heating Time (Hours)	Proportion Positive	
		Fraction	Decimal
OR4191A	1	67/74	0.905
OR4191B	2	55/74	0.743
OR4192A	3	53/74	0.716
OR4192B	4	25/74	0.338
OR4197A	6	3/74	0.040
OR4190A	8	2/74	0.027
OR4180B	10	2/74	0.027

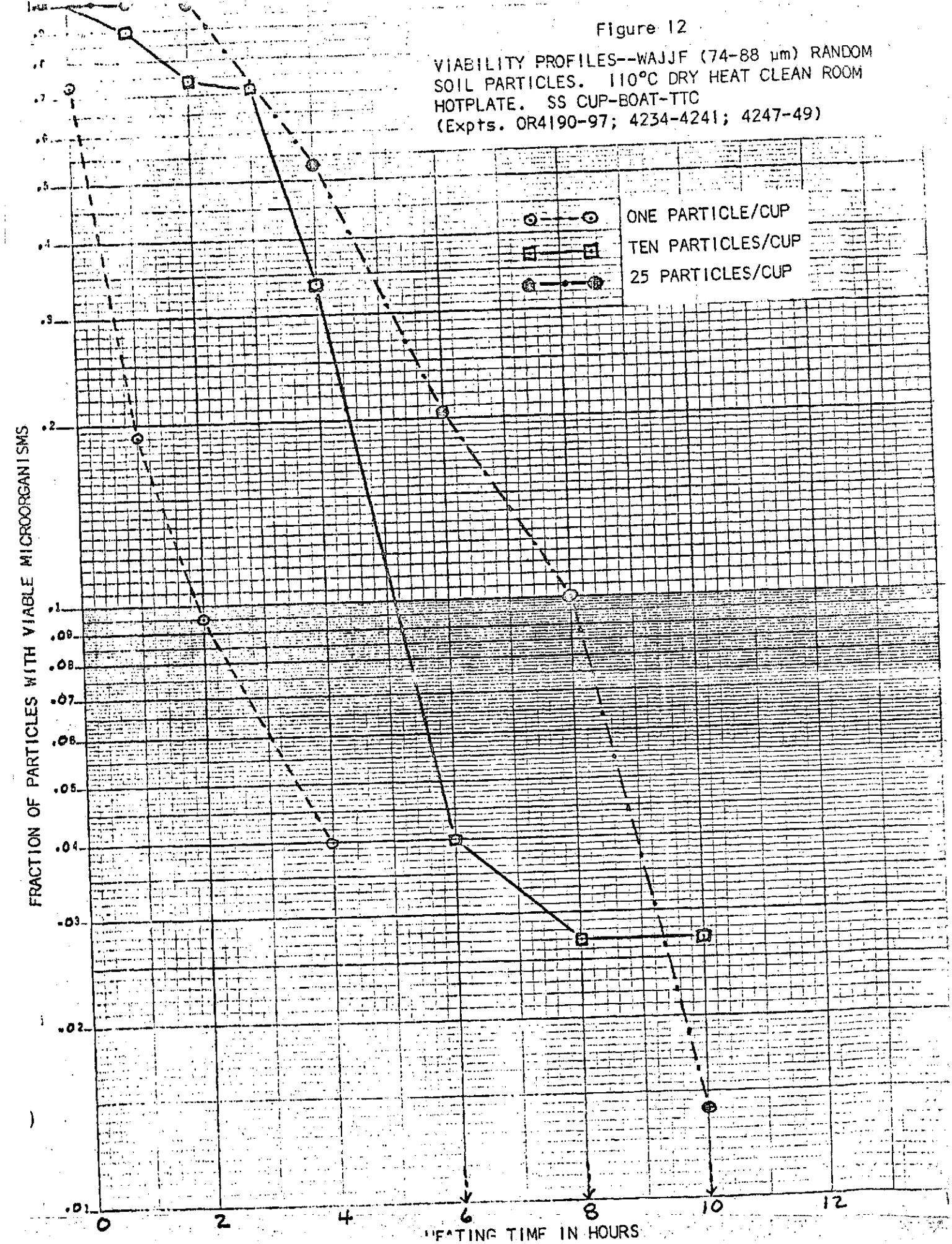
Table XII

Soil Particle Viability After Dry Heat Treatment On Clean Room Hotplate at 110°C. WAJJF (74-88 µm) Random Particles--25 Particles/Cup Proportion Positive Data

Experiment Number	Heating Time (Hours)	Proportion Positive	
		Fraction	Decimal
OR4235A	1	74/74	1.000
OR4235B	2	73/74	0.986
OR4234A	4	39/74	0.527
OR4234B	6	15/74	0.203
OR4241A	8	7/74	0.095
OR4241B	10	1/74	0.014
OR4241C	12	0/74	0.000

Figure 12

VIABILITY PROFILES--WAJJF (74-88 μm) RANDOM SOIL PARTICLES. 110°C DRY HEAT CLEAN ROOM HOTPLATE. SS CUP-BOAT-TTC
(Expts. OR4190-97; 4234-4241; 4247-49)



currently tested may extend the lag effect and prolong the time for inactivation.

CONCLUSIONS

The laboratory studies completed recently with Kennedy Space Center soil particles have provided additional data regarding the response of *in situ* particle microflora to heat treatment. Results from these microbiological analyses of soil particles support the following conclusions:

1. Data from soil particle viability analyses suggest that as the particle size increases, a viable microflora is retained for a longer time under dry heat treatment conditions.

2. Viability profiles for random *versus* dark WAKMF (74-88 µm) soil particles were similar. No microbial growth was observed from these particles after dry heat treatment at 110°C for ten hours. Analyses of the WAJJF series of random *versus* dark particles yielded viability profiles indicating that the dark particles manifested a more prolonged particle inactivation time than the random particles. Whether or not the observed difference in response is due to variability in particle selection, soil type or some other factor is not known.

3. Replicate experiments with three separate series of WAKMF (74-88 µm) dark particles treated at 110°C showed good reproducibility of the viability profile for these soil particles.

4. Analyses of unheated WAJJF soil fractions showed that 75 per cent of the individual dark particles retained viable microorganisms after several years storage in the laboratory. After 120 minutes of heat treatment at 125°C none of the particles showed evidence of microbial growth. At 110°C approximately one per cent of particles tested still retained viable microorganisms after eight hours heat treatment.

5. Soil particles which had been stored in the laboratory for 2.5 years and tested at 110°C were found to produce a viability profile similar to the profile obtained with more recently acquired soil particles in the 74-88 µm size fraction.

6. Experimental data from studies using one, ten and 25 particles per cup suggest that the particle load may influence the configuration of the particle viability profile for dry heat treatments. With an increase in particle load per unit area, a lag in the particle inactivation was observed and the time required for inactivation was extended.

FUTURE WORK

1. Further investigation concerned with the effect of particle load on the viability profiles for soil particles.

2. Analyses of particles to obtain viability profiles for anaerobic, mesophilic microflora.

3. Experiments to obtain viability profiles for soil particles from the 44-53 μm and 105-125 μm size fractions treated at 110°C

4. Exploration of statistical procedures for treatment of the viability profile data.