A QUALITATIVE SURVEY OF THE AIRBORNE ALGAE, PROTOZOA, AND BACTERIA AT THE DENTON SEWAGE TREATMENT PLANT

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A QUALITATIVE SURVEY OF THE AIRBORNE ALGAE, PROTOZOA,
AND BACTERIA AT THE DENTON SEWAGE TREATMENT PLANT

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By

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CHAPTER I

INTRODUCTION

Viable airborne microorganisms have been found by many workers. A few have studied the addition of bacteria to the air by the treatment of sewage. The algae and protozoa play an important role in sewage treatment, but no one has reported the emission of these organisms into the atmosphere by wastewater treatment processes.

Purpose

This study had a three-fold purpose. First, it was decided to determine if algae and protozoa were emitted to the air at the Denton sewage treatment plant. The information obtained could be of future importance in the fields of algal and protozoan ecology and public health. Second, it was decided to make a survey of the airborne bacteria at this plant. Some researchers have described bacterial air contamination at similar sewage treatment plants, but the one in Denton has not been studied. Third, it was hoped that in this research some relationships could be found between the bacteria and the algae and protozoa in the air in the vicinity
of the sewage aeration basin. It was hypothesized that pathogenic bacteria were carried in the air with these other organisms.

History

There has been an interest in atmospheric organisms since very ancient times. According to Gregory (14), Hippocrates, the "Father of Medicine," who lived in the late Fifth Century, B. C., felt that epidemic fevers were caused by breathing air infected with hostile pollutants. Gregory observed that Lucretius, in 55, B. C., watched dust particles in a sunbeam and speculated that minute living particles in the air accounted for the origin of plagues and other phenomena. In more recent times, germ theories of disease have been proposed, many of them founded on the proposition that microbes are carried in the air.

Fracastorius described various means of disease transmission and, in 1546, speculated that some diseases were transmitted "ad distans"—or through the air (41). With the aid of some of the first crude microscopes, Antony van Leeuwenhoek (41) in 1676 described tiny "animalcules"—bacteria and protozoa—and concluded that the air was one source of these creatures. Leeuwenhoek did not consider
these organisms to be of any consequence in regard to diseases. By 1650 the first theories of spontaneous generation of living from non-living matter were advanced (14). Throughout the ensuing abiogenesis controversy, evidence for microscopic contamination through the air was found. The use of cotton stoppers in flasks and test tubes was known as an effective means of filtering these microbes out of the air (41). In 1860 and 1861, Louis Pasteur (14, 41) performed several experiments to demonstrate that bacteria were found in dust. He also took air samples (14) and found bacteria at different elevations and at several locations which demonstrated the variety and ubiquity of these microorganisms. In 1844 Ehrenberg (9) reported diatoms in dust from air obtained from Charles Darwin (5,6) who speculated that one explanation for the distribution of species might be found in dust clouds, as cited by Gregory (14) and Schlichting (40). Although interest in airborne microbes began to wane toward the end of the Nineteenth Century, the French microbiologist Pierre Miquel continued to make extensive studies of the air flora (14). Miquel was especially interested in the number of bacteria and fungal spores to be found in the atmosphere.
New interest in aerobiology has developed in more recent years. Extensive air sampling for bacteria began anew in the 1930's. Airplanes became a popular means of obtaining atmospheric samples and Proctor (32,33,34,35) was one of the first to describe species of bacteria collected in the upper air. Meier and Lindberg (26) in 1935 reported the collection of a number of bacteria in flights over the arctic regions of Greenland. In the late 1930's, ZoBell (46) was studying populations of airborne bacteria over the Pacific Ocean. By the early 1950's, the list of atmospheric bacteria was again lengthened by reports of microbes found over Canada by Kelly and Paddy (17,31). Few of these workers in the field of aerobiology have been interested in organisms other than bacteria, fungi, and the pollen of higher plants. Reports by persons attempting to collect, identify, and culture airborne algae or protozoa are relatively few.

According to Schlichting (38), the Italian priest and scientist Lazaro Spallanzani probably isolated and studied the first protozoa from the atmosphere by exposing sterile media to the air in 1777. According to Gregory (14), Ehrenberg reported finding great quantities of protozoa in the air between 1849 and 1872 (10). In 1883, Miquel (14) found protozoan cysts in his aerial samples. Gregory (14)
has also reported that in 1913 Puschkarew found amoebae, flagellates, and a ciliate in air sampled in extensive studies along the Neckar River in Germany. Lackey, greatly interested in the microbiology of water found standing in tree-holes, reported great numbers of protozoa and algae present in these habitats in 1939 (19). Between 1955 and 1964, Schlichting (38,39,40) cultured many species of algae and protozoa from air samples collected in Michigan and Texas. Stevenson and Collier (43) reported airborne flagellates and diatoms in the air along the Texas Gulf coast in 1962. Brown, Larson, and Bold (4) reported a few airborne flagellates along with numerous algae in various air samples obtained in 1963.

It has been well documented that algae are often airborne. According to Schlichting (38), Ehrenberg (9) had identified about thirteen genera of algae in dust samples collected at sea in 1844 and Salisbury (37) in 1866 sampled air in the United States to collect "disease producing algoid" organisms which he called "Palmellae." H. Molish (27), as cited by van Overeem (30), described many airborne diatoms in 1920 and coined the term "aeroplankton." A number of unclassified algae were collected from airplane flights over Greenland in 1935 by Meier and Lindberg (26). Marie van Overeem
made a number of studies on airborne algae in 1936 and 1937 (29,30). Over forty genera were described from air samples taken by stationary sampling devices at Leiden and samplers on airplane flights. Gregory, Hamilton, and Sreeramula (15) reported the blue-green alga *Gleocapsa* and other members of the Chrococcaceae in air samples obtained during the summer of 1954 in England.

In June, 1956, Schlichting (39) exposed sterile media to the air and obtained a number of algae and protozoa. In 1961 he reported seven viable species cultured from air samples collected weekly over a one year period in Michigan (40). In 1962, Stevenson and Collier (43) reported airborne marine phytoplankton by taking air samples on the Texas Gulf Coast. During 1964, Brown and others (4) in Texas reported over sixty genera of viable algae collected by exposing agar plates at stationary locations and holding plates out of moving automobile and airplane windows, in addition to obtaining samples from twenty-one states taken by air pollution monitoring stations. In March, 1964, Schlichting (38) presented the results of his extensive airborne algae research in Michigan and Texas; thirty-three species of algae and seven species of protozoa were cultured from the Texas air samples. In 1965 Brown (2,3) reported collections of airborne algae
in Hawaii and suggested that biology classes might perform laboratory experiments in airborne algae collection. In 1967, Mahoney (23) reported finding the algae *Phormidium* and *Ankistrodesmus* in short air sampling periods at a trickling filter sewage treatment plant in Denton, Texas.

Several studies of air pollution and bacterial air contamination by waste and sewage treatment have been made. As early as 1907, Horrocks (16) had experimented with conditions that would permit infectious bacteria to remain viable in the air in London sewers. Fair and Wells (12) measured bacterial air contamination at a variety of sewage treatment plants in 1934. After a 1957 outbreak of psittacosis in Oregon, Spendlove (42) used certain marker bacteria to trace the emission of bacteria to the air by a rendering plant. Woodcock (44) reported research into the release of fine droplets and bacteria into the air by bubbling sewage in 1955. Albrecht (1) made detailed studies of bacterial air contamination in 1958 at several Florida trickling filter sewage treatment plants. Reports of odor control (8) and air pollution resulting from the treatment of sewage were common by 1960. In 1964 Ledbetter (20) published his observations on the addition of fine aerosols and odors to the atmosphere. He speculated that this problem would
increase in importance with "the growth of the population, advance of industrial technology, and the public awareness of air pollution" (20,p. 62).

Ledbetter and Randall (21,36) in 1965 and 1966 reported sampling the air upwind and downwind of an activated sludge aeration unit for coliform bacteria. They found large numbers of coliforms in the air and made proposals as to the effect of certain environmental conditions on bacterial air contamination. Air contamination was compared at different types of sewage treatment plants in 1966 by Napolitano and Rowe (28). They found that the aeration method emitted three times as many bacteria as did the trickling filter method. Glaser and Ledbetter (13) have made a detailed study of the mechanical and physical factors in the production of aerosols in sewage aeration, quantifying the particles by size, weight, and other characteristics. None of these workers have searched for or reported the finding of algae or protozoa in their aerial sampling, although there have been many studies of the organisms usually found in sewage (7,8,18,25)--algae, protozoa, viruses, bacteria, and fungi as well as many of the higher plants and animals.

Some evidence has been presented that airborne algae or protozoa might cause allergies and fevers. In 1866,
Salisbury (37) attempted to show that a \textit{Palmella}-like alga was the cause of fevers. In 1948, A. H. Woodcock (45) showed that some respiratory irritations occurring in coastal communities were caused by inhaling mist containing high concentrations of marine plankton or their waste products. McElhenny and others (24) have demonstrated in 1962 laboratory tests that green algae, as common air contaminants, are a possible cause of some inhalant allergic sensitivities. Mackenthum and Ingram (22) have cited fourteen studies of human respiratory disorders associated with algae.
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CHAPTER II

MATERIALS AND METHODS

Air Sampling Location

The Denton, Texas sewage treatment plant is located at Mayhill Road and Woodrow Lane, three and one-half miles southeast of the Denton County courthouse. The plant receives sewage from the north, south, and west parts of Denton. This plant, built in 1962, uses the sludge activation method in the treatment of sewage. Aeration of the sewage-sludge mixture is effected by bubbling air through this mixture by submerged jets in the center of a large basin (151 feet long, 61 feet wide) near the center of the plant.

The treatment plant is situated on a grassy, clay sand hill, bordered on the north, east, and west sides by trees and pasture. Open pasture with a few trees extends for some distance to the south of the plant. Pecan Creek, which receives the effluent of the treatment plant, is located about 200 yards north and northeast of the aeration basin. Engineer's drawings and construction details (5) indicate
that the elevation at the aeration basin is 570 feet and that the creek and land surrounding the 18 acre plant is generally less than 555 feet. The grounds of the plant are covered with grasses and ornamental shrubs. Only a few large trees are found on the plant grounds and many small ones have recently been planted.

With the exception of two small equipment buildings and utility poles, there are no structures taller than ten feet within 400 feet of the aeration basin (fig. 1). The return sludge lift station and operator's office is located 40 feet north of the eastern end of the basin. Meteorological instruments were placed on the roof and inside this building during this study. The blower building, where equipment was stored between sampling periods, is 40 feet north of the western end of the basin. These buildings are 11 feet high and 102 feet apart. The space between the two structures consists of a small, paved parking area and grass lawn. Other large structures at the plant are the raw sewage pump station, about 400 feet north of the basin, and the sludge digester and drying beds, about 500 feet southwest of the aeration basin. Immediately east and west of this basin are the open settling units or clarifiers.
The 55-foot diameter primary clarifier for raw sewage is centered 80 feet west of the aeration basin (figs. 1,2). Sewage that has been screened and chopped to eliminate large, undissolved solids enters the clarifier at its center. As suspended solids settle to the bottom where they are removed for digestion and drying, the liquid portion passes over a peripheral weir and is piped to the aeration basin. This mixture of liquid sewage and unsetttable particles is agitated by air jets in the large basin. This aeration and agitation with the aid of microorganisms in the sewage increases the size of the particles and converts them to insoluble, non-putrescible solids known as activated sludge (19). The activated sewage then passes a weir and is piped to the center of the final clarifier, which is 65 feet wide and centered 60 feet east of the aeration basin. The activated sludge-sewage mixture is settled; sewage plant effluent passes a peripheral weir, is chlorinated, and discharged into Pecan Creek. The settled activated sludge or return sludge is sent back to the aeration basin or to the digester and drying beds. On the average, 2,800,000 gallons of sewage are processed daily at the Denton sewage treatment plant.
Fig. 2 - Air sampling stations, Denton sewage treatment plant. A, B, sampling stations; 1, paved roads; 2, blower building; 3, return sludge lift station; 4, primary clarifier; 5, aeration basin; 6, final clarifier. (scale: 5 mm. = 10 ft.)
Air passing over this aeration basin was sampled using a Gelman Model 24001 Sequential Sampler by the liquid impingment method. Samplers were placed on portable structural aluminum stands and sampled air at a height of eighty-seven inches above the ground. The sampling stations (fig. 2) were on opposite sides of the basin on a north-south line at the center of the basin, seventy-five and a half feet from either end. The stations were fifteen feet from the north or south side of the aeration unit. Power for the samplers was provided by electrical cables terminating in the blower building.

Air Sampling Procedure

Low velocity liquid impingers were used throughout this study. These "bubblers" were constructed with eight dram vials fitted with two-hole, rubber stoppers and glass tubes (fig. 3). The longest tube served as the air intake during operation. The shorter tube was connected to the sampler vacuum pump and sequential valve with rubber tubing. The orifice of the intake tube was submerged in the collecting medium and adjusted to a distance of fifteen millimeters from the vial bottom. Fifteen bubblers were prepared prior to each sampling run. The outer tube openings were plugged with
cotton and the empty bubblers sterilized in the autoclave at 121 degrees centigrade and 15 pounds pressure for 15 minutes. After sterilization and cooling, fifteen milliliters of the collection medium were added aseptically to each bubbler sampler. Twelve of these were used in each sampling run; one was designated as a sampling medium control; the remaining two were used to replace any bubblers damaged in transit to the sewage plant, if necessary.

The collection medium used was Bristol's Solution (3). Six stock solutions, 1000 milliliters each in volume, were prepared. Each contained one of the following salts in the amount listed:

<table>
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<th>Salt</th>
<th>Amount</th>
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<tr>
<td>Sodium nitrate</td>
<td>25.0 grams</td>
</tr>
<tr>
<td>Calcium chloride</td>
<td>2.5</td>
</tr>
<tr>
<td>Magnesium sulfate</td>
<td>7.5</td>
</tr>
<tr>
<td>Dipotassium phosphate</td>
<td>7.5</td>
</tr>
<tr>
<td>Potassium phosphate</td>
<td>17.5</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>2.5</td>
</tr>
</tbody>
</table>

Ten milliliters of each stock solution were added to 940 milliliters of glass distilled water. One drop of a 1.0 per cent ferric chloride solution was then added to the mixture. The solution was then sterilized by filtration (Millipore, pore size 0.22 microns) into a sterile, double
side-arm, 1500 milliliter flask. The lower are was connected to a sterile syringe set-up that was used to deliver fifteen milliliters of the solution to each bubbler.

Each week a sampling run was made, beginning March 22, 1967. The two sequential samplers were loaded with six bubblers each in the laboratory and taken to the sewage plant. There, the samplers were placed on their stands at the sampling stations (fig. 2). These were connected to their power supply and simultaneously started after the cotton plugs were removed from the exposed intake tubes. Each run consisted of six, two-hour air sampling periods, separated by two-hour wait periods. The total time for each run was twenty-two hours. The air sampling rate was 0.25 cubic feet of air per minute. For each two-hour period, 30 cubic feet of the air was sampled at each station. After completion of the run, the openings of the intake tubes were sealed with autoclave tape. The samplers and bubblers were then returned to the laboratory for analysis.

Microbiological Analysis

The contents of each bubbler were aseptically added to individual 125 milliliter flasks of Soil-water Medium. The soil-water culture medium, modified after Pringsheim (13),
was prepared as follows: One-quarter-inch of sieved loam soil (pH 6.5 to 7.0) was placed in forty-eight clean, 125 milliliter flasks. Flasks were then filled to about two inches from the top with glass-distilled water and plugged with cotton. All flasks were then sterilized by steaming at atmospheric pressure for one hour on three consecutive days. For each group of soil-water culture flasks prepared, three were chosen at random as culture medium controls.

For bacteriological analysis, triplicate one-tenth milliliter samples were taken from the soil-water medium cultures and plated out in triplicate on plates of Bacto-Tryptic Soy agar, Eosin Methylene Blue (EMB) agar, and Brain Heart Infusion agar, using sterile pipets and the spread-plate method. For each set of twenty plates that was poured, three were set aside as controls. The collection medium control bubbler and soil-water medium control flask were also plated out at this time. Tryptic Soy agar plates were incubated at room temperatures, 28 to 30 degrees centigrade, for 24 to 48 hours. EMB and Brain Heart Infusion agar plates were incubated at 35 to 37 degrees centigrade for 24 hours.
Bergey's Manual of Determinative Bacteriology (4) was used to identify bacteria collected. Colony morphology of bacteria appearing after the incubation period was noted. Colonies representing the majority of the bacterial population were isolated by streaking on additional plates. Gram stains (1) and agar slant cultures were prepared of these organisms. Bacteria were isolated and identified using staining techniques, differential and selective media, and microscopic examination. Coliform bacteria were readily isolated by their characteristic colony morphology on EMB agar plates (6). Other differential tests used were motility, carbohydrate fermentation reaction, indole production, the methyl red test, Voges-Proskauer reaction, growth on Simmon's Citrate agar, urea decomposition, litmus milk reaction, gelatin liquifaction, nitrate reduction, production of hydrogen sulfide in Kligler's Iron Agar, and characteristic growth on Bismuth Sulfitite or Salmonella-Shigella agars (1,2, 4,6,8,16,21).

After removing samples for bacteriological determinations, soil-water media cultures were kept in an algal culturing unit (12) at 21 to 30 degrees centigrade for nine months. Throughout this period, the laboratory temperature rose on four occasions due to air conditioning failures, but the
temperature remained at 21 degrees most of the time. The cultures were examined at the end of the first, second, and third week by preparing wet-mount slides from each flask. Then, cultures were routinely examined every five weeks. When possible, unialgal or uniprotean cultures were made in additional flasks of soil-water medium. Cells were sketched and measurements made using the compound microscope and an ocular micrometer. Iodine-potassium iodide stain (21) was used to test for starch production. India ink was used as a negative stain for matrix and other organelles (15). Living protozoa were studied with the aid of several reagents: nickel sulfate, methyl cellulose, butacaine sulfate solution, neutral red, Lugol's solution, and iodine-potassium iodide (9,10,15,21). Six-three-one solution was used occasionally on slides to kill organisms for study (15). Permanent slide preparations following Kudo's techniques were unsuccessful (10). Algae were identified using Smith, Prescott, Fritsch, and Tiffany and Britton (17,12,7,18) Protozoa were identified using Kudo, Jahn, Ward and Whipple, and Ludin and West (10,9,20,11).
Monitoring Metrological Conditions

As an essential part of this research, weather instruments were installed at the sewage treatment plant to measure several parameters. These were mounted on the roof and inside the return sludge lift station. Wind direction was obtained using a Gelman-Gill bivane and recorder. Wind velocity was obtained using an anemometer and recorder. Both instruments were mounted on the roof of the building at a height of fourteen feet above the ground, attached to a vent pipe about three feet above the building. Recorders for these instruments and a recording barograph were installed in the chlorinator room of the station. A Taylor hygrothermograph was installed and was supposed to record the temperature and relative humidity during the air sampling periods, but was not operational or erratic throughout the study. As a result, this information was obtained from the Texas A and M University Agricultural Experiment Station, Substation 6, located about five and one-half miles west-northwest of the sewage treatment plant. The amount of precipitation at the plant was determined using a rain gauge. Reports on the general Denton weather conditions on radio station KDNT were used to supplement personal observations and recorded data.
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CHAPTER III

RESULTS

In Table I, thirteen genera of algae and protozoa and twelve groups of bacteria identified from air sample cultures are listed.

Organisms were enumerated only on the basis of the frequency of samples in which they were found. No attempt has been made to determine the number of cells present in the air during the sampling period because the collecting and culturing methods employed did not permit quantitative estimates. The collection medium was not examined immediately after sampling but was added to soil-water medium to culture algae and protozoa. Descriptions of the viable algae, protozoa, and bacteria are based on this second medium and represent the majority populations present. Because of the large number of samples and the difficult taxonomic problems of certain groups, only generic classifications have been attempted in the case of most of these organisms. Genus-like names were given to those organisms closely resembling a certain organism but differing in some aspects. Organisms
<table>
<thead>
<tr>
<th>Organism</th>
<th>Number of Samples*</th>
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<td><strong>Algae and Protozoa</strong></td>
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<tr>
<td>Chlamydomonas sp.</td>
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<tr>
<td>Chlorella ellipsoidea</td>
<td>4</td>
</tr>
<tr>
<td>Chlorella sp.</td>
<td>5</td>
</tr>
<tr>
<td>Chlorococcum-like sp.</td>
<td>4</td>
</tr>
<tr>
<td>Chromulina sp.</td>
<td>2</td>
</tr>
<tr>
<td>Cryptoglena sp.</td>
<td>1</td>
</tr>
<tr>
<td>Cryptomonas sp.</td>
<td>3</td>
</tr>
<tr>
<td>Euglena sp.</td>
<td>3</td>
</tr>
<tr>
<td>Microcystis sp.</td>
<td>1</td>
</tr>
<tr>
<td>Peranema sp.</td>
<td>2</td>
</tr>
<tr>
<td>Pleuromonas sp.</td>
<td>1</td>
</tr>
<tr>
<td>Uronema-like sp.</td>
<td>3</td>
</tr>
<tr>
<td>Westella botryoides</td>
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</tr>
<tr>
<td>unidentified hypotrich.</td>
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</tr>
<tr>
<td>unidentified protozoan cyst</td>
<td>4</td>
</tr>
<tr>
<td><strong>Bacteria</strong></td>
<td></td>
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<tr>
<td>Gram negative bacilli</td>
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<tr>
<td>Achromobacter-Alcaligenes spp.</td>
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<td>Aerobacter sp.</td>
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<td>Escherichia sp.</td>
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<td>Flavobacterium sp.</td>
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<td>Serratia sp.</td>
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<td>uncertain.</td>
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<td></td>
<td>121</td>
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<tr>
<td><strong>Gram positive cocci</strong></td>
<td>146</td>
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*Total number of samples = 192.
were studied in mixed and unialgal or pure culture. Several keys and reference works were consulted for the identification of the algae (5,8,10,12,13,14), protozoa (7,8,9,10,12,14), and bacteria (1,2,3,4,6,11).

Chlorococcum-like cells (Table I) were the most frequently encountered alga in this survey. These cells were in large masses of hundreds of cells that easily covered the soil surface in the soil-water medium flasks. Strict identification could not be made because colony cell numbers were usually higher than that described. Cell wall and chloroplast characteristics and the formation of biflagellated zoospores identified this as a species of Chlorococcum or similar organism (5,10). The flagellates reported were easily identified using several keys, but the ciliates encountered presented some difficulty. The Uronema-like organism appeared to be rather uniformly ciliated, lacked a caudal filament or cilium, and had a rather indistinct oral ciliature (8,14). The hypotrich found was characterized by an unusually small size (25 microns long), typical flattened body form, and sparse, irregular, ventral cirri. In general, this ciliate closely resembled both Balladyna and Psilotricha species (8,14). A number of unidentifiable protozoan cysts were found in four collections, usually
occurring in the same collection with the Uronema-like ciliates. These cysts were spherical or ovoid and were about eight microns in diameter.

Great numbers of bacterial colonies developed from media that was plated out. Studying plates of EMB agar allowed for quick identification and isolation of coliform bacteria. Species of *Escherichia* obtained included both citrate positive (*Citrobacter*, 2) and citrate negative forms. *Klebsiella* was identified and distinguished from *Aerobacter* by capsule formation and lack of motility. Urease and methyl red positive organisms were classified as *Proteus* species. One collection on May 18, 1967 contained several paratyphoid *Salmonella* organisms that were determined by growth on bismuth sulfite agar plates and by slide agglutination with Group B antiserum. *Serratia*, *Pseudomonas*, *Flavobacterium*, *Achromobacter*, and *Alcaligenes* organisms were identified by colonial characteristics and reactions in various media. Organisms grouped as uncertain included a number of *Paracolobactrum*-like organisms with slow and variable biochemical reactions. Gram positive organisms were of the aerobic, spore-forming *Bacillus* type and a great variety of cocci, including *Sarcina*, *Staphylococcus*, *Micrococcus*, and others. Colonies representing less than five per cent of those observed on similar plates
for each sample were not identified. Molds and yeasts, as well as an occasional actinomycete, were also cultured from the air samples; these were disregarded in this survey.

The frequency with which the algae, protozoa, and bacteria were found in the air with respect to wind direction and time of day is presented in Table II.

A total of 192 samples were taken during the four month survey. Of the samples positive for algae and protozoa, eight were taken on the upwind side of the aeration basin. Seven samples taken at night contained these organisms, whereas five were positive during the day collections. Only twelve of the 192 samples contained algae or protozoa. More genera of algae and protozoa were identified from the upwind-day collections (Table II). Only one sample was positive on the downwind side of the basin during the day. This collection was made on July 13 and contained only the Chlorococcum-like alga. On only one occasion, June 22, algae and protozoa were found in both the upwind and downwind air samples.

Bacteria were found in all of the 192 samples taken in this survey. More genera were found in the downwind samples than in upwind samples (Table II). Genera found most often during the day were Flavobacterium and Escherichia. At night,
<table>
<thead>
<tr>
<th>Organism</th>
<th>Upwind Samples</th>
<th>Downwind Samples</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day</td>
<td>Night</td>
</tr>
<tr>
<td>Chlamydomonas sp.</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Chlorella ellipsoidea</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Chlorella sp.</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Chlorococcum-like sp.</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Chromulina sp.</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Cryptoglena sp.</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Cryptomonas sp.</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Euglena sp.</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Microcystis sp.</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Peranema sp.</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Pleuromonas sp.</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Uronema-like sp.</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Westella botryoides</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>unidentified hypotrich</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>unidentified protozoan cyst</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Achromobacter-Alcaligenes spp.</td>
<td>5</td>
<td>14</td>
</tr>
<tr>
<td>Aerobacter sp.</td>
<td>16</td>
<td>31</td>
</tr>
<tr>
<td>Escherichia sp.</td>
<td>24</td>
<td>29</td>
</tr>
<tr>
<td>Flavobacterium sp.</td>
<td>37</td>
<td>33</td>
</tr>
<tr>
<td>Klebsiella sp.</td>
<td>12</td>
<td>15</td>
</tr>
<tr>
<td>Proteus sp.</td>
<td>1</td>
<td>7</td>
</tr>
<tr>
<td>Pseudomonas sp.</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Salmonella sp.</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Serratia sp.</td>
<td>21</td>
<td>58</td>
</tr>
<tr>
<td>uncertain Gram negative</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Gram positive bacilli</td>
<td>18</td>
<td>28</td>
</tr>
<tr>
<td>Gram positive cocci</td>
<td>19</td>
<td>28</td>
</tr>
</tbody>
</table>

*Total number of samples = 192.*
Serratia, Flavobacterium, Escherichia, and the Achromobacter-Alcaligenes group were found most often. Serratia was the only organism found more often in upwind samples than in downwind ones. The groups found most often in downwind collections were the Escherichia, Flavobacterium, and Gram positive cocci.

A summary of prevailing meteorological conditions during the air sampling runs is given in Table III.

Three samples contained algae and protozoa on March 30 during the morning hours. These organisms were found in five collections during the June 22 and 23 sampling period. On July 13 and 14, the first three sequential samples contained algae and protozoa. Only one collection on July 21 contained these microorganisms. No samples were taken on April 28 and August 5 as a result of equipment failure. Controls for the cultures taken May 10 and 11 were found to be badly contaminated with Microcystis and bacteria; the results of samples taken on this date were discarded. For the majority of samples the wind was out of the south at a velocity between 10 and 20 miles per hour. Thus, most of the downwind samples were those taken on the north side of the aeration unit. The skies were partly cloudy throughout the survey and traces of rain were recorded at the sewage treatment plant on only
### TABLE III

**METEROLOGICAL CONDITIONS DURING AIR SAMPLING**

<table>
<thead>
<tr>
<th>Sample Run&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Dates 1967</th>
<th>Time&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Temperature&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Relative Humidity&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Wind Direction and Velocity</th>
<th>Sky&lt;sup&gt;d&lt;/sup&gt;</th>
<th>Precipitation</th>
</tr>
</thead>
<tbody>
<tr>
<td>S1</td>
<td>Mr 22-23</td>
<td>5:30pm</td>
<td>55-80 (65)&lt;sup&gt;°&lt;/sup&gt;F.</td>
<td>20-70 (55)%</td>
<td>SSE 15-20 mph</td>
<td>PC</td>
<td>..</td>
</tr>
<tr>
<td>S2 X</td>
<td>Mr 29-30</td>
<td>7:00</td>
<td>59-86 (72)</td>
<td>32-79 (48)</td>
<td>SSE 20-25</td>
<td>C</td>
<td>..</td>
</tr>
<tr>
<td>S3</td>
<td>Ap 6-7</td>
<td>8:00</td>
<td>65-89 (75)</td>
<td>35-79 (48)</td>
<td>SSW 15-20</td>
<td>PC</td>
<td>..</td>
</tr>
<tr>
<td>S4</td>
<td>Ap 13-14</td>
<td>8:00</td>
<td>55-84 (68)</td>
<td>24-78 (50)</td>
<td>S</td>
<td>10-15</td>
<td>C</td>
</tr>
<tr>
<td>S5</td>
<td>Ap 20-21</td>
<td>7:30</td>
<td>69-80 (72)</td>
<td>40-90 (76)</td>
<td>SSE 15-20</td>
<td>Cl</td>
<td>0.25in.</td>
</tr>
<tr>
<td>S6 D</td>
<td>Ap 28-29</td>
<td>7:30</td>
<td>60-75 (65)</td>
<td>74-85 (78)</td>
<td>SSE 15-20</td>
<td>PC</td>
<td>..</td>
</tr>
<tr>
<td>S7</td>
<td>My 2-3</td>
<td>7:30</td>
<td>55-65 (61)</td>
<td>47-58 (52)</td>
<td>SSW 10-15</td>
<td>PC</td>
<td>..</td>
</tr>
<tr>
<td>S8 D</td>
<td>My 10-11</td>
<td>8:00</td>
<td>75-98 (82)</td>
<td>39-58 (52)</td>
<td>SSW 10-15</td>
<td>C</td>
<td>..</td>
</tr>
<tr>
<td>S9</td>
<td>My 17-18</td>
<td>6:00</td>
<td>65-91 (75)</td>
<td>19-59 (46)</td>
<td>SSW 10-15</td>
<td>C</td>
<td>..</td>
</tr>
<tr>
<td>S10</td>
<td>My 24-25</td>
<td>6:00</td>
<td>65-85 (78)</td>
<td>30-65 (37)</td>
<td>SE 15-20</td>
<td>PC</td>
<td>..</td>
</tr>
<tr>
<td>S11</td>
<td>Jn 7-8</td>
<td>3:30</td>
<td>72-87 (79)</td>
<td>45-82 (60)</td>
<td>PC</td>
<td>..</td>
<td></td>
</tr>
<tr>
<td>S12</td>
<td>Jn 14-15</td>
<td>3:30</td>
<td>70-91 (82)</td>
<td>42-79 (57)</td>
<td>SSE 10-15</td>
<td>PC</td>
<td>..</td>
</tr>
<tr>
<td>S13 X</td>
<td>Jn 22-23</td>
<td>3:00</td>
<td>80-94 (84)</td>
<td>47-67 (60)</td>
<td>SSW 5-10</td>
<td>PC</td>
<td>0.16</td>
</tr>
<tr>
<td>S14</td>
<td>Jn 29-30</td>
<td>3:30</td>
<td>78-96 (90)</td>
<td>36-74 (57)</td>
<td>WSW 5-10</td>
<td>PC</td>
<td>1.14</td>
</tr>
<tr>
<td>S15</td>
<td>Jl 6-7</td>
<td>4:00</td>
<td>74-88 (79)</td>
<td>62-83 (75)</td>
<td>NE 5-10</td>
<td>PC</td>
<td>..</td>
</tr>
<tr>
<td>S16 X</td>
<td>Jl 13-14</td>
<td>4:00</td>
<td>69-91 (79)</td>
<td>38-65 (52)</td>
<td>NE 5-10</td>
<td>PC</td>
<td>0.16</td>
</tr>
<tr>
<td>S17 X</td>
<td>Jl 21-22</td>
<td>4:30</td>
<td>74-90 (83)</td>
<td>50-83 (64)</td>
<td>SSE 5-10</td>
<td>PC</td>
<td>..</td>
</tr>
<tr>
<td>S18</td>
<td>Jl 28-29</td>
<td>4:30</td>
<td>81-89 (90)</td>
<td>32-62 (45)</td>
<td>SSE 5-10</td>
<td>PC</td>
<td>..</td>
</tr>
<tr>
<td>S19 D</td>
<td>Ag 5-6</td>
<td>4:30</td>
<td>79-98 (89)</td>
<td>24-63 (41)</td>
<td>SSE 5-10</td>
<td>PC</td>
<td>..</td>
</tr>
</tbody>
</table>

<sup>a</sup>X, Samples positive for algae or protozoa; D, all samples discarded.

<sup>b</sup>Starting time for sampling run.

<sup>c</sup>Minimum, maximum, and average for entire sampling run.

<sup>d</sup>Sky conditions during sampling run: C, clear; PC, partly cloudy; Cl, cloudy.

*Data obtained from Tex. A & M University Agricultural Experiment Station.*
four occasions during the weekly sampling runs. In between sampling runs, traces of rain that could not be recorded occurred in the last part of May, early June, and the last part of July. The weather was quite variable during July. The prevailing winds were from the north and unusually low temperatures were recorded throughout the North Central Texas area in the second week of that month.
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CHAPTER IV

DISCUSSION

It is well documented in the literature that algae, protozoa, and bacteria are found in the air. Gregory (7) has discussed the physical environmental phenomena of air movements that cause dust and microscopic organisms to become airborne. The atmosphere is layered, that is, at different elevations there exist differences in the air temperature, density, viscosity, and pressure. These differences cause the motion of air in our environment. One common mechanism for the addition of dust particles to the air is the action of winds and small eddy currents in the air flow as it passes over and around objects. These currents lift dust and the possible microorganisms contained therein into the atmosphere. Other mechanisms are simple splashing of water or the bursting of bubbles at a water surface, as reported by Mason (15) and Woodcock (29). Tiny droplets form at the water surface because of the cohesive properties of the water molecules. These droplets and any objects that might be contained in them are carried into the air. Gregory (7) suggests that even drying
and crumbling of the thallus of an alga or the soredia of a lichen may contribute to the algal flora of the air. Airborne microbes have been found in practically all parts of the atmosphere. Bacteria have been collected from the air only inches over the surface of sewage aeration basins (11,22). Algae and protozoa have been found in the air over a football stadium (24) and along highways (3). Airborne microbes have also been cultured from airplane collections at thousands of feet in the atmosphere (18,20). However, most of the many workers in aerobiology have neglected the algae and protozoa in their search for airborne microorganisms. Although these organisms are also found in sewage (1,4,12), none of the recent studies of airborne organisms at sewage plants (11,19, 22) have reported these organisms in the air. A crude preliminary survey has been made (14) in which two genera of algae were found in the air near a sewage treatment trickling filter.

Methods of survival of these airborne microbes have been extensively studied. The adverse effects of drying, radiation, and temperature are quite different among the various organisms because of survival mechanisms like cysts, spores, and gelatinous sheaths around cells. Loss of water from proteins and increased survival time with lower or higher humidity is reported as one
factor to be considered by Wells (26). Gislen (6) maintains that bacteria survive less often when high temperature and high humidity are combined. Ultra-violet radiations are most detrimental to airborne organisms on clear days when no natural atmospheric shield exists (6). According to Gislen (6), many bacteria and protozoa are capable of withstanding ultra-violet longer on cloudy days. In the survey presented in this research, most of the algae and protozoa were found on cloudy days or at night when the humidity was high and the temperatures were moderate.

The case for disease transmission through the atmosphere has been well documented (26). Algae have become important as possible causitive agents in various respiratory ailments, as shown by several persons (8,9,16,23,29) and reported by Mackenthum (13). It has not been conclusively shown that some of the reported symptoms are not produced by the synergistic action of the algae and bacteria. Many blue-green algae that have been found in the air (14,24) have a matrix which may contribute to the dispersal of bacteria embedded in this matrix. Perhaps the algae and protozoa in sewage can become airborne like the bacteria (11,19,22) and constitute a health hazard themselves. Wells reports that organisms of less than five microns in size are responsible for most of the
respiratory infections (26). Many of the non-bacteria organisms isolated in the air near the sewage aeration basin are in this size range.

Several weaknesses are evident in this survey of the airborne organisms at the sewage treatment plant. First, quantitative data as to the number of cells in the air has not been determined. One reason for this is that the liquid impingement method of air sampling breaks up clusters of cells and dust (28). Other sampling techniques might have been used (24, 28) to give a quantitative estimate of the airborne microorganisms. No single sampling device has been shown to be of equal quality in collecting and culturing different types of cells. In qualitative surveys, the low velocity bubbler impinger has been reported to give satisfactory results (24,28). Also, the original samples were not plated out for cell counts but were immediately subcultured for the determination of viable algae and protozoa.

Next, the location of the samplers and sampling stations should be considered, since this would effect the numbers and kinds of organisms collected. Survival distances of airborne organisms vary greatly within short distances from the source of emission (11,19,22) generally depending on the wind velocity. Eddy currents in the air created by the
aeration basin would affect the dispersal direction and distance traveled by these living aerosols. More quantitative background and experimental counts might be obtained by using a number of samplers and different locations for sampling the air. Another factor is the length of the sampling period and length of time that the samples remain at the sampling station before analysis. In this study, the first sample taken remained at the sewage plant until the twenty-two hour run was completed. During this time, changes in sample content could certainly occur. Temperature fluctuations on the inside of the sequential sampler case might have been detrimental to some organisms collected. The sampler intake tubes remained open to the atmosphere throughout the sampling run, perhaps permitting additional organisms to enter the samplers. Not only could these parameters affect the number of viable cells but they could also change the kinds of organisms found on analysis.

The choice of the collection and culture media determine which organisms are reported as viable (28). Bristol's solution and the soil-water medium were used in this study because they were convenient and have been successfully used by previous investigators (2,24) for the cultivation of the algae and protozoa. Perhaps some organisms actually removed
from the air were not viable in these media. In this study, control media were also cultured as a check for sterility. This precaution proved that on one occasion algal and bacterial contamination had occurred.

Some other sources of error may have occurred at the sewage plant. For example, no record of frothing or foaming due to detergents in the sewage—or control of this—was kept at the plant. To control this action, water is sprayed on the surface of the aeration tank (21,25). This fine spray could cause additional turbulence that could affect in turn the air sampled and its content. Sewage treatment plant personnel working in the vicinity of the sampling stations could have some effect on the result of the aerial sampling. Disturbances such as mowing, as suggested by Brown (3), would greatly alter the natural air flora and fauna. At the Denton sewage plant, little activity occurred near the air samplers during the sampling periods. This type of variable was not recorded, but certainly not overlooked, in this survey.

Many of the algae, protozoa, and bacteria cultured from the air samples in this survey have also been found in the air by previous investigators (3,22,24). At least one isolation of Salmonella was made in this study; recent surveys by others have not reported this organism. The non-enteric bacteria
found in this survey appear to be rather common in studies of this type. In his 1962 studies, Schlichting (24) reported twenty-three genera of algae and protozoa in his air samples obtained during a similar period (March 22 to August 6). He was working at a different air sampling height and location and did not find many of the organisms cultured from the sewage plant air samples, such as *Westella*, *Chlamydomonas*, *Peranema*, *Cryptoglena*, *Chromulina*, *Cryptomonas*, *Pleuromonas*, *Uronema*-like species, and hypotrich-like organisms. Perhaps this difference was a result of sampling air near the sewage aeration unit, as well as the other differences in the sampling techniques and procedures.

At the Denton sewage plant, samples of the activated sludge-sewage mixture from the surface at various places in the aeration basin contain large numbers of the organisms collected in the air near the basin, as well as many not found in the samples. The sewage in the basin contains about twice as many flagellates as it does ciliates or amoeba. No coccoid algae have been observed in these sewage samples. Five-minute air exposure agar plate collections have been made on the service walk in the center of the basin. On a cool, humid night after a few days of rain, high concentrations of coliform and other bacteria were collected in this manner.
A wide variety of different media was used, including EMB agar, Brain Heart Infusion agar, Tryptic Soy agar, bismuth sulfite agar, modified synthetic sewage agar, and sterilized sewage agar. These cultures and those made by direct plating of sewage produced bacteria that were also isolated from the air collections performed in this survey. This means that the sewage aeration basin could have been the source of microorganisms found in the air samples.

In this survey, no associations among airborne organisms could be demonstrated. Bacteria were found more often than the algae or protozoa. As more genera were collected at night, it would seem that daylight, humidity, and temperature had affected the viability and dispersion of these organisms. Fairly consistent weather conditions throughout this survey cannot be reflected in the air samples. Under similar conditions at similar times, dissimilar populations were found. Rainfall is usually thought to greatly change the number of bacteria in the air (7) for a period after the rain. At the Denton plant some samples were positive for algae and protozoa and bacteria after short showers. Perhaps the sampling stations were close enough to the aeration basin that collection of microorganisms emitted by the aeration could be affected after the rain had stopped. Downwind samples containing
algae and protozoa were found when the wind was from the north. Perhaps the changing wind direction and eddy currents caused by the sewage treatment plant buildings caused an increase in the amount of dispersion of these organisms.

Although not quantitative, this survey has shown that airborne algae and protozoa can be found in the air at this type of sewage plant under these sampling conditions. It does appear that the aeration unit can emit these organisms to the air as they can be found in the liquid at the surface of the aeration basin. More extensive quantitative studies of this phenomenon should be made to obtain a better idea of the role of sewage treatment in the dispersion of these organisms.


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CHAPTER V

SUMMARY AND CONCLUSIONS

1. This survey has shown that airborne algae and protozoa are found in the air at the Denton sewage treatment plant and that these may be emitted by the activated sludge aeration basin.

2. Thirteen genera of algae and protozoa and twelve groups of bacteria were identified from cultures of the air samples taken at the sewage treatment plant.

3. Algae and protozoa were found in twelve of the 192 air samples taken over the four month survey; bacteria were found in all samples.

4. Algae and protozoa were collected more often in air samples taken at night and on the upwind side of the aeration basin.

5. A greater variety of bacteria was collected in air samples taken at night and on the downwind side of the aeration basin.

6. There was no apparent relationship found between the airborne bacteria and the algae and protozoa at this location.
7. The algae, protozoa, and bacteria cultured from the air samples were also found in surface samples taken from the aeration basin and on plates of sterile media exposed for five minutes on the service walk in the center of the basin, twelve inches above the bubbling mixture of sewage and activated sludge.

8. Many studies have shown that bacteria are emitted to the air at sewage treatment plants; this survey has shown that algae and protozoa may also be found in this habitat.

9. In addition to the other microorganisms, the algae and protozoa have been shown to be of medical importance as a cause of some respiratory irritations and other ailments; it is suggested by this survey that more extensive and quantitative studies need to be made in areas of suspected high airborne concentrations of these organisms.

10. More thorough studies should be made of the role of sewage treatment in the dispersal of algae and protozoa.
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