QUANTITATIVE ECOLOGY OF PSYCHROPHILIC, MESOPHILIC AND THERMOPHILIC MICROORGANISMS IN THERMIC, MESIC AND FRIGID SOILS

by

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ABSTRACT

Soil samples were taken from five different elevations in the Santa Catalina mountain range to assess the affect of environmental factors on microbial numbers. The relative numbers of psychrophiles, mesophiles, and thermophiles were determined for three classes of microbes, bacteria, actinomycetes, and fungi. Soil moisture was found to have the most effect on microbial numbers. Temperature response studies were also explored with 150 actinomycete isolates.

INTRODUCTION

Soil is populated by many organisms including animals and microorganisms. The latter are of considerable importance in the mineralization of organic matter. Soil environment controls to a great extent microbial growth and relative numbers of different microorganisms. For instance, fungi are dominant in many acidic forest soils, while bacteria predominate in more neutral to alkaline soils and in waterlogged muds. Actinomycetes are seldom numerically dominant (10).

Three important environmental factors which affect microbial populations are soil moisture, soil temperature, and soil reaction or pH. The soil moisture regime is the keystone in the complex of interlocking soil physical factors. Soil water contains many solutes including minerals, organic substances and gases which form the liquid nutrient medium for microorganisms. Soil water is subject to extreme fluctuations causing the concentration of its solutes to change, thus affecting microbial habitats. Osmotic gradients and soil aeration also depend on moisture (10). Shameemullah, Parkinson and Burges (26) suggested that soil moisture content was probably the most important factor controlling microbiological activity in pinewood soils. Seifert (24, 25) also demonstrated that moisture affected the numbers of bacteria in soil to the greatest degree. Temperature had little effect. Soil moisture has in addition been shown by other investigators to be the most important factor determining microbial numbers in soil (4, 5, 6, 7, 11, 20, 28, 35).

Temperature of soil is directly related to moisture content but is also influenced by other factors. For example, the amount of solar energy absorbed by soil depends on the direction and degree to which the land surface slopes, the soil color, and the density of vegetation cover (10, 15). In the northern hemisphere, southward sloping land receives more solar energy than northward sloping land. White soils may absorb only 20 percent of the radiant energy from the sun while dark soils absorb as much as 86 percent of the sun's radiant energy (15). Vegetation can reduce the amount of heat reaching the soil. Plants such as conifers not only intercept radiant energy but also form a thick layer of litter which insulates the soil.

Heat waves are formed in soils as a result of the fluctuation of warming and cooling. This is most pronounced in the surface layers. Wilkins and Harris (34) found that average monthly temperatures at the surface of a forest soil varied from 2C to 19C. Increased moisture affects the temperature of a soil as it decreases heat absorbing capacity.

Soil temperature affects the metabolic activity as well as numbers and types of soil microflora. This is demonstrated in certain soils where thermophilic and psychrophilic organisms co-exist. Thermophiles are able to grow above 40C and sometimes up to 75C and 85C and are common organisms in desert communities and compost piles. Psychrophiles, as defined by Ingraham and Stokes (13), are microorganisms which have the ability to grow at 0C within one to two weeks. Lochhead (19) has shown, however, that bacteria of frozen soil tend to be psychroduric

rather than psychrophilic. In contrast, Stokes and Redmond (27) maintain that psychrophiles are ubiquitous. They found that psychrophiles made up 86 percent of the bacterial population in uncultivated soil near Pullman, Washington. Less than one percent of the population was composed of thermophiles. They found that uncultivated soil differed markedly from garden and cultivated soil. Cultivation appears to favor mesophilic growth while psychrophilic bacteria appear to be the predominant bacterial flora in uncultivated soils. Larkin (16) studied the distribution of psychrophiles in a more temperate climate. Numbers of psychrophilic bacteria were determined in samples of Louisiana soil, mud, and water. He found no psychrophiles in the summer. In winter he found psychrophiles present in water and mud but not in soil.

The pH of a soil is determined by a number of factors, including the concentration of salts and carbon dioxide in the soil solutions and the exchangeable cations present. As these factors fluctuate in time and space, so does the pH of the soil, usually within the limits of one to two units (10). As water moves through a soil, bases tend to be leached out and replaced by hydrogen ions. Thus constant leaching leads to the formation of an acid soil. These changes can affect the chemical properties of soil by altering the solubility of certain substances. In acid soils toxicity to microbes could occur if the solubility of aluminum, nickel, and other components increased while nutrients such as calcium and phosphorous compounds solubilized and washed away. Alkaline soils are formed under arid conditions. The light rainfall is insufficient to

leach away the salts formed by progressive weathering. Evaporation during the dry season brings the salts to the soil surface.

Generally, soil bacteria and actinomycetes are less tolerant of acid soils than are fungi. The critical pH level for most bacteria and actinomycetes is around 5.0, below this many cease to grow (10). Davies and Williams (4) observed that low pH was a limiting factor on actinomycete numbers, but once values approached neutrality numbers fluctuated according to other factors. Mukerji (22) showed that with a rise in pH from 6.8 to 7.5 bacteria varied in number while fungi steadily decreased in Usar soils. At pH 10.9 bacteria were still high in number but fungi were no longer recovered.

The occurrence of a microbe in a soil with a certain gross pH does not mean that it is living and carrying out its activities at that pH. In micro-environments the reaction is not always the same as that of the macro-environment. Soil organisms and plant roots contribute to localized changes in pH.

Van Groenewoud (29) presenting data on the pH of grey wooded podsols in Canada, demonstrated a wide variation among large numbers of small samples of a soil. Corke and Chase (3) stated that their data did not indicate the actual range of soil pH where "less-acid pockets" would allow active proliferation of soil organisms.

Most factors in the soil environment vary with climatic changes, especially temperature, moisture content, and nutrient supply. Often clear seasonal patterns do not emerge since much of the soil is buffered against large scale changes and the methods used to detect changes in

microbial numbers are not sensitive to small scale fluctuations. In soils subjected to extreme climatic conditions, such as long periods of drought which occur regularly every year, clear seasonal changes in the microflora have been detected (10).

Waksman (31) in studying cultivated and uncultivated loams in New Jersey, found different bacterial population maxima in each soil at different times during a year. He stated that moisture and temperature have a bearing upon bacterial numbers, but these changes in numbers cannot be completely explained. Other non-seasonal factors such as soil type, vegetation, condition of organic matter of soil, and soil pH have to be considered to explain changes in the soil. Waksman suggests that seasonal variation does not exist due to the variation of these factors. Hiltner and Störmer (as cited by Waksman [31]) found that seasonal variation of soil temperature had relatively little influence upon bacterial numbers which rose and fell in summer along with the water content of the soil. Davies and Williams (4) indicated that little could be concluded concerning the seasonal fluctuation of the actinomycete population since the variation was not statistically significant between temporally separated samples and spatially separated samples taken at the same time. Hattori (12) states that the numbers of microbes vary daily and annually but remain comparatively stable.

Burges (2) maintains a seasonal variation in numbers and types of microorganisms exists, particularly in forest soils of cool temperate climates. Numbers of microorganisms corresponded with a raise in nitrogen content. He found, for example, an increase in bacterial numbers

from 3-10 x 10^7 to 15-40 x 10^7, actinomycete numbers from 3 x 10^7 to 4-10 x 10^7, and fungal numbers from 2 x 10^4 per gram to 4 x 10^5 organisms per gram in spring. Low winter temperatures and summer soil drying most probably affect numbers and types (21). Krasil'nikov (15) observed that total microbial numbers in soil in winter were smaller than in summer. The number of microbes in temperate soils is greatest in spring, smaller in summer, and increases somewhat in autumn. Larkin (16) indicated that the level of psychrophilic, mesophilic and thermophilic bacteria also change with the season.

Previous reports have concentrated on the quantitative determination of bacteria, actinomycetes, and fungi soil. Numbers vary depending on the soil. Krasil'nikov (15) and others agree that non-fertilized soil has a smaller microbial population than fertilized soil. A podsol from a Moscow forest contained 1-3 x 10^5 bacteria and 7-10 x 10^4 actinomycetes per gram, while in a Moscow garden counts of 1-10 x 10^6 bacteria and 5-10 x 10^5 actinomycetes per gram were obtained. Burges (2) reported bacterial numbers of greater than 10 x 10^6 bacteria per gram if the pH was greater than 6.0 in fertile agricultural soil. Hattori (12) reported that in a fertile agricultural soil 2 x 10^7 bacteria per gram were observed. Gray and Parkinson (9) give values of 10^9 organisms per gram for a field soil. Actinomycetes occur in lower numbers. Hattori observed 8 x 10^5 per gram in agricultural soil and Davies and Williams (4) reported 1-7 x 10^5 per gram of pine forest soil. Hattori observed 7 x 10^5 fungi per gram of soil, while Burges noted 2-10 x 10^4 per gram of a fertile soil.

Several investigators have studied the numbers of the three temperature groups (psychrophiles, mesophiles, thermophiles) in soil. Lochhead (19), in studying psychrophilic or psychroduric organisms, found that at 20C, 70 percent of the organisms present were bacteria (2×10^7 per gram) and 30 percent actinomycetes (9×10^6 per gram). At 3C only bacteria were recovered at a level of 1.8×10^6 per gram. Forester (as cited by Lochhead [19]) found 1.4×10^5 psychrophilic microorganisms per gram of garden soil in summer. Stokes and Redmond (27) determined numbers of bacteria and fungi in cultivated and uncultivated soils at 0C, 20C, and 55C. In cultivated soils 2.3×10^4 psychrophilic bacteria (0.5-15 percent), $4.6\text{-}6.7 \times 10^6$ mesophilic bacteria (84-98 percent), and $3\text{-}7 \times 10^4$ thermophilic bacteria (0.4-1.6 percent) were isolated per gram of soil. No thermophilic fungi were found in any of the soils. Of the fungi 30 percent were psychrophiles (690 per gram) and 70 percent were mesophiles (1600 per gram). In an uncultivated soil psychrophiles made up a large portion of the bacterial numbers. Psychrophilic bacterial numbers ranged from 4.2 to 31.0×10^5 per gram (49-86 percent), mesophiles ranged from 4.3 to 10×10^5 per gram (13-50 percent), and thermophiles ranged from 4 to 7×10^3 per gram (0.2-0.7 percent). Psychrophilic fungi ranged from 4.1 to 5.1×10^3 per gram (24-27 percent) and mesophilic fungi ranged from 1.2 to 1.6×10^4 per gram (73-76 percent).

The present investigation was undertaken to observe the relative numbers of three classes of microorganisms in soil: bacteria, fungi, and actinomycetes in soils representing several climatic conditions. Viable

numbers of psychrophilic, mesophilic and thermophilic microorganisms were determined in soil samples obtained at various elevations in the Santa Catalina Mountain range.

The Santa Catalina mountain range located approximately 40 miles northeast of Tucson, Arizona, offers a variety of soil environments ranging from desert to sub-alpine. The soil and vegetation patterns of the Santa Catalina Mountains were extensively studied by Whittaker et al. (33). The soil types on the south slope are gray-brown podsolics with mor in coniferous regions, gray-brown podsolics with mull and shantung brown soil in oak woodlands, reddish brown soils in desert grassland, and red desert soils in desert areas. These soils are predominantly shallow lithosols. The illuvial horizon is often absent with the A horizon resting on the R horizon. Exposed rock surface cover varies with elevation from 0.3 to 2.4 percent exposed rock in coniferous forests to 30-60 percent rock exposure in desert.

The Santa Catalina Mountain range is in addition characterized by increasing precipitation and decreasing temperature with increasing elevation. Diurnal soil temperature fluctuations are marked, especially at lower elevation. Soil temperatures of the desert fluctuate daily from 3.5 to 9.0C, open woodland and desert grassland 2.5 to 6.0C, pine and pine-oak 0.8 to 2.5C, and fir forests may fluctuate less than 0.4C. Increased moisture and vegetative cover also reduce temperature contrasts in the higher elevations. The following is a series of trends corresponding to increasing elevation: (a) productivity and biomass increases,

(b) vegetation coverage increases (30-50 percent in deserts to 100 percent in fir forests), (c) nitrogen content increases, and (d) soil pH decreases (33).

MATERIALS AND METHODS

Materials

Three different media were used to enumerate fungi, bacteria or actinomycetes from soils. Bacteria were isolated on Peptonized Milk Agar (PMA), a medium described by Larkin (17). It contains one g Difco-Peptonized Milk, 15 g Bacto-agar, 0.1 g actidione (cycloheximide, Upjohn Laboratories) in one 1 double-distilled water. Fungi were isolated on acidified Potato Dextrose agar (PDA) as recommended by Stokes and Redmond (27). The medium contained 35 g Difco PDA and 10 ml of ten percent tartaric acid in one 1 double-distilled water. The final pH varied from pH 3.5 to 4.0. Actinomycetes were isolated on Czapek's agar described by Waksman (32). This medium contained 30 g sucrose, 3 g sodium nitrite, 1 g dipotassium phosphate, 0.5 g magnesium sulfate, 0.5 g potassium chloride, 0.01 g iron sulfate, 15 g Bacto-agar, 40 μg actidione in one 1 double-distilled water.

Trypticase Soy Broth (TSB) was employed as an election medium. This medium contained 15 g Trypticase peptone, 5 g Phytone peptone, 5 g sodium chloride in one 1 double distilled water. Trypticase Soy Agar (TSA) included the addition of 15 g Bacto-agar to TSB.

Methods

Soil samples were collected from five elevations in the Santa Catalina Mountain range northeast of Tucson, Arizona. Soil samples were

taken beginning September 1973 and every two months thereafter for one year. The elevations, soil type, and surrounding vegetation are described in Table 1.

Prior to sampling, surface litter was removed at each site. Approximately 300 g of soil were taken from shaded areas at three locations within a four square foot area using an ethanol-flamed spatula. The soil samples were placed in labelled plastic bags and processed within six hours in the laboratory. At each sample site the temperature of the soil (surface 5 cm) and the air was taken and the general weather conditions were observed.

In the laboratory the three soil samples from one site were sifted together through a sieve with a one mm square mesh. From the sifted material three separate aliquots (1-3 g) were weighed in preweighed aluminum pans and dried 24 hours at 105C in order to determine soil moisture and dry weight. Soil pH was measured with a Coleman metrion IV pH meter using a 1:1 soil-water suspension (1).

Numbers of viable microorganisms were determined as follows: 10 g of soil were measured and added to a Waring blendor which contained 90 ml sterile distilled water, agitated 15 sec to break particulate material, and was further diluted serially. Three soil dilutions, in triplicate, were either pour plated or spread plated. The above procedure was repeated for samples from each site.

Culture Conditions

Numbers of psychrophiles, mesophiles, and thermophiles were determined as follows: For psychrophiles, triplicate plates were

Table 1. Description of sampling areas.

Elevation	Vegetation Type	Shade Provided By	Soil Type*
1. 3500 ft.	Desert Shrub	Cercidium microphyllum (Yellow Palo Verde)	gravelly sandy loam, arid
2. 4300 ft.	Oak Woodland	Quercus oblongifolia (Mexican Blue Oak)	gravelly sandy loam, arid
3. 5400 ft.	Pinyon-Juniper	Quercus arizonica (Arizona White Oak)	loamy, medium textured semi-arid to mesic
4. 7000 ft.	Ponderosa Pine	Pinus ponderosa var. arizonica (Ponderosa Pine)	stony sandy loam, mesic
5. 8200 ft.	Mixed Conifer	Quercus gambelii (Gamble Oak) Pseudotsuga taxifolia (Douglas Fir)	loamy, moderately fine textured, frigid

* Data from USDA Soil Conservation Service (23).

incubated for 14 days at 0C; for mesophiles, 4 to 5 days at 20C; and for thermophiles, 2 days at 50C. This followed the convention of Stokes and Redmond (27). All colonies isolated at 0C represented psychrophiles and colonies isolated at 50C represented thermophiles. Numbers of mesophiles were determined by subtracting the 0C count from the 20C count.

Psychrophile Election

No psychrophiles were recovered during the sampling period from soil suspensions plated on solid media. Therefore psychrophile election with a liquid culture was employed. Duplicate, one gram, soil samples from each of the five sites plus one additional sample from 9,000 ft were inoculated into 9 ml of TSB and incubated at 0C for two months. Samples were removed from the broth (every two weeks), plated on TSA and incubated for two weeks at 0C. Ten gram soil samples were added to 50 ml of TSB in flasks and incubated at 0C. Samples were removed from the broth enrichment and plated on TSA plates every two weeks and incubated at 0C for two weeks.

Temperature and Soil Response Studies of Isolated Actinomycetes

The effect of temperature on the growth of actinomycetes was investigated. Isolated colonies from enumeration plates were transferred onto Czapek's media to obtain pure cultures. Pure cultures were transferred to slants of Czapek's medium and stored at 20C. Stock cultures were transferred once a month.

Cardinal growth temperatures of each isolate were determined on Czapek's medium and TSA, at seven different temperatures: 4, 15, 20, 25, 37, 45, and 55C. Cultures at each temperature were examined grossly and microscopically for changes in colony and cell appearance, sporulation, and pigment production. The isolates grew equally well on Czapek's and TSA media.

The response of actinomycetes to different soils was observed. This was a qualitative test to observe growth of actinomycetes on soil from each of the sampling sites. Soil-water (equal amounts of each) pastes were prepared using soils from each site and placed in petri dishes. The pastes were then overlayed with 1.5 percent agar in water or Czapek's salts (no sucrose) in 1.5 percent agar or Czapek's complete media in 1.5 percent agar. Actinomycetes isolated from each of the five study sites were inoculated onto plates of soil from each site.

Statistical Analysis

A computerized regression analysis was performed using environmental factors and organism numbers as variables. The multiple-regression model involves a single criterion variable which is predicted from a set of predictor variables, yielding a multiple-correlation coefficient. The square of the multiple-correlation coefficient may be interpreted as the proportion of the variance of the criterion variable that is explained by the predictors. The solution of a multiple-correlation problem involves the determination of a set of weights, one for each predictor variable, which can be applied to each

subject's set of predictor scores to yield a series of composite predicted criterion scores. The equation used to compute a subject's criterion score may be expressed as:

$$Y = B_1V_1 + B_2V_2 + \ldots B_nV_n + RC$$

where

Y = predicted criterion score

B = raw score weight

V = raw score for a predictor variable

RC = regression constant (30).

RESULTS

Evaluation of Primary Isolation Media

Both media used to determine actinomycete and bacterial populations contained the antibiotic actidione. This antifungal agent effectively inhibited the growth of fungi on Czapek's (18) and PMA agar plates. Larkin (17) showed PMA to be superior to TSA and soil extract agar for bacterial enumeration. Czapek's medium is a synthetic medium, providing reproducible results. Actinomycete enumeration is enhanced as the microorganisms produce a typical colony morphology.

Potato Dextrose Agar acidified with tartaric acid was used to enumerate fungi. With the exception of an occasional bacterial colony only fungal colonies were found to grow on this medium. Spreading of fungal colonies on PDA was largely inhibited by the low acidity.

Distribution of Bacteria, Actinomycetes, and Fungi in Relation to Environmental Factors

Table 2 shows the distribution of bacteria, actinomycetes, and fungi found at each elevation. At 20C the numbers of fungi varied from 10^4 to 10^6 organisms per gram, actinomycetes 10^5 to 10^6 organisms per gram, and bacteria 10^6 to 10^8 organisms per gram depending on the conditions of the soil. These values correspond to numbers of organisms determined by other investigators.

Table 2. Numbers of microorganisms in relation to sampling period and elevation.

Month	Elevation (feet)	Numbers of Organisms per Gram Dry Soil								
		Bacteria			Actinomycetes			Fungi		
		0C	20C	50C	0C	20C	50C	0C	20C	50C
September	3500	-	1.6×10^{7}	4.2×10^{5}	-	1.2×10^{7}	2.9×10^{5}	-	4.9×10^{4}	-
	4300	-	8.2×10^{6}	-	-	5.5×10^{6}	-	-	5.2×10^{4}	-
	5400	-	1.5×10^{7}	5.0×10^{3}	-	1.3×10^{7}	-	-	1.5×10^{5}	-
	7000	-	2.3×10^{6}	-	-	6.9×10^{5}	-	-	1.2×10^{6}	-
	8200	-	2.2×10^{7}	2.0×10^{5}	-	2.2×10^{7}	2.6×10^{4}	-	8.4×10^{5}	-
November	3500	-	1.8×10^{7}	1.1×10^{6}	-	2.4×10^{5}	1.1×10^{6}	-	3.4×10^{4}	-
	4300	-	1.5×10^{7}	8.9×10^{4}	-	4.1×10^{5}	2.9×10^{4}	-	1.8×10^{5}	-
	5400	-	1.3×10^{7}	7.3×10^{3}	-	2.1×10^{6}	4.1×10^{2}	-	4.8×10^{4}	-
	7000	-	2.5×10^{6}	-	-	1.6×10^{5}	-	-	7.0×10^{5}	-
	8200		1.9×10^{7}	1.2×10^{5}	-	2.6×10^{5}	-	-	1.1×10^{5}	-
January	3500	-	6.2×10^{7}	1.6×10^{6}	-	2.1×10^{6}	8.1×10^{5}	-	8.1×10^{3}	1.2×10^{4}
	4300	-	1.6×10^{7}	1.6×10^{6}	-	4.3×10^{6}	3.3×10^{4}	-	7.0×10^{4}	-
	5400	-	1.1×10^{8}	1.5×10^{5}	-	3.7×10^{7}	3.9×10^{2}	-	1.2×10^{6}	-
	7000	-	4.9×10^{7}	3.8×10^{2}	-	2.2×10^{5}	-	-	9.2×10^{5}	-
	8200	-	1.0×10^{8}	1.1×10^{5}	-	1.9×10^{6}	-	-	5.8×10^{5}	-

Table 2. -- continued.

Month	Elevation (feet)	Numbers of Organisms per Gram Dry Soil								
		Bacteria			Actinomycetes			Fungi		
		0C	20C	50C	0C	20C	50C	0C	20C	50C
March	3500	-	2.0×10^7	1.2×10^6	-	1.6×10^5	9.2×10^4	-	4.5×10^4	6.8×10^2
	4300	-	8.5×10^6	2.8×10^5	-	6.0×10^5	2.0×10^4	-	6.5×10^4	-
	5400	-	3.2×10^7	4.5×10^5	-	2.4×10^6	1.2×10^3	-	9.3×10^5	-
	7000	-	7.3×10^6	2.5×10^3	-	1.9×10^5	-	-	1.2×10^6	-
	8200	-	1.2×10^8	7.8×10^4	-	4.7×10^6	1.7×10^3	-	1.1×10^6	-
May	3500	-	2.1×10^7	4.1×10^5	-	6.0×10^5	6.9×10^4	-	1.2×10^4	2.3×10^1
	4300	-	1.4×10^7	2.3×10^5	-	9.4×10^5	3.6×10^4	-	1.2×10^5	3.6×10^1
	5400	-	1.6×10^7	9.3×10^3	-	4.3×10^5	2.7×10^2	-	1.2×10^5	-
	7000	-	1.7×10^6	-	-	3.7×10^5	-	-	7.8×10^5	-
	8200	-	1.9×10^7	5.6×10^5	-	3.1×10^5	1.0×10^4	-	6.4×10^5	-
July	3500	-	9.7×10^7	2.9×10^6	-	6.7×10^5	1.2×10^6	-	5.4×10^4	-
	4300	-	1.1×10^8	5.7×10^6	-	6.4×10^5	5.6×10^5	-	7.8×10^4	7.5
	5400	-	6.6×10^7	1.4×10^6	-	4.6×10^6	7.9×10^3	-	1.1×10^5	1.6×10^2
	7000	-	1.0×10^7	5.2×10^3	-	3.7×10^5	1.2×10^2	-	1.0×10^6	2.8
	8200	-	1.5×10^8	2.4×10^5	-	7.5×10^6	6.6×10^4	-	8.8×10^5	11.9

Frequently a greater number of organisms was recovered in January and July. Thermophilic and mesophilic bacteria exhibited prominent peaks. This was most likely due to the increase in soil moisture. The March readings were often high because of spring thaw producing more available water and nutrients. Mesophilic actinomycetes showed peaks in January and July similar to bacteria. Thermophilic actinomycetes showed one prominent peak in July. Even though January provided available water the low temperatures inhibited growth. Mesophilic fungi did not seem to rely on the presence of soil moisture as the bacteria and actinomycetes did. March produced peaks most likely due to spring thaw and availability of nutrients. The peaks in March occurred only at the upper elevations where there was snow cover. The two lower elevations had no snow or rain in March and therefore no increase in numbers occurred.

At the 7000 foot elevation where the soil does not support good growth it is interesting to note that thermophilic fungi and actinomycetes were isolated only at one time when soil moisture and warm temperatures were greatest in July. Thermophilic bacteria were recovered only in January, March, and July when water and nutrients were readily available.

At the upper elevations fewer numbers of thermophiles were isolated. This was particularly true of the actinomycetes as July numbers show an increase when both temperature and moisture increased.

Table 3 shows soil moisture, soil pH, and soil and air temperature. Soil moisture appeared to have the most effect on numbers

Table 3. Environmental factors in relation to sampling period.

Month	Elevation (feet)	Soil Moisture (%)	Soil pH	Soil Temperature (C)	Air Temperature (C)
September	3500	0.78	6.5	41	35
	4300	1.19	6.3	32	32
	5400	13.30	6.45	19	26
	7000	3.5	5.4	17	22
	8200	19.00	5.8	15	18
November	3500	0.86	6.5	20	21.5
	4300	1.39	7.0	19	19
	5400	2.00	7.3	13	11
	7000	1.16	5.0	14	8
	8200	4.93	7.2	7	6
January	3500	6.43	6.8	12	16
	4300	16.22	7.2	12	21
	5400	22.31	7.2	5	12.5
	7000	13.84	5.5	4	18
	8200	26.21	7.0	2	8
March	3500	1.12	6.9	21.1	22.2
	4300	1.83	7.0	16.6	15.5
	5400	11.49	6.8	12.2	10
	7000	7.53	6.8	10.5	7.7
	8200	27.89	7.0	8.3	6.6
May	3500	0.82	6.9	35	28
	4300	1.72	6.8	24	26
	5400	2.69	6.5	17	20
	7000	2.42	6.8	16	18
	8200	4.86	6.8	12	15
July	3500	10.81	7.5	29	28
	4300	16.12	7.3	20	26
	5400	19.62	7.2	18	22
	7000	18.68	6.0	16	21
	8200	30.18	6.8	13	16

of microorganisms. The greatest percentage of soil moisture was recorded in January and July which is reflected in the increased numbers of microbes isolated. Soil pH changed no more than two units for all samples. These units were near optimal growth values. Soil and air temperature reflected seasonal changes. The incubator temperature however had a more visual effect on soil microbial numbers than environmental temperatures. Soil taken from periods of extremes in temperatures such as January and July, produced more colonies at 20C than soil taken from periods of more optimal temperatures.

Temporal Fluctuation of Microbial Numbers

With each season a significant difference in bacterial, actinomycete, and fungal numbers was observed as shown in Table 2. Seasonal variations are a result of environmental changes and moisture appears to have the most effect on numbers and types of microbes. Figure 1 shows the relationship between bacteria isolated at 20C from soil at 8200 feet and rainfall, temperature, and soil moisture. Rainfall data was obtained from a U.S. Forest Service ranger station located at 7900 feet. Snow cover occurred from November through March. The melting snow in March was most likely responsible for elevated soil moisture and a possible increase in available nutrients, thus contributing to the higher bacterial counts, since there was little rainfall recorded for the area during this period. It can be seen that the bacterial count is a reflection of soil moisture which depends for the most part on the amount of rainfall. For example, in January and July the bacterial

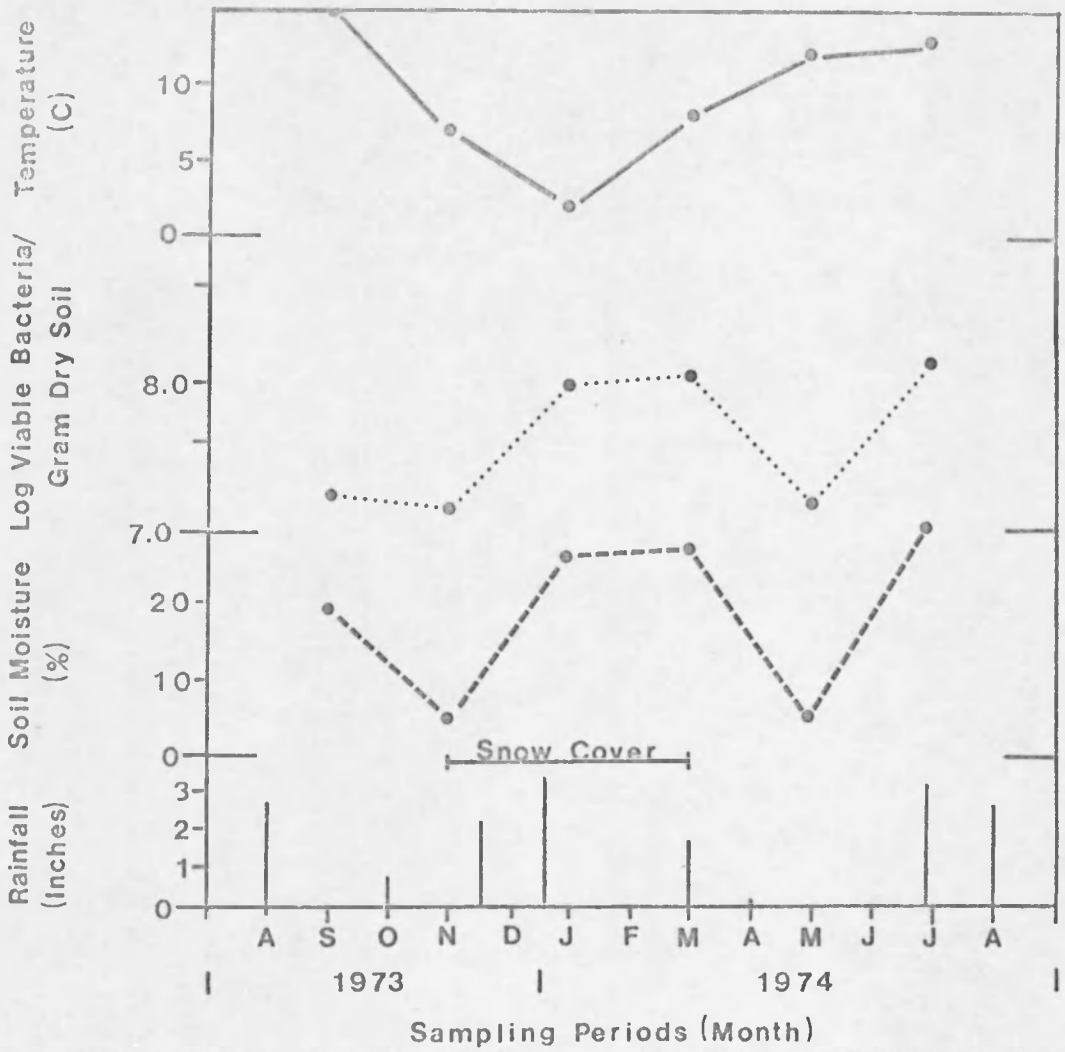

Figure 1. Number of bacteria (at 20°C from 8200 feet), soil moisture, temperature and rainfall in relation to monthly sampling periods.

counts were the highest as was soil moisture and rainfall greatest. Numbers were lowest in November and May when there was no rainfall.

Isolation of Psychrophiles and Thermophiles

Psychrophilic organisms were isolated during the first two sample periods. However, the small incubator used for 0C incubation was found to be unreliable. Subsequently, plates of each of the three media were incubated in a larger incubator with a better temperature control system. No psychrophiles were isolated from serially diluted soil samples irrespective of site.

Psychrophiles were isolated however, but only by elective culture. For example, following a six week incubation period at 0C, growth did occur in tubes of TSB inoculated with one gram of soil taken at the 5400 ft, 8200 ft, and 9000 ft sampling sites. By definition these organisms would not be psychrophilic but psychroduric. Growth did occur within two weeks at 0C from a ten gram soil sample inoculated into TSB. According to Ingraham and Stokes (13) the latter are most likely psychrophiles. Upon subculture growth occurred within eight days at 0C. Gram stains revealed that the organisms were gram negative rods. This demonstrated that psychrophilic microorganisms were present but in numbers too low to be detected by conventional direct plate count methods.

Thermophilic bacteria and actinomycetes were relatively common. However, thermophilic fungi were isolated only when the temperature of incubation was lowered from 55C to 50C after the November sampling period and then only occasionally. Table 4 indicates the ratio of

Table 4. The ratio of thermophilic to mesophilic bacteria and actinomycetes.

Month	Elevation (feet)	Bacteria Ratio*	Actinomycetes Ratio	Month	Elevation (feet)	Bacteria Ratio	Actinomycetes Ratio
Sept.	3500	2.5	2.2	March	3500	5.47	36.2
	4300	-**	-		4300	3.18	3.3
	5400	.03	-		5400	1.36	.05
	7000	-	-		7000	.03	-
	8200	.89	-		8200	.06	.04
Nov.	3500	5.76	121.8	May	3500	1.92	10.3
	4300	.59	6.6		4300	1.56	3.7
	5400	.06	.02		5400	.06	.06
	7000	-	-		7000	-	-
	8200	.63	-		8200	2.87	3.3
Jan.	3500	2.52	27.8	July	3500	3.12	152.7
	4300	9.1	.76		4300	4.83	88.6
	5400	.14	.01		5400	2.18	.17
	7000	.001	-		7000	.05	.03
	8200	.11	-		8200	1.57	.87

* Ratio of thermophiles to mesophiles.

** No thermophiles were isolated.

thermophilic to mesophilic bacteria and actinomycetes isolated at each sampling site. Generally, the ratio of thermophilic to mesophilic organisms decreased as elevation increased. Thermophilic actinomycetes represented a significant portion of the population isolated, at times outnumbering mesophilic actinomycetes. For example, at the lowest elevation, 3500 ft, there is a significant thermophilic actinomycete population. In November and July thermophilic actinomycetes outnumbered mesophilic actinomycetes. The number of thermophilic actinomycetes dropped considerably with increased elevation.

Variation Among Elevation Levels and Numbers and Types of Microorganisms

Data in Figure 2 show trends among elevations and numbers and types of microorganisms indicating that soil characteristics may be important factors. Numbers of mesophilic fungi increased with elevation and were highest at the 7000 ft site. Numbers of bacteria and actinomycetes were lowest at this elevation. At 7000 ft the pH of the soil was consistently lower than the other four elevations, ranging from pH 5.0 to pH 6.8, while the pH varied from 6.3 to 7.5 at the other levels. Thermophilic bacteria showed a more pronounced reaction to soil conditions as compared to mesophilic bacteria. Thermophilic actinomycetes decreased with elevation as was shown previously (Table 4).

Actinomycetes

One hundred fifty actinomycete isolates were obtained from soil samples collected at the various elevations. Cardinal growth temperatures were determined for each isolate. No psychrophilic actinomycetes

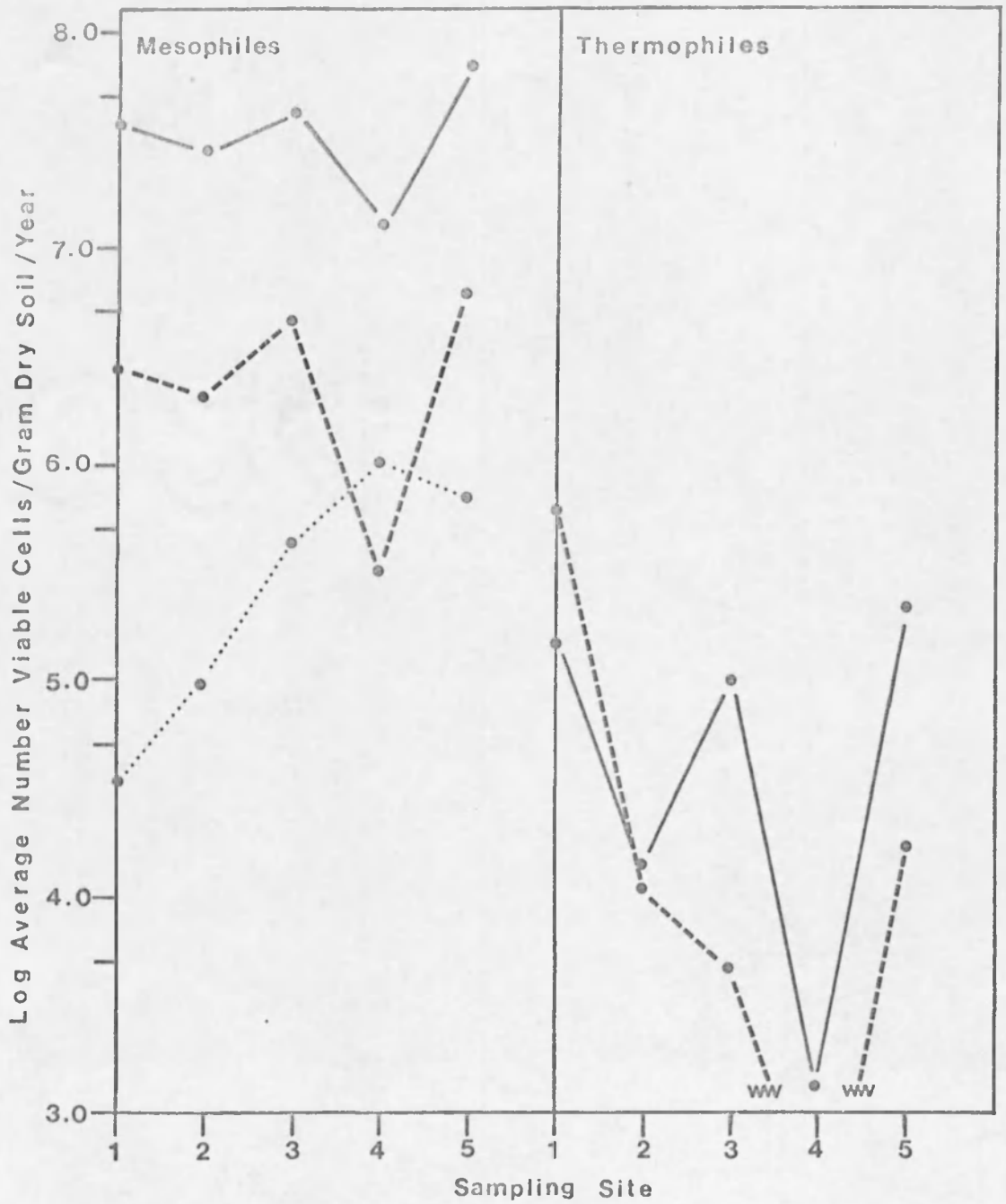

Figure 2. Numbers of bacteria, actinomycetes and fungi in relation to elevation.--Bacteria ·———— Actinomycetes ·- - - Fungi · · · · ·

were isolated. None of the actinomycetes grew below 4C nor above 60C. Mesophilic actinomycetes grew from 15C to 37C with an optimum of 20C to 25C. Thermotolerant species grew from 20C to 50C with an optimal temperature of 37C to 45C. Table 5 lists the numbers of actinomycetes capable of growth either at 20C or 50C or both as correlated with elevation. Very few of these organisms would be classified as thermophiles by Emerson and Cooney (8) as most of those that grow at 50C also had the capability of growth at 20C. Those actinomycetes able to grow at both 20C and 50C would be classified thermotolerant.

The actinomycetes were grouped according to color of the aerial mycelium. Table 6 lists several of the more common groups. Listed also are the predominant temperature responses and elevations of the individuals within each group. The grey shaded species are thermotolerant and were generally found at the lower elevations. The species exhibiting brighter and more varied colors were mesophilic and often found at the higher elevations. This suggests that pigment production may be temperature sensitive.

An effort was made to determine if the soil from each elevation contained some factor that would prevent growth of organisms isolated from elevations other than the soil being tested. Table 7 shows the results of these experiments. The data are probably a reflection of soil type and not elevation. Growth is poor at the 7000 ft level where the soil is more acidic and sandy. An actinomycete from 7000 ft was the only isolate to grow. Soil from 5400 ft supported the best growth of all the isolated actinomycetes. Why growth on soil from 8200 ft was poor is

Table 5. Growth of actinomycetes at elevated temperature in relation to sample site elevation.

Elevation (feet)	Total Number of Isolates	Number of Isolates Growing at Incubation Temperature of:		
		20C	50C	20 and 50C
3500	49	45*	40*	45**
4300	27	27	3	3
5400	30	30	17	17
7000	12	12	0	0
8200	19	19	1	1

* Numbers of isolates of the total isolates obtained from each sample site, showing growth at each respective temperature.

** Numbers of isolates capable of growth at both 20C and 50C.

Table 6. Actinomycetes grouped in relation to the color of the aerial mycelium.

Aerial Mycelium Color	Number of Isolates	Temperature Range	Sample Elevation (ft)
Grey Shades:			
Pale olivaceous grey	10	20C-50C	3500
Pale olivaceous grey and white	11	20C-50C	3500,5400
Pale olivaceous grey and white and silver exudate	8	20C-50C	3500
Lavender Shades:			
Rosy vinaceous	9	15C-37C	3500,5400
Rosy vinaceous and white	6	15C-37C	5400
Pale vinaceous	6	15C-37C	4300
Pale vinaceous grey	10	15C-37C	8200
Others:			
White	4	15C-50C	3500,4300
Greenish glaucous	4	15C-37C	7000

Table 7. Growth of actinomycetes isolated from each elevation on soil obtained from each elevation.

Test Soil at Elevation (feet)	Test of Actinomycete Isolate Growth on Soils at Elevation (feet)				
	3500	4300	5400	7000	8200
3500	2+	2+	2+	1+	2+
4300	2+	2+	2+	1+	2+
5400	3+	3+	3+	1+	3+
7000	-	-	-	1+	1+
8200	2+	2+	2+	-	2+

unclear as this soil appeared to be the richest in organic matter and soil moisture.

Statistical Analysis

The regression analysis program, based on covariation, begins by computing an intercorrelation matrix as shown in Table 8. Perfect correlation is expressed by 1.0000. The values of soil temperature, pH, moisture, month, elevation, and microbial numbers at 20C and 50C for each sampling period were correlated. For example, there was a high degree of correlation, 0.8093, between the number of fungi isolated at 20C and elevation (Table 8). This indicated that as elevation increased the number of fungi isolated at 20C also increased.

Regression analysis is primarily predictive in that a single criterion variable can be predicted from a set of variables. In predicting this criterion variable the program uses an iterative procedure which builds a regression equation by adding variables to the predictor set or adjusting weights of variables already in the set in order to maximize the increase in the square of the multiple correlation coefficient. The iteration process begins by selecting the variable with the highest validity from those available as predictors. As shown in Table 9 where the criterion variable is the number of bacteria isolated at 20C the variable having the greatest effect is the percent moisture (0.6844). The second iteration selects the variable, in this sample elevation, which will maximally increase the square of the multiple correlation coefficient when used together with the first variable to

Table 8. Intercorrelation analysis.

	1	2	3	4	5	6	7	8	9	10	11
Temperature	1.000										
pH	.0201	1.000									
Month	-.0146	.4076	1.000								
Elevation	-.6734	-.2830	.0000	1.000							
% Moisture	-.5020	.1525	.2787	.5010	1.000						
Bacterial # at 20C	-.1987	.5098	.3601	-.0148	.6844	1.000					
Actinomycete # at 20C	.0975	.1578	-.2185	.1166	.4676	.3746	1.000				
Fungal # at 20C	-.5619	-.3930	.0272	.8093	.4894	-.0843	.0734	1.000			
Bacterial # at 50C	.0430	.6551	.3466	-.3384	.2687	.7040	.2210	-.4045	1.000		
Actinomycete # at 50C	.3992	.4342	.3286	-.5878	-.0049	.4561	.1724	-.5508	.7932	1.000	
Fungal # at 50C	.2603	.3138	.4235	-.4905	-.0090	.3923	-.0905	-.5078	.4092	.5261	1.000

Table 9. Iteration sequence in regression analysis.

Criterion 6: The Number of Bacteria at 20C

Predictors = 1-5*

P = 5	RSQ = 0.4683**
P = 4	RSQ = 0.6392
P = 2	RSQ = 0.7012
P = 1	RSQ = 0.7075
P = 3	RSQ = 0.7084
P = 4	RSQ = 0.7087
P = 1	RSQ = 0.7088
P = 2	RSQ = 0.7089
P = 4	RSQ = 0.7089
P = 1	RSQ = 0.7090
P = 3	RSQ = 0.7090
P = 4	RSQ = 0.7090
P = 2	RSQ = 0.7090

Variables	B weights
1	0.0480
2	-0.0587
3	0.0063
4	0.0986
5	-0.0226

Regression Constant = 6.8145

* The environmental variables: Temperature, pH, Month, Elevation, Moisture.

** The square of the multiple correlation coefficient.

form a set of two predictors and so on. The B weights indicate the extent to which each variable is utilized in the regression equation.

With this analysis many hypotheses may be tested. Table 10 shows the results of one hypothesis. The question was: Can the number of bacteria isolated at 20C be predicted using (a) all the variables programmed and (b) only the environmental variables.

Computing this question gives the square of the multiple correlation coefficient as 0.8109 for the full model and 0.7090 for the environmental model. This indicates that the full model can predict within 81 percent of the true value and the environmental model can predict within 70.9 percent. Actual data from the month of January at an elevation of 5400 ft were inserted into the equation. The results of 7.1195 and 6.4415 for the full and shortened models, respectively, indicate a prediction within 81 percent and 70.9 percent of the real value of 8.0414 (log number of organisms from bacteria at 20C in the month of January at 5400 ft). All computations were performed on a CDC 6400 computer.

Table 10. Prediction of numbers of bacteria at 20C from environmental variables.

Criterion: Number of Bacteria at 20C

Predictors 1-5, 7-11* RSQ = 0.8109		Predictors 1-5 RSQ = 0.7090	
V	B weights	V	B weights
1	0.0356**	1	0.0480
2	-0.0572	2	0.0587
3	-0.0095	3	0.0063
4	0.0031	4	0.0986
5	0.0112	5	-0.8145
7	0.0000		
8	-0.0849		
9	0.1508		
10	-0.0152		
11	-0.0410		
Regression Constant = 6.8726		Regression Constant = 6.8145	
$V_6 = V_1B_1 + V_2B_2 \ . \ . \ . + RC$			
$V_6 = 7.1195$		$V_6 = 6.4415$	

* Predictors represent environmental factors (1-5) and organism numbers (7-11). Refer to Table 8.

** $V_1 - V_{11}$ is actual data obtained from month of January at an elevation of 5400 ft.

DISCUSSION

The causes underlying the variations in numbers of microorganisms in soil have been examined by numerous investigators. Opinions have varied regarding positive or negative effects of moisture, temperature, and pH. There appears to be some measure of agreement, however, that temperature has relatively little effect (28). Waksman (31) recorded his inability to show any correlation between bacterial numbers and moisture or temperature. Jensen (14) concluded that bacteria, actinomycetes, and fungi showed no correlation with the temperature. Seifert (24, 25) also showed that temperature had little effect on the number of bacteria in soil. Eggleton (7) found that changes in bacterial numbers were a reflection of moisture and not temperature.

Only fungi isolated at 20C appeared to show a significant correlation with temperature. The correlation coefficient of -0.5619 indicates an inverse relation of fungi with temperature. However the high correlation of fungi with elevation (0.8093) must also be considered. It is difficult to tell whether the number of fungi varies due to elevation effects or temperature only as temperature is an integral part of a change in elevation.

Actinomycetes isolated at 50C also showed a degree of correlation with temperature (0.3992). This is easily seen as in January (winter) no thermophilic actinomycetes were isolated at the two upper elevations.

At the three lower elevations a log decrease in numbers of thermophilic actinomycetes isolated as compared to the July (summer) results was observed.

The highest numbers of microorganisms obtained from media incubated at 20C were observed when the environmental temperature was the coldest in January and the warmest in July. These greater microbial numbers are most likely due to an increase in soil moisture. Bacteria isolated from plates incubated at 20C showed a very significant correlation with the percent moisture in soil, with a correlation coefficient of 0.6844. Actinomycetes and fungi isolated from 20C also showed a significant correlation (0.4676 and 0.4894, respectively) with moisture content. Jensen (14) showed a significant correlation of bacteria with soil moisture (0.520) but the fungi and actinomycete coefficients were not as relevant, being 0.272 and 0.210, respectively. This greater degree of correlation may be due to the dryness of the year in southern Arizona. Any change in moisture was felt acutely. Eggleton (7) also utilized regression analysis in studying environmental factors. He concluded that of all the environmental factors the association of bacteria with soil moisture was greatest. With regard to the fluctuations of actinomycetes and fungi, neither showed the same clear relationship with moisture.

Eggleton (7), working in the limited pH range of 6.1 to 6.8, showed no evidence of soil pH exercising any effect on numbers of bacteria, actinomycetes, or fungi. Jensen (14) also indicated that the soil pH shows no significant correlation with numbers of microbes.

There was a significant correlation with bacteria and fungi isolated at 20C and bacteria, actinomycetes, and fungi isolated at 50C. The pH of all soils throughout the year varied from 5.0 to 7.5. For each elevation the pH rarely varied more than one unit. However, at the times of increased microbial numbers, the pH also increased slightly, this being reflected in the correlation coefficient.

Soil from the 7000 ft site had a consistently lower pH. This influenced the relative numbers of fungi to numbers of bacteria and actinomycetes. Numbers of fungi increased with lowered pH while bacterial and actinomycete numbers decreased. Jensen (14) also noted this occurrence in similar coarse, acid sand soils that were generally poor in humus. The lower pH (average 5.2) and humus content of Jensen's soils related to lower numbers of bacteria and actinomycetes but not to fungi.

Seasonal variation of microbial numbers is a controversial subject. Eggleton (7) suggested that seasonal changes in temperature and moisture are not the direct cause of the seasonal changes in numbers of organisms but that in controlling the growth of surface vegetation these climatic factors control the amount of energy material reaching the microorganisms. Jensen (14) wrote that no distinct seasonal changes in the numbers, apart from results from the changes in the moisture content, are noticeable in any of the groups of organisms.

There was some correlation of month with the bacteria isolated from 20C and the bacteria, actinomycetes, and fungi isolated from 50C. The soil moisture which is a reflection of rainfall had a more

significant correlation with the organisms isolated from 20C. Temperature correlated with fungi isolated from 20C. This suggests that seasonal variation does exist to some degree among these organisms. Microbial numbers were considerably lower during drier portions of the year such as May and November. Numbers were highest during the months of January and July when rainfall was greater than three inches. Numbers were also high during March because of the spring thaw providing moisture and nutrients to the microorganisms.

The levels of moisture and availability of nutrients were important factors influencing numbers and types of microbes isolated from soils collected at the different elevations. Smaller numbers of organisms were obtained from soils taken at lower elevations, not only because of less moisture but also because vegetative cover was sparse. The upper elevations were forested with oak and pine trees which provided both shade and soil leaf litter.

Data on the Santa Catalina Mountain range collected by Whittaker et al. (33) show some characteristics of the soil. There is a 3.23 percent increase in organic matter with each 3200 ft up to 6560 ft. There is a higher rate of increasing organic matter in the coniferous forests of the upland above 6560 ft. With the increase in organic matter in the surface soil there is an increasing thickness and coverage of litter toward higher elevations. There is a 0.05 percent increase in nitrogen content with each 3200 ft increase in elevation.

Throughout the investigation no psychrophiles were isolated by conventional methods of enumeration. This was in contrast to results of

investigators such as Stokes and Redmond (27) who maintained that psychrophiles constitute up to 86 percent of the bacterial population in uncultivated soils.

Growth did occur at 0C however within two weeks when larger soil samples were inoculated into broth culture. In the more temperate Arizona climate, it is therefore suggested that psychrophilic organisms do not play as large a part in the environment as in colder climates.

After incubation for two weeks at 0C looking for psychrophilic organisms, the agar plates were placed at room temperature prior to discarding. Growth occurred after several days. Numbers of colonies were equal to those on plates prepared from the same material and held continuously at 20C. This suggested that low temperatures inhibited growth but did not cause death.

Thermophilic organisms are probably a more important part of the Arizona desert environment, as a significant correlation exists between numbers of thermophilic microorganisms isolated and elevation. Stokes and Redmond (27) found that thermophilic bacteria usually comprised one percent or less of the bacterial population. This was generally true of the upper elevations of the Santa Catalina Mountain range.

The lower desert elevations depend on some thermophilic action to decay the dead vegetation. For the sampling year the low temperature at 3500 and 4300 ft was 12C (54F) and the high was 41C (106F) which indicates that thermophilic or thermotolerant organisms are a necessity.

One hundred fifty actinomycete isolates were examined. Most of the isolates belonged to the genus Streptomyces which may be a reflection on the selectivity of the medium, one which selects for aerobic heterotrophs.

The elevation experiments (Table 7) indicated that the organisms were not restricted by elevation, although data from Table 6 suggest otherwise. The available nutrients may be an important elevation factor as shown in data from Table 7 where growth of all actinomycetes on soil from 7000 ft was poor.

The apparent delineation between mesophilic (lavender shades) and thermotolerant (grey shades) organisms is more likely a result of pigment production than elevation. Although thermotolerant organisms are generally found at the lower elevations some were found at the 5400 ft site and some of the mesophilic organisms were found at the lower (3500 and 4300 ft) elevations.

LITERATURE CITED

1. Black, C. A. (ed.). 1965. Methods of Soil Analysis, Part 2, Chemical and Microbiological Properties. Madison, Wisconsin: American Society of Agronomy, Inc.

2. Burges, A. 1958. Microorganisms in the Soil. London: Hutchison and Co., Ltd.

3. Corke, C. T. and F. E. Chase. 1964. Comparative Studies of Actinomycete Populations in Acid Podzolic and Neutral Mull Forest Soils. Soil Science Society Proceedings 28:68-70.

4. Davies, F. L. and S. T. Williams. 1970. Studies on the Ecology of Actinomycetes in Soil. I. The Occurrence and Distribution of Actinomycetes in a Pine Forest Soil. Soil Biol. Biochem. 2: 227-238.

5. Dayal, R. and O. S. D. Gupta. 1967. The Soil Fungi of Varanosi, India in Relation to Edaphic Factors. Oikos 18:76-81.

6. Dayal, R. and L. Giri. 1971. Soil Fungi in Leguminous Fields of Varanosi, India. Oikos 22:122-127.

7. Eggleton, W. G. E. 1938. The Influence of Environmental Factors on Numbers of Soil Microorganisms. Soil Science 46:351-363.

8. Emerson, R. and D. Cooney. 1964. Thermophilic Fungi. San Francisco: Freeman.

9. Gray, T. R. G. and D. Parkinson (eds.). 1968. The Ecology of Soil Bacteria. Ontario: University of Toronto Press.

10. Gray, T. R. G. and S. T. Williams. 1971. Soil Microorganisms. New York: Hafner Publishing Co.

11. Griffin, D. M. 1963. Soil Moisture and the Ecology of Soil Fungi. Biological Reviews 38:141-166.

12. Hattori, T. 1973. Microbial Life in the Soil. New York: Marcel Dekker, Inc.

13. Ingraham, J. L. and J. L. Stokes. 1959. Psychrophilic Bacteria. Bacteriological Reviews 23:97-108.

14. Jensen, H. L. 1934. Contributions to the Microbiology of Australian Soils. I. Numbers of Microorganisms in Soil, and Their Relation to Certain External Factors. Linnean Society of New South Wales Proceedings 59:101-116.

15. Krasil'nikov, N. A. 1958. Soil Microorganisms and Higher Plants. Moscow: Academy of Science of the USSR.

16. Larkin, J. M. 1970. Seasonal Incidence of Bacterial Temperature Types in Louisiana Soil and Water. Applied Microbiology 20: 286-288.

17. Larkin, J. M. 1972. Peptonized Milk Agar as an Enumeration Media for Soil Bacteria. Applied Microbiology 23:1031-1032.

18. Lawrence, C. H. 1956. A Method of Isolating Actinomycetes from Scabby Potato Tissue and Soil with Minimal Contamination. Canadian Journal of Botany 34:44-47.

19. Lochhead, A. G. 1925. The Bacterial Types Occurring in Frozen Soil. Soil Science 21:225-231.

20. Meiklejohn, J. 1957. Bacteria and Actinomycetes in a Kenya Soil. Journal of Soil Science 8:240-247.

21. Miller, J. H., J. E. Giddens and A. A. Foster. 1957. A Survey of the Fungi of Forest and Cultivated Soils of Georgia. Mycologia 49:779-808.

22. Mukerji, K. G. 1966. Ecological Studies on the Microorganism Population of Usar Soils. Mycopathologia et Mycologia Acta 29:339-349.

23. Richardson, M. L. and M. L. Miller. 1974. Report and Interpretations for the General Soil Map, Pima County, Arizona. USDA: Soil Conservation Service.

24. Seifert, J. 1960. The Influence of Moisture and Temperature on the Number of Microorganisms in the Soil. Folia Microbiologica 5:176-180.

25. Seifert, J. 1961. The Influence of Moisture and Temperature on the Number of Bacteria in the Soil. Folia Microbiologica 6:268-271.

26. Shameemullah, M., D. Parkinson and A. Burges. 1971. The Influence of Soil Moisture Tension on the Fungal Population of a Pinewood Soil. Canadian Journal of Microbiology 17:975-986.

27. Stokes, J. L. and M. L. Redmond. 1966. Quantitative Ecology of Psychrophilic Microorganisms. Applied Microbiology 14:74-78.

28. Tresner, H. D., M. P. Backus and J. T. Curtis. 1954. Soil Microfungi in Relation to the Hardwood Forest Continuum in Southern Wisconsin. Mycologia 46:314-333.

29. Van Groenewoud, H. 1961. Variation in pH and Buffering Capacity of the Organic Layer of Grey Wooded Soils. Soil Science 92:100-105.

30. Veldman, D. J. 1967. Fortran Programming for the Behavioral Sciences. New York: Holt, Rinehart and Winston.

31. Waksman, S. A. 1916. Bacterial Numbers in Soils, at Different Depths, and in Different Seasons of the Year. Soil Science 1:363-380.

32. Waksman, S. A. 1967. The Actinomycetes, A Summary of Current Knowledge. New York: Ronald Press.

33. Whittaker, R. H., S. W. Buol, W. A. Niering and Y. H. Havens. 1968. A Soil and Vegetation Pattern in the Santa Catalina Mountains, Arizona. Soil Science 105:440-450.

34. Wilkins, W. H. and G. C. M. Harris. 1974. The Ecology of Larger Fungi. V. An Investigation into the Influence of Rainfall and Temperature on Seasonal Production of Fungi in a Beech and a Pine Wood. Ann. Appl. Biol. 33:179-188.

35. Witkamp, M. 1966. Macroflora, Mycoflora, and Soil Relationships in a Pine Population. Ecology 47:238-244.