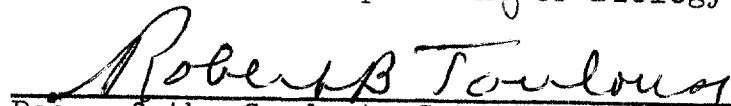A BACTERIOLOGICAL SURVEY

OF A

FRESHWATER RESERVOIR

APPROVED:

_____
Major Professor

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Minor Professor

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Director of the Department of Biology

_____
Dean of the Graduate School

A BACTERIOLOGICAL SURVEY

OF A

FRESHWATER RESERVOIR

THESIS

Presented to the Graduate Council of the

North Texas State University in Partial

Fulfillment of the Requirements

For the Degree of

MASTER OF ARTS

By

John Clayborn Stiles, B. A.

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CHAPTER I

INTRODUCTION

Many attempts have been made to enumerate and identify
the groups of bacteria that can usually be found in im-
pounded waters (1, 2, 3, 4, 5, 6). In each reported listing
of the organisms detected, there are groups of bacteria that
are common to nearly every other report. The presence of
common listings of genera of bacteria seems to indicate a
consistency in the various reports. Genera such as Pseudo-
monas, Azotobacter, Achromobacter, Flavobacterium, Alcali-
genes, Sarcina, Micrococcus, and Brevibacterium make up the
list of some of the commonly found organisms in water. This
investigation indicates the same common organisms being pre-
sent. However, an expansion is attempted in that not just
names of organisms are listed, but relative counts for each
group are included.

In attempting to interpret data collected from doing a
bacteriological survey of a large body of water, several con-
siderations must be taken into account. Definition of the
scope of the study must be of considerable importance. In
this study organisms that can be subcultured from lake water,
using a prescribed procedure, limit, to an extent, the popu-
lation, or portions of the population, that can be monitored.

1

In essence, what is taking place is that a set of conditions is set forth and a study is made of the bacteria that will grow under these prescribed conditions. The conditions are such that, by no means is the entire bacterial population monitored, but only that portion which will grow under the prescribed outline of sampling. The greater part of these organisms are called by Welch (7), the indigenous bacteria of a lake. These bacteria are said to be the normal, regularly occurring inhabitants of a lake.

Because of the limitations of such a study, only tendencies and generalities can be concluded from observations. Those arrived at must necessarily apply to the situations under which they arose. Any extrapolation or application of these tendencies would be speculative and reasonably unreliable.

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CHAPTER II

MATERIALS AND METHODS

Source of Samples

Lake Hefner is a water supply reservoir of Oklahoma City, Oklahoma. Figure 1 is an outline map of Lake Hefner which shows the sampling stations and the inlet canal. This lake is unique in that appreciable amounts of water can enter the lake only by way of the filling canal.
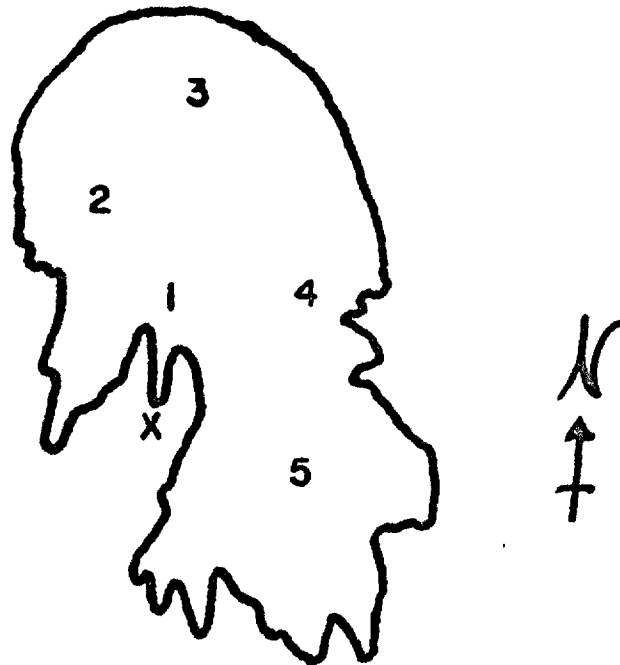
Fig. 1--Map of Lake Hefner showing sampling stations. Entrance of the filling canal denoted by X.

Water samples

Water samples were collected at the indicated stations in Figure 1. Samples were taken using a Kemmerer sampling device (5). At stations 1, 2, 4, and 5, samples were taken at a depth of one meter. At station 3, samples were taken at one, thirteen, and twenty-five meters. All samples were placed in sterile, glass containers until used.

Culturing of Samples

Each sample obtained was cultured on Plate Count Agar (Difco-1) after making the appropriate dilutions (2). Each sample from the five respective stations was plated out in triplicate at two dilutions. The dilutions selected were pre-determined so that from thirty to three hundred colonies were observed. Several incubation schedules were tried after the plates were prepared. Attempts were made to determine which of the schedules would yield the most colonies with a minimal amount of overgrowth. The first attempted schedule was to incubate the cultures at thirty-five degrees centigrade for one to two days. A second schedule was to incubate for two days at room temperature (thirty degrees centigrade) followed by two days at thirty-five degrees centigrade. The third schedule was to incubate the plates at room temperature for four to five days. After comparing the countability of the culture plates grown on the three schedules, the latter was chosen.

The culture medium used was not as prescribed by standard methods (2). Standard Methods for the Examination of Water and Waste Water prescribes the use of a beef extract medium for doing plate counts of bacteria in water. Plate Count Agar affords a greater variety of nutrients which is assumed to give a wider spectrum of organisms on the culture plates. Plate Count Agar gives a shorter incubation period with more luxuriant growth of bacterial colonies.

After the completion of the incubation period, the culture plates were examined for colonies of bacteria and these colonies were counted with a Quebec Colony Counter. Differential counts, based on macroscopic colonial morphology, were made and the colonies were subcultured onto test tube slants of Plate Count Agar.

Identification of each subculture was carried out by schemes outlined in Manual of Microbiological Methods (4). An additional identification scheme was supplied from the personal notes of Dr. R. K. Guthrie (3). To facilitate the differential counting and identifying of each subculture, nine groups were selected, such that each subculture could be placed in one of the nine groups. These groups are listed in Table I.

TABLE I

DIFFERENTIAL COUNTS BASED ON THE FOLLOWING GROUPINGS:

1. <u>Bacillus</u>
2. Fungi
   a. Molds
   b. Yeasts
3. Actinomycetes
4. <u>Pseudomonas</u>
5. <u>Flavobacterium</u>
6. <u>Brevibacterium</u>
7. Other gram-negative rods
   a. <u>Alcaligenes</u>
   b. Coli-aerogenes groups
   c. Colon-like organisms
   d. <u>Achromobacterium</u>
   e. Soil forms (<u>Azotobacter</u>, <u>Rhizobium</u>)
   f. Vibrio-like
8. <u>Micrococcus</u>
9. Others
   a. <u>Sarcina</u>
   b. <u>Gaffkya</u>
   c. <u>Corynebacterium</u>

How Counts Were Determined

The data based on the bacterial counts were determined by using mean numbers. The sample from each of the five stations was plated out on six culture plates, three at one dilution and three at a second dilution. The total colonies that appeared on the three plates at a single dilution were counted. This total was then divided by three to give the average total number of organisms per milliliter of water at that dilution. After both dilutions were counted, these totals were then averaged to give the number of organisms per milliliter for that station. The differential counts at each station were based on similar manipulations. After all five stations were complete, averages

were calculated for the total number of organisms and for each of the nine groups.

The data for each of the summer months represent averages of weekly samplings of each of the five stations. The monthly averages presented in the graphs are averages of weekly samplings. The average counts presented are relative, not absolute, numbers.

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CHAPTER III

RESULTS AND DISCUSSION

Total and differential counts were made of samples taken
from the sampling stations over a fifteen month time period
beginning in June, 1966 and ending in August, 1967. Average
monthly total counts are presented in Figure 2. The highest
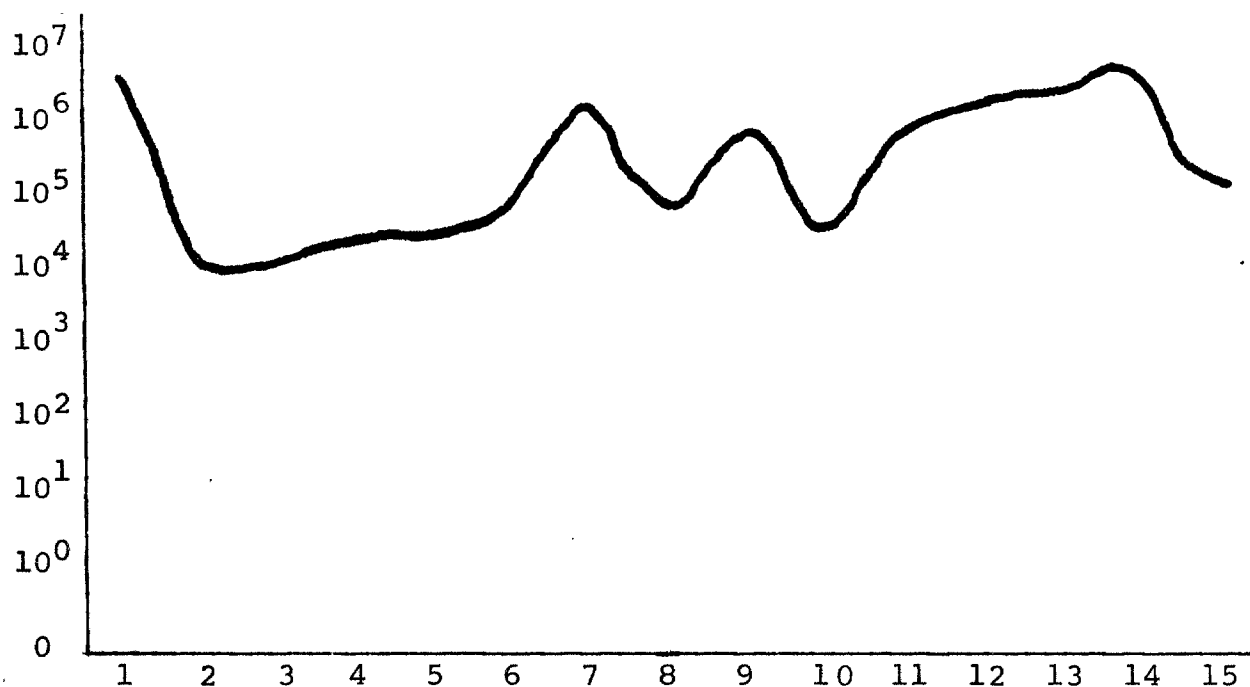total counts are seen in June, 1966 and July, 1967. Both

Fig. 2--Monthly averages of total number of organisms per
milliliter. The vertical axis shows number of organisms per
milliliter, while the horizontal axis shows the time in months.

of these seemingly higher than normal counts were recorded

during filling periods. At both times there was much agitation

of the lake because of the inrushing waters.

Monthly averages based on the differential counts of the
nine subdivisions are presented in Figures 3-7. Relative per-
centages of each subdivision are presented in Table II.

Bacillus

In Figure 3, the monthly averages show that the Bacillus
genus had highest counts in June, 1966 and July, 1967. Corres-
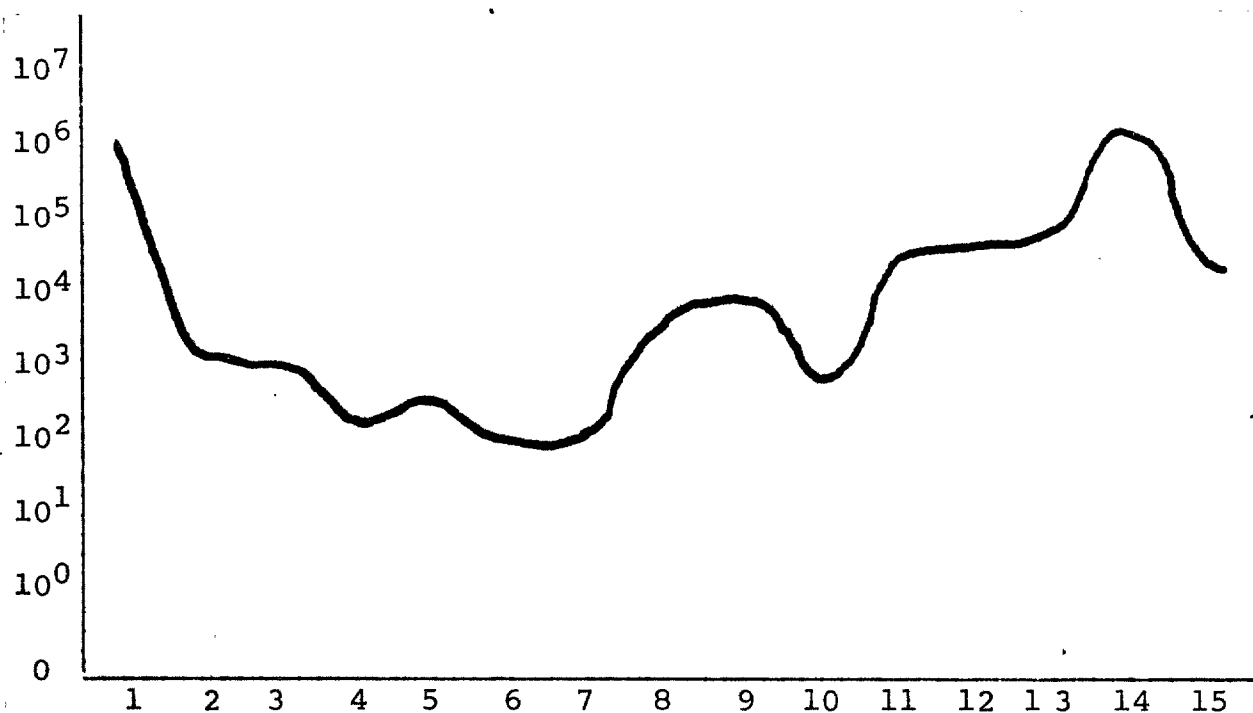pondingly, at these periods this group maintained its highest

Fig. 3--Monthly average counts of the genus Bacillus
relative percentages of the total population (Table II). A
general tendency seems to be indicated for the genus Bacillus,
in that the highest counts and relative percentages of this
group fall in warm weather months.

TABLE II

RELATIVE PERCENTAGES OF THE TOTAL COUNTS OF THE DIFFERENT
GROUPS OVER THE FIFTEEN MONTH SAMPLING PERIOD
(ONLY VALUES OF 5% OR GREATER ARE SHOWN)

Month	1	2	3	4	5	6	7	8	9	10	11	12*	13	14	15
Bacillus	33	12	12					8	19		7			39	13
Actinos**	7	7		10					25						22
Fungi	33	8	55					8	8	7			5	19	6
Pseudomonas						12					5				
Flavobacterium		26			6		21			28	19				14
Brevibacterium	5	13				42		24							15
O.G.N.***		14	17	75	48	41	53	51	6	53	43		60		17
Micrococcus	8	6		5	42		22		9					7	6
Others	8	13		8						5	22			7	6

*No samples received or taken for this month
**Most of these thought to be Streptomyces spp.
***Other gram negative rods

Actinomycetes

No attempts were made to identify the members of this
subdivision. The colonial forms tentatively placed in this
subdivision are, however, in accord with the classical de-
scription of Streptomyces species (1, 2). Monthly averages
for this subdivision are presented in Figure 4. Like the
Bacillus group, these organisms seem to be predominant in

the warmer months. Unlike the Bacillus group, the actino-
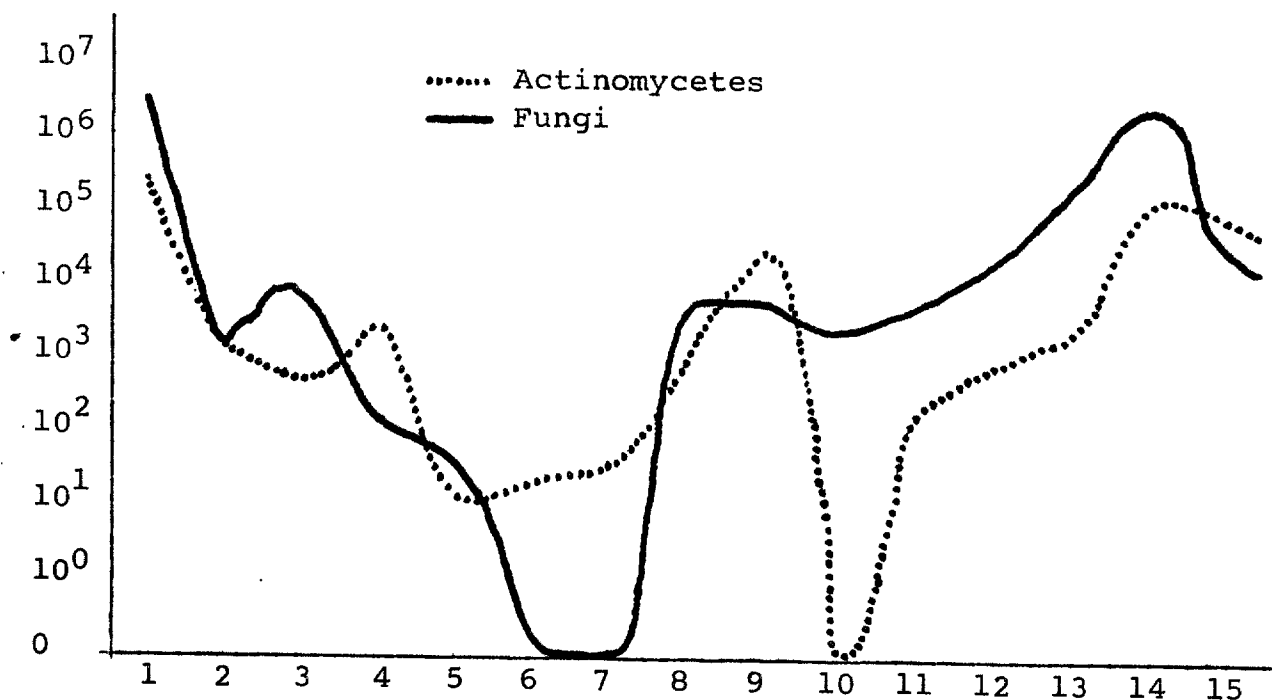mycetes had a more drastic decline in the colder months.

Fig. 4--Monthly average counts for the subdivisions
Actinos and Fungi.

It should be noted also that during the sampling period, steps
were being taken by the Oklahoma City Water Department to
decrease the population of these organisms.

Fungi

Classification of the Fungi group was not attempted.
Two major categories were noted, however. The group of or-
ganisms commonly called molds were present consistently
throughout the sampling period, excepting the months of
November and December, 1966. Colonial morphology enabled
the ease of classification of the molds. The second major

group included in this subdivision were the yeasts. Micro-
scopic examination of gram stained smears of these organisms
allowed the tentative classification of an organism as a
yeast. Most of the organisms classified as yeast produced
chromogenic colonies that varied somewhat in color. The
greater numbers were pink colonies, but hues of yellow and
tan to brown colonies were observed. Many of the yeast
colonies observed lacked the typical "yeasty" odor produced
by the more familiar baking yeast. In most cases odors re-
sembling rotting materials were noted.

The monthly average counts for the Fungi subdivision
are presented in Figure 4. The rather sharp increase in the
number of organisms in January, 1967 is due almost entirely
to the presence of yeasts. Very few molds were noted at this
time. The rest of the sampling period consisted of a mixed
grouping of molds and yeasts with the molds being the more
numerous.

<p style="text-align:center">Pseudomonas</p>

Counts of organisms of the Pseudomonas subgroup are pre-
sented in Figure 5. Although the counts are somewhat high,
the relative percentage of the total count never reached a
high level (as seen in Table II). The predominant number
of colonies counted were observed at the deep water station
(Station 3). Most of these came from the bottom sample at
this station. Identification of the pseudomonads was based
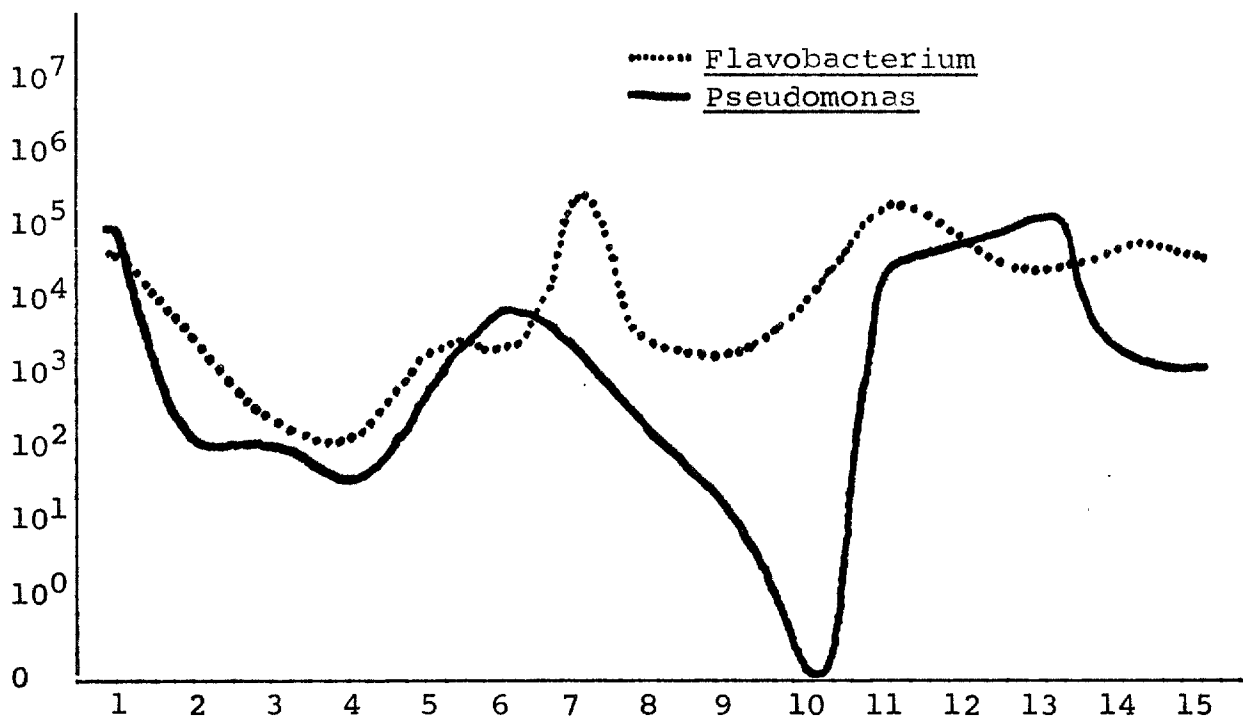mainly on the water soluble pigment produced by the colony,

Fig. 5--Monthly average counts of the genera Pseudomonas and Flavobacterium

as well as the inert reaction of these organisms to carbohydrate media. A few non-pigment forming colonies were observed and tentatively identified by the carbohydrate reactions and ability of the organism to produce oxidase.

Flavobacterium

Monthly averages for the genus Flavobacterium are presented in Figure 5. Organisms of the genus Flavobacterium were consistently present throughout the sampling period. This group seemed to have the highest counts in the cooler months of the sampling period while also having a higher relative percentage of the population at this time.

The colonies produced by <u>Flavobacterium</u> species were consistently chromogenic. This is in accord with classical descriptions of the genus (1). The predominant pigmentation was yellow, while many different hues of tan, orange, and very light pink were also observed. Like the pseudomonads, the flavobacteria produced little, if any, reaction on carbohydrate media.

Brevibacterium

The organisms tentatively identified as belonging to the genus <u>Brevibacterium</u> seemed to be somewhat erratic as to the counts observed. In Figure 6, high counts can be observed both in warm months and in cold months. The highest relative percentage of the population is observed, however, in a winter month (Table II).

The organisms tentatively identified as brevibacteria were classified mainly on chromogenesis and gram reaction, as well as microscopic morphology.

Micrococcus

The counts of the organisms identified as belonging to the genus <u>Micrococcus</u> show a degree of consistency in maintaining a relatively higher count throughout the sampling period. In Figure 6, counts vary enough so that no general tendency seems to be indicated as to warmer or cooler months affecting drastic changes. In two of the cooler months,
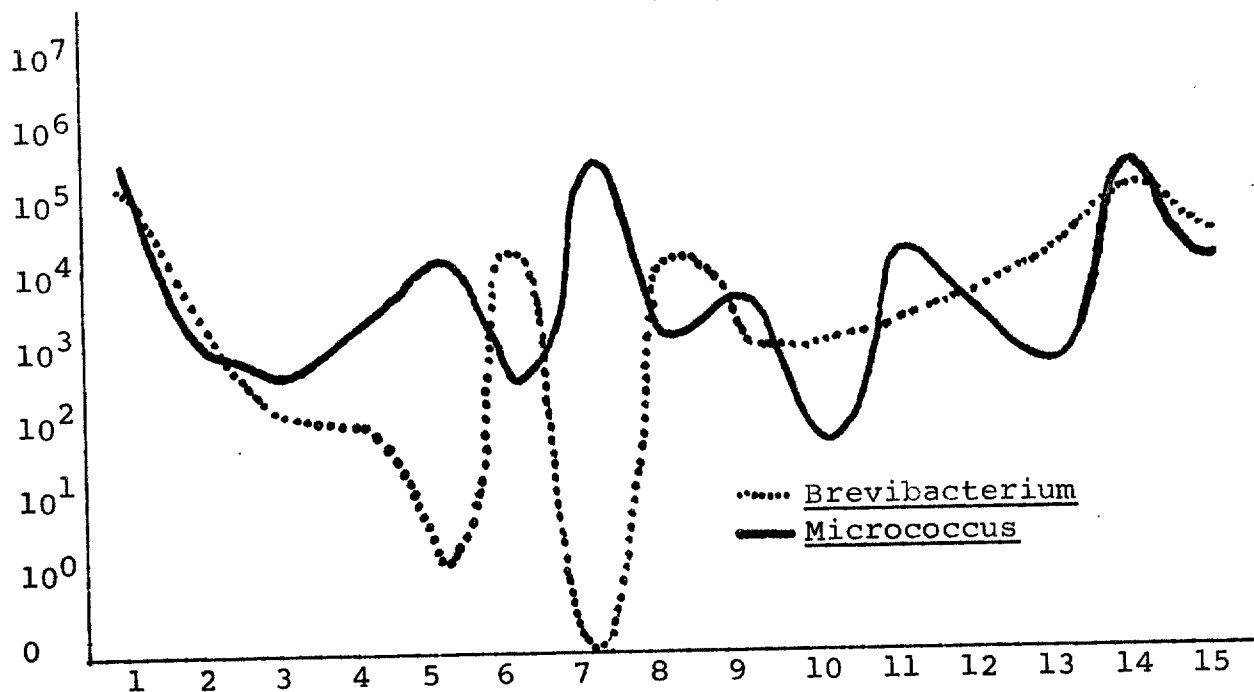
Fig. 6--Average monthly counts of the genera Micrococcus and Brevibacterium

October and December, 1966, Micrococcus maintained a fairly high relative percentage of total organisms.

The distinguishing characters of this subdivision are the chromogenic colonies and microscopic morphology and staining character of the cells. Varied colors such as yellows, oranges, and pinks were the most commonly observed colonies. The characteristic staining reaction (1) is indicative of this genus as well.

Other Gram Negative Rods

This subdivision maintained the most consistently high
relative percentage of total organisms (Table II). It is
shown in Figure 7 that this group maintained the consistently
higher counts of the nine subgroups. One reason for this is
the increased number of genera observed within this one sub-
division.

The reasons for placing organisms in this subdivision
are varied. The simplest and most direct reason is that these
organisms did not classify in any of the other subdivisions.
Other characteristics used were the carbohydrate reaction,
microscopic cellular morphology, colony formation, and other
biochemical reactions.
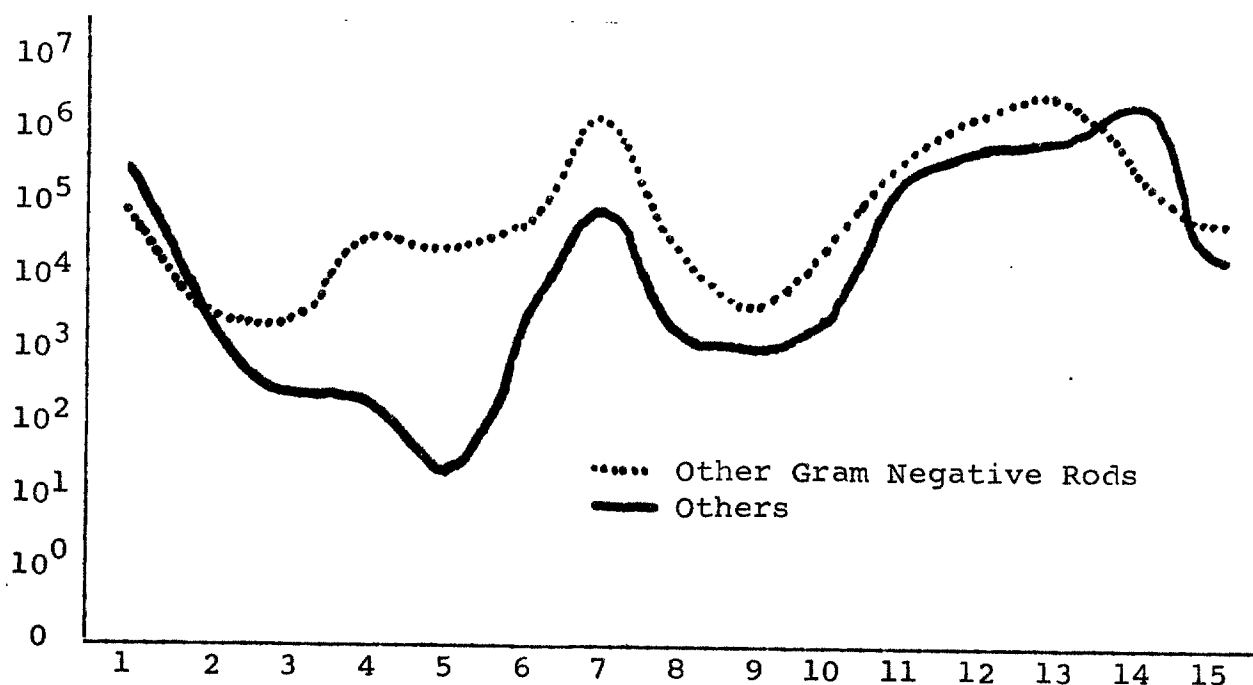
Fig. 7--Average monthly counts for the subdivisions
Other Gram Negative Rods and Others

Others

The average monthly counts for this group appear in
Figure 7. These counts seemingly follow closely those of
Other Gram Negative Rods. Comparing the relative percent-
ages of the total counts, however, shows that in very few
months does the subdivision Others correspond.

This group contains all the organisms that could not
be classified as belonging to any of the other subdivisions
listed. This group contained perhaps the widest variety of
organisms, ranging from coccoid forms to bacillary forms with
varied staining reactions.

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CHAPTER IV

CONCLUSION

This investigation has attempted to provide a somewhat limited description of the bacteriological flora of a freshwater reservoir. An attempt was made to show some general tendencies exhibited by some of the groups of organisms observed. These tendencies may or may not be consistent; however, a cyclical effect seems to be indicated regarding certain groups.

Gram negative organisms seem to predominate in colder weather, while gram positive organisms and fungi, especially mold-forms, predominate in the warmer months of the sampling period. The presence of variations in the counts exhibited by certain of the various groups of organisms would seem to disallow any constant rules regarding the presence, numbers, or relative percentages of these groups.

The Bacillus genus exhibits high counts at times when the surface waters of the lake are agitated. This occurs during warmer months of the fifteen month period. This might seem to indicate a correlation of temperature and water movement as two factors effecting the number of Bacillus species being present. Since members of this group are spore forming bacteria,

it is not determined by this investigation whether the colonies appearing are derived from vegetative cells or spores.

The actinomycete group tends to follow the same pattern, as to counts, as the Bacillus group. The temperature seems to affect this group more so than it does the <u>Bacillus</u>. During colder temperatures the counts decreased rapidly. A correlation of actinomycete presence and blue-green algae blooms has been suggested by Allison (1). Since these organisms are also spore forming bacteria, a possibility of airborne spores present on or near the surface of the water could affect the counts of both of these groups (<u>Bacillus</u> and Actinos). Because of the relatively dry circulating air in the summer months, there is a better chance of the spores being carried by air currents. During the cooler times of the sampling there could possibly be enough humidity to "wash" the air free of spores.

The pseudomonads and flavobacteria were found to be a greater part of the total population observed during cooler months of the sampling period. The flavobacteria were present in water samples from all stations consistently throughout the sampling period. The highest count occurred in December during cold weather. The pseudomonads were found inconsistently at the sampling stations. When found in higher numbers these

organisms were found in deep water at station 3. The flavo-
bacteria seem to fit Welch's description of indigenous bacteria.
The pseudomonads observed were probably soil forms which, by
being associated with soil, would be found in samples taken
from near the bottom.

The inconsistency of the counts of the brevibacteria would
make this group fit in as one of the unpredictable subdivisions.
The micrococci were consistently present at all stations. Prob-
ably these coccoid forms are indigenous bacteria.

Gram negative rod-shaped organisms, being of such a wide-
spread number of genera, could not be called indigenous as a
whole group. The likelihood of having indigenous genera within
this group is fairly certain. Because of this, the consis-
tently high counts of the group would be maintained without too
much deviation caused by the appearance of transient forms such
as coliform-like organisms.

The only genus seen consistently as a member of the sub-
division Others was Sarcina. The consistent appearance of this
genus, especially in colder periods, would seem to indicate
that this organism is an indigenous form. Streptococcus and
some of the other forms classified in this group appeared incon-
sistently.

It is concluded from this investigation that the organisms
observed are not unexpected as to the numbers present and the
varieties observed. The counts reported are similar to previous

reports of similar work done on this reservoir (1,2). The tentatively identified organisms are also like those previously reported (2).

The types and numbers of organisms present at any given sampling seems to be related to a seasonal factor. This seasonal factor, according to Welch (3), is related to temperature, pH, dissolved salts, and organic material present. Additional factors should probably be added to this list.

Specific conclusions regarding a survey report of this kind would necessarily be very hard to conceive. Because of the many factors affecting the results of any extended sampling period, no two sets of results might be expected to correspond. This would indicate a need of carrying out a survey of this nature over extended periods of time that would exceed fifteen months.

Since such a variety of organisms is observed to occur in impounded waters, a method of less involved identification procedures might be very useful. Conventional methods tend to be lacking as to time and expense involved. At the present time serological methods of identification are being worked out using a millipore filtering technique and fluorescent antibody. Limited information regarding the bacteria that can be expected to be found inhabiting a freshwater reservoir is a definite hindrance in conducting a survey of this nature.

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CHAPTER V

SUMMARY

This investigation has attempted to present a survey of
a part of the bacterial population of Lake Hefner, Oklahoma
City, Oklahoma.

This survey includes the total and differential counts
of organisms cultured from the water samples. An attempt was
made to show the relative percentages of the different groups
as related to the total observed population.

Standard procedure for sampling, culturing, counting, and
identifying organisms found in Lake Hefner was established.
The data resulting from this standard procedure have been pre-
sented in graphic form with explanation. Certain groups of
bacteria, such as Bacillus, Actinos, and Flavobacterium, exhibit
a tendency to appear consistently in the sampled water. Other
groups, such as Micrococcus, Others, and Brevibacterium lack
consistency in counts and time of appearance.

The counts observed, as well as the species observed were
not surprising when compared to earlier work. The tendencies
speculated about each group were not surprising nor were they
wholly expected, due to the lack of earlier investigations on
which to base an opinion. This work does give a semi-quantitative

presentation as to some of the organisms present in Lake Hefner water, as well as the relative numbers present.

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