A BACTERIOLOGICAL SURVEY
OF A
FRESHWATER RESERVOIR

APPROVED:

[Signatures]

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A BACTERIOLOGICAL SURVEY
OF A
FRESHWATER RESERVOIR

THESIS

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By

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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 impounded 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 Pseudomonas, Azotobacter, Achromobacter, Flavobacterium, Alcaligenes, 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 present. 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 considerations 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 population, or portions of the population, that can be monitored.
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 predetermined 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. **Bacillus**
2. **Fungi**
   a. Molds
   b. Yeasts
3. **Actinomycetes**
4. **Pseudomonas**
5. **Flavobacterium**
6. **Brevibacterium**
7. **Other gram-negative rods**
   a. **Alcaligenes**
   b. **Coll-aerogenes groups**
   c. **Colon-like organisms**
   d. **Achromobacterium**
   e. **Soil forms (Azotobacter, Rhizobium)**
   f. **Vibrio-like**
8. **Micrococcus**
9. **Others**
   a. **Sarcina**
   b. **Gaffkyya**
   c. **Corynebacterium**

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

![Graph showing 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.](image)
Monthly averages based on the differential counts of the nine subdivisions are presented in Figures 3-7. Relative percentages 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. Correspondingly, at these periods this group maintained its highest 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)

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

*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 description 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 actinomycetes had a more drastic decline in the colder months.

![Graph showing monthly average counts for the subdivisions Actinos and Fungi.](image)

**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 organisms 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. Microscopic 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 resembling 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.

Pseudomonas

Counts of organisms of the Pseudomonas subgroup are presented 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 *Flavobacterium* 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 *Brevibacterium* 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 *Micrococcus* 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 subdivision.

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.

![Graph showing average monthly counts for the subdivisions Other Gram Negative Rods and Others](image)

**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 percentages 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 coccoïd 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 Bacillus. 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 (Bacillus 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 flavobacteria 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. Probably these coccoid forms are indigenous bacteria.

Gram negative rod-shaped organisms, being of such a widespread 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 consistently 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 subdivision 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 inconsistently.

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 presented 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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