AN ECOLOGICAL STUDY OF THE VIABLE AIRBORNE ALGAE OF THE TUCSON AND THE SANTA CATALINA MOUNTAIN AREAS

by

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ABSTRACT

An investigation was conducted to determine the algal air spora of the Santa Catalina Mountains and the Tucson area.

Sites for studies of algal air spora were selected at various elevations along the Mt. Lemmon Highway near Tucson. The first technique involved quantitative sampling of air. Weekly sampling for airborne algae was undertaken by drawing air through a membrane-type filter. During the 8-month sampling period, a total of 12, 720 liters of air were drawn through the membrane-type filters of a 0.5μ porosity at a sampling rate of 2.1 liters/min. The membrane filters so exposed were placed on Modified Bristol's Medium supplemented with soil-water and the algal disseminules were cultured under a light intensity of 4, 305 lux at a temperature of 22$\pm$2 C.

In order to obtain spores of airborne algae using a qualitative sampling technique, hand-held petri dishes containing nutrient agar were exposed to the air for 10 to 12 min from an automobile traveling approximately 30-35 mph.

Of the two sampling methods employed, the qualitative sampling technique yielded by far the greater quantity and diversity of algae. Examination of the cultures revealed a predominance of unicellular chlorophytan and cyanophytan genera.

INTRODUCTION

In the U. S. government publication Sampling Microbiological Aerosols (1959), biological contaminants that occur in the air as aerosols were defined as solid or liquid particles suspended in the air. Particles in these biological aerosols usually vary in diameter from less than 1μ to approximately 50μ or larger. These particles may consist of a single unattached organism or may occur in the form of clumps or filaments of cells. Further, the organisms may adhere to a dust particle or may exist as a free floating particle surrounded by a film of dried organic or inorganic material.

Algae occur in and have been recovered from the atmosphere. In general, however, information in the literature is suitable only for numerical determinations of air contaminants. Because accurate taxonomic determination of algae requires intensive study of the organisms in unialgal culture, extensive work has not been done with respect to determining the heterogeneity of airborne algal genera (Deason and Bold, 1960).

Knowledge of the air spora has depended on developing suitable techniques for air sampling. All convenient methods depend on apparatus to collect the spores onto a surface where they can be examined directly under the microscope or after growth in culture

(Gregory, 1961). In order to obtain information about the air spora using a quantitative sampling technique, it is necessary to use apparatus that removes spores efficiently from a measured volume of air. Such apparatus requires a means of drawing a measured volume of air through a filter. The qualitative analysis of air spora generally involves impaction upon some sticky surface exposed to the atmosphere. Impaction rate depends upon the size, shape and specific gravity of the airborne particles (Gregory and Stedman, 1953). Results from this method are difficult to interpret quantitatively because the impactions of spores differ markedly with time and place.

Success in identification necessitates growth of algal cultures having morphologically normal cells. This can only be accomplished by working with the cultures and observing them under different growth conditions over a period of time (Starr, 1955).

The objective of the present research was to determine the algal air spora in the Santa Catalina Mountains and the Tucson area. Climatological information such as wind velocity, temperature and relative humidity was collected to determine if a correlation existed between environmental conditions and the number of algal impactions.

REVIEW OF LITERATURE

Gregory (1961) credits Pasteur with pioneering the studies on the distribution of microorganisms in the air. In attempting to resolve the controversy on spontaneous generation, Pasteur was the first to demonstrate visually the existence of an air spora by means of filtering air through gun cotton.

Gregory (1961) writes further that when the H. M. S. Beagle was near the Cape Verde Islands, Darwin found the atmosphere hazy with dust from North Africa. In samples of this dust, Ehrenberg (in 1872) found large quantities of protozoan and plant spores and gradually he became convinced that viable microorganisms could survive transport through the atmosphere.

Cunningham (1874), an English physician in Calcutta, wrote, "Spores and other vegetative cells are constantly present in atmospheric dust, and usually occur in considerable numbers: the majority of them are living and capable of growth and development; the amount of them present in the air appears to be independent of conditions of velocity and direction of wind; and their numbers are not diminished by moisture."

Van Overeem (1936) was one of the early investigators to recover successfully and culture the algal disseminules. Gregory

(1961) reported that Van Overeem, using an air pump, sampled the air from the roofs at Leiden. At least 40 species of algae were obtained from a total of 20 m^3 of air sampled including: Chlorococcum, Chlorella, Pleurococcus, Stichococcus, and Navicula.

Methods of spore liberation and adaptations facilitating take-off into the air are unknown in the algae even though algal cells get into the air regularly. According to Gregory (1961), Pettersson suggested that Chlamydomonas nivilis is carried away from its habitat on snow fields and glaciers in melt water and becomes airborne by splash in mountain torrents. Gregory maintains that lichen soredia may be a possible aid for the dispersal of some algae.

Evidence for a varied species distribution, though indirect, was presented by Lackey (1939) in studying the flora and fauna of water-filled tree holes. Lackey found 140 different species of algae inhabiting such holes. Gislen (1948) had indicated that distribution of species may be due to an organism's ability to form spores or to encyst. According to Gislen, microorganisms are constantly being driven up into the air to return again to earth in rain showers or downward air currents. Some spores of organisms, however, are sensitive to atmospheric radiation and thereby are limited in their distribution.

Gregory (1961) maintains that the process of wind dispersal of spores has three principal stages: (1) spore liberation by which pollen grains or spores "take off" into the air from the structure

where they have been formed; (2) dispersion of spores by means of gentle air currents or strong winds and (3) depositions whereby spores leave the air and land on a surface. Gregory believes that most of the air spora come from ground sources on the surfaces of plant and vegetable debris rather than from the soil itself. He maintains that soil and surface dust raised by wind and splash droplets from marine and fresh water is the source of most of the air spora.

According to Proctor (1935), the ability of living organisms to attain altitudes of 20,000 feet or more through the chance action of air currents is particularly significant as it suggests the almost limitless possibilities of travel in a horizontal direction. Tiffany (1938) states soil algae may exist in extremely unfavorable moist environments for long periods of time in so-called resting stages. Thus the survival of such forms despite the many influences which are unfavorable to their existence also is significant in view of the length of time for which they may remain viable.

Schlichting (1961) sampled approximately 3 cubic miles of air by drawing it through modified impingers and membrane-type filters. Sampling was confined to short periods in order to restrict variability in temperature, relative humidity, and other environmental conditions thereby producing more uniform results. The exposed membrane filters and collecting medium were cultured for a 3-month period. Schlichting found that a longer period of culture (3-9 months) under

variable conditions seemed preferable for a more complete analysis of the aerial biota. Of the 153 flasks innoculated by him, 12 contained positive algal isolates. The viable genera found were: Chlorella, Chlorococcum, Navicula, Paranema, and an unclassified zooflagellate. The organisms were found in the atmosphere at heights of 15 and 27 feet in clear and cloudy weather with relative humidities of 28-98%, temperatures of -2.8 to 28.9 C, and wind velocities of 1-15 mph.

The most comprehensive investigation on airborne algae was completed by Brown, Larson and Bold (1964) at the University of Texas. Their data disclosed the following information:

(1) A surprising number of species of soil algae is constantly present in the air and can survive transport.

(2) The origin of airborne algae is primarily from the soil.

(3) Cultivated soil when blown as dusts yields a greater quantity and diversity of algae than do undisturbed soils.

(4) The specific composition of the airborne algal flora is dependent upon proximity to various soil algal populations and upon meteorological conditions as they vary in time and place.

(5) Species of the Chlorophyta and Cyanophyta seem to be most abundant in the air sampled, but members of the Bacillariophyceae and Xanthophyceae also are present.

MATERIALS AND METHODS

Since no previous research in Arizona concerned itself with studying air spora on the basis of elevational limits, six sampling sites of variable elevation were selected.

Although media preparations exist which are primarily suited to a particular algal group (e.g., Cyanophycean Agar) it was believed that if only a single medium preparation was used the variables arising from using different media preparations would be removed. Further, the objective was to select a medium which did not appear to exhibit any selectivity to one or more genera of the many genera collected.

The culturing procedures followed utilized primarily the suggestions of Starr (1960) in regard to temperature and light intensities. Identification was made from agar and liquid media using living material. A variety of references (Bold, 1949; Deason and Bold, 1960; Herndon, 1958; Prescott, 1954; Smith, 1950) were required in the identification procedure.

Medium Preparation. Modified Bristol's Medium was prepared in the following manner: 10 ml of each of the following stock solutions were added to 940 ml of demineralized water. To this a drop of 0.1% $FeCl_3$ solution was added.

Stock Solutions

$NaNO_3$	10.0 g in 400 ml water
$CaCl_2$	1.0 g in 400 ml water
$MgSO_4 \cdot 7H_2O$	3.0 g in 400 ml water
K_2HPO_4	3.0 g in 400 ml water
KH_2PO_4	7.0 g in 400 ml water
NaCl	1.0 g in 400 ml water

In addition, for each 960 ml of Modified Bristol's solution, 40 ml of soil-water supernatant were added. This was obtained by placing in a 1/2 pint milk bottle, 1/4-1/2 inch of garden soil and adding demineralized water until the bottle was 3/4 full. The bottle was then capped and steamed for 1 hr on 2 consecutive days. The supernatant was then used. In order to solidify the above inorganic medium, 15 g of Difco agar were added. The medium was then autoclaved at a temperature of 120 C for 20 min. The pH of the uninoculated liquid culture medium was 6.1.

Sampling Sites and Air Sampling Procedure. The first sampling site was located near 12th and Cherry streets within the city limits of Tucson, Arizona, at an elevation of 2,400 ft. The five other sampling sites were selected along the Mt. Lemmon Highway located on the southern slope of the Santa Catalina Mountains, 30 miles NE of Tucson. The elevations of the sites selected along the highway were: 3,600 ft,

4, 500 ft, 5, 500 ft, 6, 620 ft, 6, 750 ft. Beginning November 17, 1963, samples were taken weekly. After March 8, 1964, semimonthly samples were taken.

Wind velocity was determined by using an anemometer held 6 ft above ground level. Readings were made for 1-3 min depending on the steadiness of the wind velocity prior to the sampling period. Temperature readings and relative humidity were taken with a sling psychrometer. These data were collected at each of the sampling sites and represented the wind and temperature conditions for the duration of the sampling period, at any particular sampling site. In addition, sky condition and the presence of snow or rainfall were recorded.

In order to obtain information on airborne algae of both a qualitative and quantitative nature, two methods of sampling were used. In using the quantitative sampling technique, samples were taken by means of drawing air through a membrane-type filter. The orifice of the sampling apparatus was a 23 gage hypodermic needle with an opening diameter of 230μ. Anisokinetic sampling errors were reduced by facing the orifice toward the wind (Watson, 1954). The sampled air was then passed through a flowmeter set at its maximum sampling rate of 2.1 liters/min. By means of plastic tubing, the air was then passed over a 0.5μ membrane-type filter enclosed within a filter holder. The sampling apparatus was connected by means of plastic tubing to a

vacuum pump operated by an electric generator. The duration of a sampling period at each site was 30 min.

Information using the qualitative sampling technique was obtained by means of exposing hand-held petri dishes, 15 x 100 mm, containing nutrient agar medium, to the air for 10 to 12 min from an automobile traveling approximately 30-35 mph.

Culturing and Identification. The membrane filters exposed during the quantitative sampling were removed from the filter holder in the field and placed on the petri dish containing nutrient agar medium. In the laboratory, 3 ml of sterile water were placed on the agar surface in order to have a more uniform distribution of particles. The petri plates and filter used during the quantitative and qualitative sampling intervals were then placed upon a culturing rack. The light intensity within the culturing unit was approximately 4, 305 lux from 40-watt cool-white fluorescent bulbs. The cultures received 12 hrs of light and 12 hrs of dark each day. The temperature in the algal culturing unit was approximately 22$\pm$2 C. In general, much of the growth appeared within a 2-week culturing period, however, additional growth occurred up to a 3-month period. As algal growth appeared, the material was transferred aseptically onto an agar slant (Modified Bristol's Medium supplemented with soil-water) until it was convenient to identify the material. Because of the occasional large quantity of

heterotrophic contaminants and/or the overlapping of one algal colony with another, it was necessary at times to spray the growth out onto an agar surface in order to obtain unialgal colonies (Pringsheim, 1946). This was accomplished by making a liquid suspension of the growth and drawing the suspension into a micro-pipette. Micro-pipettes were made using a small flame and drawing out the glass pipette from a 1 mm bore at the narrow end to a bore of approximately 0.1 mm. Filtered air passing through a narrow bore (approximately 0.5 mm) pipette over the end of the micro-pipette containing the algal growth produced a fine aerosol. With the aerosol directed onto an agar surface, sufficient separation of the growth produced widely dispersed unialgal colonies.

Identification of the algal isolates was accomplished by observing the growth under a variety of environmental conditions. Growth upon the agar surface gave general morphological characteristics while growth in liquid culture (Modified Bristol's supplemented with soil-water and without any addition of agar) gave the more detailed taxonomic criteria upon which identification was made, including: (1) the type of zoospore, (2) comparative length of flagella, (3) a walled or naked zoospore, (4) the type of chromatophore, and (5) the presence or absence of a pyrenoid in the vegetative cell. Cultures of the blue-green genera found were sent to Dr. Francis Drouet, The Academy of Natural Sciences of Philadelphia, for identification and/or verification.

RESULTS

Algae Found in the Atmosphere. Viable spores of algae were found in the atmosphere of the Tucson area and the Santa Catalina Mountain range. Table 1 lists 16 species of algae found to be airborne during the period of this study. Besides representatives of the Chlorophyta, Chrysophyta and Cyanophyta, an unclassified fern prothallus (Fig. 15) and a great abundance of fungi and bacteria were found. The green algae (Chlorophyta) are exemplified by Chlorella, Chlorococcum and Stichococcus. The cells of these genera are either solitary or gregarious in the form of compact packets or united end to end in short filaments. The genus Stichococcus was found to be of wide morphological variation. These variations, no doubt, are due to the morphological forms an organism might assume with changes in the environment. In some forms of Stichococcus, the filaments may become dissociated into individual cells. In Hantzschia (Chrysophyta), unlike most other pennate diatoms, the girdle and valve sides are not at right angles to one another. The blue-green algae (Cyanophyta) include such forms as Oscillatoria and Scytonema. In Oscillatoria, the straight trichomes have no observable sheaths. Members of this Suborder reproduce by means of hormogonia only and do not form heterocysts or akinetes. In contrast, the filaments of Scytonema are

TABLE 1

Algae recovered from the atmosphere and cultivated in or on Modified Bristol's Medium supplemented with soil-water.

Chlorophyta	Cyanophyta	Chrysophyta
Bracteococcus	Microcoleus	Hantzschia
Chlorella*	Nostoc	
Chlorococcum*	Oscillatoria	
Chlorosarcinopsis	Schizothrix	
Hormidium	Scytonema	
Oocystis		
Planktosphaera		
Stichococcus		

*Indicates those genera most frequently encountered.

enclosed by a firm sheath and are generally colored when old. Photographs showing some characteristics of the algae in culture are shown in Figs. 1-14.

Airborne Algae and Atmospheric Conditions. The atmospheric conditions under which airborne algae were found are shown in Table 2. The interaction of these conditions makes it difficult, however, to delimit the specific conditions under which airborne algae occur. It appears then that the air stratum which is constantly changing with respect to many physical factors is at the same time a vehicle for the dispersion and transmission of many types of microorganisms and dust particles. To what extent they are dispersed, however, and under what specific environmental conditions and altitudes they are transported could not be determined from this study.

Seasonal Fluctuations of Airborne Algae. The seasonal fluctuations in numbers of colonies of the identified genera are listed in Table 3. It is apparent that the greatest numbers and diversity of algae were found during the winter months of December, January and February. Also, some algae were found only at certain times of the year, e.g., Oscillatoria was found only in late January. It would be difficult to make any predictions relative to algal succession in the atmosphere on the basis of the results obtained in this study. In nature, succession may result from inherent differences of specific growth rate as

well as from different responses to environmental factors during active growth.

TABLE 2

Summary of atmospheric conditions under which airborne algae were collected.

Code #	Date	Elevation in ft.	Sky condition*	Air temp. °C.	Relative humidity %	Max. gusts mph	Organisms (genera) collected
3A	11/17/63	2400	Cl	65	31	2	None
3B	11/17/63	3600	Cl	70	36	5	None
3C	11/17/63	4750	Cl	66	32	9	None
3D	11/17/63	5550	Cl	60	48	3	None
3E	11/17/63	6620	Cl	58	46	3	None
3F	11/17/63	6750	Cl	45	64	6	None
4A	11/24/63	2400	Cl	62	59	5	None
4B	11/24/63	3600	Cl	65	39	7	None
4C	11/24/63	4750	Cl	61	31	9	None
4D	11/24/63	5550	Cl	51	56	8	None
4E	11/24/63	6620	Cl	50	64	8	None
4F	11/24/63	6750	Cl	44	71	9	None
5A	12/1/63	2400	Cl	70	40	3	None
5B	12/1/63	3600	Cl	70	36	5	None
5C	12/1/63	4750	Cl	64	47	10	None
5D	12/1/63	5550	Cl	56	44	2	None
5E	12/1/63	6620	Cl	55	54	2	None
5F	12/1/63	6750	Cl	50	61	2	None

*C, cloudy; PC, partly cloudy; Cl, clear.
[a]Collected by means of quantitative sampling technique.
[b]Collected by means of qualitative sampling technique.

TABLE 2--Continued

Code #	Date	Elevation in ft.	Sky condition*	Air temp. °C.	Relative humidity %	Max. gusts mph	Organisms (genera) collected
6A	12/8/63	2400	C	59	42	3	None
6B	12/8/63	3600	C	70	22	8	None
6C	12/8/63	4750	C	65	24	2	None
6D	12/8/63	5550	C	58	27	2	None
6E	12/8/63	6620	C	57	21	2	None
6F	12/8/63	6750	C	58	27	2	None
7A	12/15/63	2400	Cl	58	39	2	None
7B	12/15/63	3600	Cl	65	27	9	None
7C	12/15/63	4750	Cl	62	32	2	None
7D	12/15/63	5550	Cl	55	43	2	None
7E	12/15/63	6620	Cl	54	27	2	None
7F	12/15/63	6750	Cl	56	30	2	None
8A	12/22/63	2400	Cl	61	27	2	None
8B	12/22/63	3600	Cl	58	27	19	Chlorosarcinopsis[b], Planktosphaera[b]
8C	12/22/63	4750	Cl	50	38	23	Chlorella[a], Chlamydomonas[b]
8D	12/22/63	5550	Cl	42	69	13	Chlorococcum[b], Hormidium[b], Chlorella[b], Planktosphaera[b], Spongiochloris[b]
8E	12/22/63	6620	Cl	40	60	15	Chlorococcum[b], Chlorella[b], Oocystis[b], Chlorosarcinopsis[b], Nostoc[b]
8F	12/22/63	6750	Cl	35	54	15	None
9A	12/29/63	2400	Cl	65	31	2	Chlorococcum[b]
9B	12/29/63	3600	Cl	67	26	5	Stichococcus[b]
9C	12/29/63	4750	Cl	62	20	10	Chlorosarcinopsis[b]

TABLE 2--Continued

Code #	Date	Elevation in ft.	Sky condition*	Air temp. °C.	Relative humidity %	Max. gusts mph	Organisms (genera) collected
9D	12/29/63	5550	Cl	56	27	27	Chlorella[b], Chlorococcum[b], Stichococcus[b]
9E	12/29/63	6620	Cl	50	22	20	None
9F	12/29/63	6750	Cl	47	20	33	Chlorella[a]
10A	1/5/64	2400	PC	57	66	2	None
10B	1/5/64	3600	C	54	48	5	Stichococcus[b]
10C	1/5/64	4750	C	50	56	5	Chlorella[b]
10D	1/5/64	5550	C	44	63	3	Chlorella[b], Chlorococcum[b], Oocystis[b], Stichococcus[b]
10E	1/5/64	6620	C	42	55	7	Chlorella[b]
10F	1/5/64	6750	C	37	65	9	None
11A	1/12/64	2400	Cl	53	78	2	None
11B	1/12/64	3600	Cl	50	79	16	None
11C	1/12/64	4750	Cl	44	85	15	None
11D	1/12/64	5550	Cl	37	65	10	Nostoc[b]
11E	1/12/64	6620	Cl	35	72	15	Oocystis[b], Stichococcus[b], Chlorella[a]
11F	1/12/64	6750	Cl	35	72	15	None
12A	1/19/64	2400	Cl	63	72	2	None
12B	1/19/64	3600	Cl	58	46	2	None
12C	1/19/64	4750	Cl	52	46	5	None
12D	1/19/64	5550	Cl	49	57	3	None
12E	1/19/64	6620	Cl	42	54	3	None
12F	1/19/64	6750	Cl	43	69	3	None

TABLE 2--Continued

Code #	Date	Elevation in ft.	Sky condition*	Air temp. °C.	Relative humidity %	Max. gusts mph	Organisms (genera) collected
13A	1/26/64	2400	PC	72	55	3	None
13B	1/26/64	3600	PC	70	21	3	Oscillatoria[b]
13C	1/26/64	4750	PC	66	15	5	Scytonema[b]
13D	1/26/64	5550	PC	58	14	8	Planktosphaera[b]
13E	1/26/64	6620	PC	58	10	8	None
13F	1/26/64	6750	PC	60	17	8	None
14A	2/2/64	2400	Cl	75	21	3	None
14B	2/2/64	3600	Cl	64	18	10	None
14C	2/2/64	4750	Cl	59	20	12	Chlorella[a], Chlorococcum[a]
14D	2/2/64	5550	Cl	50	23	8	Chlamydomonas[b], Chlorella[b], Spongiochloris[b]
14E	2/2/64	6620	Cl	48	31	8	Chlorella[b], Chlorosarcinopsis[b], Planktosphaera[b], Schizothrix[b]
14F	2/2/64	6750	Cl	45	31	3	Schizothrix[b]
15A	2/9/64	2400	PC	70	19	2	Chlorella[a], Chlorococcum[a]
15B	2/9/64	3600	PC	73	18	2	Chlorococcum[b]
15C	2/9/64	4750	PC	65	24	2	None
15D	2/9/64	5550	PC	58	32	2	None
15E	2/9/64	6620	PC	55	37	3	None
15F	2/9/64	6750	PC	54	37	2	None
16A	2/16/64	2400	C	63	37	5	None
16B	2/16/64	3600	C	56	39	12	None
16C	2/16/64	4750	C	50	43	15	None

TABLE 2--Continued

Code #	Date	Elevation in ft.	Sky condition*	Air temp. °C.	Relative humidity %	Max. gusts mph	Organisms (genera) collected
16D	2/16/64	5550	C	48	44	10	Bracteococcus[b], Oocystis[b]
16E	2/16/64	6620	C	38	54	10	Chlorella[b]
16F	2/16/64	6750	C	35	54	15	None
17A	2/23/64	2400	Cl	62	41	3	None
17B	2/23/64	3600	Cl	59	41	3	None
17C	2/23/64	4750	Cl	53	34	7	None
17D	2/23/64	5550	PC	47	48	7	None
17E	2/23/64	6620	C	49	55	4	Chlorella[b]
17F	2/23/64	6750	C	43	53	4	None
18A	3/1/64	2400	PC	51	75	3	None
18B	3/1/64	3600	PC	51	62	5	None
18C	3/1/64	4750	PC	51	50	3	None
18D	3/1/64	5550	PC	46	52	5	None
18E	3/1/64	6620	PC	44	50	5	None
18F	3/1/64	6750	PC	44	50	5	None
19A	3/8/64	2400	C	55	43	5	None
19B	3/8/64	3600	C	35	100	5	None
19C	3/8/64	4750	C	35	100	3	None
19D	3/8/64	5550	C	32	100	3	None
19E	3/8/64	6620	C	35	100	3	None
19F	3/8/64	6750	C	32	100	3	None

TABLE 2--Continued

Code #	Date	Elevation in ft.	Sky condition*	Air temp. °C.	Relative humidity %	Max. gusts mph	Organisms (genera) collected
20A	3/22/64	2400	C	68	42	12	None
20B	3/22/64	3600	C	57	35	18	Chlorosarcinopsis[b], Spongiochloris[b]
20C	3/22/64	4750	C	51	50	10	None
20D	3/22/64	5550	C	40	92	12	Chlorella[b], Chlorosarcinopsis[b]
20E	3/22/64	6620	C	38	83	12	Chlorosarcinopsis[b], Schizothrix[b]
20F	3/22/64	6750	C	38	91	16	None
21A	4/5/64	2400	Cl	68	42	3	None
21B	4/5/64	3600	Cl	63	42	5	None
21C	4/5/64	4750	Cl	58	46	10	None
21D	4/5/64	5550	Cl	51	39	10	None
21E	4/5/64	6620	Cl	45	57	5	None
21F	4/5/64	6750	Cl	44	56	15	None
22A	4/19/64	2400	Cl	78	27	22	None
22B	4/19/64	3600	Cl	72	24	22	Microcoleus[b]
22C	4/19/64	4750	Cl	70	22	16	Schizothrix[b]
22D	4/19/64	5550	Cl	66	14	16	Stichococcus[b]
22E	4/19/64	6620	Cl	56	34	18	Stichococcus[b]
22F	4/19/64	6750	Cl	54	27	16	None
23A	5/3/64	2400	Cl	78	24	3	None
23B	5/3/64	3600	Cl	76	20	7	None
23C	5/3/64	4750	Cl	72	21	7	None

TABLE 2--Continued

Code #	Date	Elevation in ft.	Sky condition*	Air temp. °C.	Relative humidity %	Max. gusts mph	Organisms (genera) collected
23D	5/3/64	5550	Cl	71	20	5	Chlorococcum[b]
23E	5/3/64	6620	Cl	61	27	7	Stichococcus[b]
23F	5/3/64	6750	Cl	67	12	3	None
24A	5/17/64	2400	Cl	90	31	3	None
24B	5/17/64	3600	Cl	85	32	3	None
24C	5/17/64	4750	Cl	83	31	3	None
24D	5/17/64	5550	Cl	81	27	5	None
24E	5/17/64	6620	Cl	79	24	5	None
24F	5/17/64	6750	Cl	79	24	3	None
25A	5/31/64	2400	Cl	88	18	3	None
25B	5/31/64	3600	Cl	88	35	3	None
25C	5/31/64	4750	Cl	83	15	3	None
25D	5/31/64	5550	Cl	80	15	3	None
25E	5/31/64	6620	Cl	75	24	3	Hantzschia[b]
25F	5/31/64	6750	Cl	75	24	3	None
26A	6/14/64	2400	Cl	96	32	3	None
26B	6/14/64	3600	Cl	88	35	3	Schizothrix[b]
26C	6/14/64	4750	Cl	85	32	5	None
26D	6/14/64	5550	Cl	80	29	3	None
26E	6/14/64	6620	Cl	78	30	5	None
26F	6/14/64	6750	Cl	77	29	3	None

TABLE 2--Continued

Code #	Date	Elevation in ft.	Sky condition*	Air temp. °C.	Relative humidity %	Max. gusts mph	Organisms (genera) collected
27A	6/28/64	2400	Cl	96	47	5	None
27B	6/28/64	3600	Cl	93	51	5	None
27C	6/28/64	4750	Cl	89	33	3	None
27D	6/28/64	5550	Cl	86	32	3	None
27E	6/28/64	6620	Cl	86	32	3	None
27F	6/28/64	6750	Cl	83	16	3	None
28A	7/12/64	2400	Cl	96	32	3	None
28B	7/12/64	3600	Cl	90	36	3	None
28C	7/12/64	4750	Cl	87	37	3	None
28D	7/12/64	5550	Cl	80	47	3	None
28E	7/12/64	6620	Cl	79	46	3	None
28F	7/12/64	6750	Cl	78	46	3	None

TABLE 3

Seasonal variations in total numbers of colonies collected at all 6 stations in the Tucson area and Santa Catalina Mountains.

Genus	Dec. 22	29	Jan. 5	12	19	26	Feb. 2	9	16	23	Mar. 1	8	22	Apr. 5	19	May 3	17	31	June 14
Bracteococcus[a]									1*										
Chlamydomonas[a]	2						1												
Chlorella[a]	6	7	7	1			7	2		3									
Chlorococcum[a]	9	4					1	2								2			
Chlorosarcinopsis[a]	4	2					2												
Hantzschia[c]																	1		
Hormidium[b]	3																		
Microcoleus[b]														3					
Nostoc[b]	1			1															
Oocystis[a]	1		1	2					1										
Oscillatoria[b]						1													
Planktosphaera[a]	3					1	1												
Schizothrix[b]						1								1					
Scytonema[b]						1													
Spongiochloris[a]	1				1														
Stichococcus[a, d]	2		3	1										3	1				

Note: From December 22 to March 8, collections were made weekly. After March 8, collections were made semimonthly.

*Refers to number of colonies

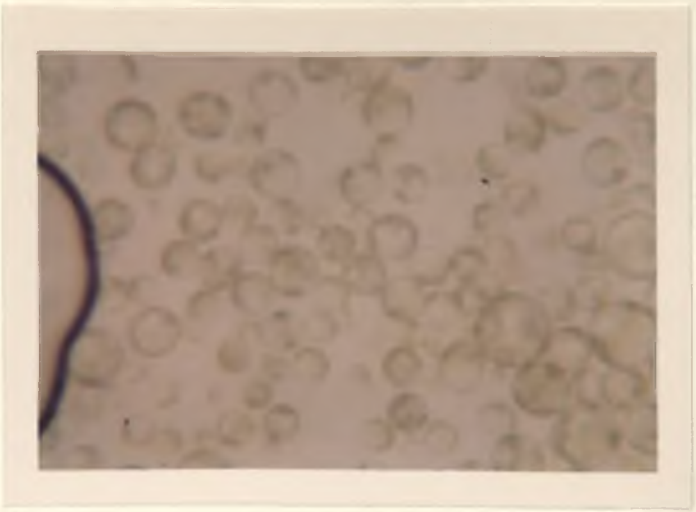
[a]Identified by E. T. Luty. [b]Identified by F. Drouet. [c]Identified by C. W. Reimer.

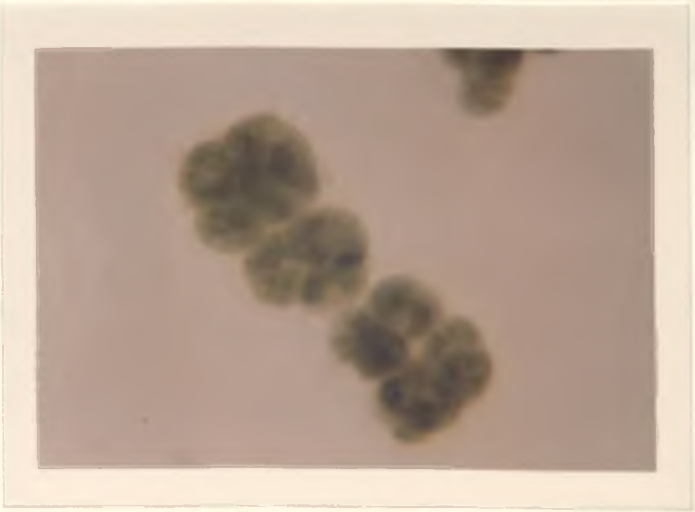
[d]Verified by F. Drouet.

Fig. 1. Chlorella Beijerinck, 1890. Approximately X 420.
Date Collected: February 2, 1964
Place Collected: Mt. Lemmon Highway, Windy Point,
Elevation 6, 620 ft.

Fig. 2. Chlorococcum Fries, 1820. Approximately X 420.
Date Collected: May 3, 1964
Place Collected: Mt. Lemmon Highway, Elevation 5, 550 ft.

Fig. 3. Stichococcus subtilis (Kuetz.) Klerek. Approximately X 420.
Date Collected: May 3, 1964
Place Collected: Mt. Lemmon Highway, Windy Point,
Elevation 6, 620 ft.





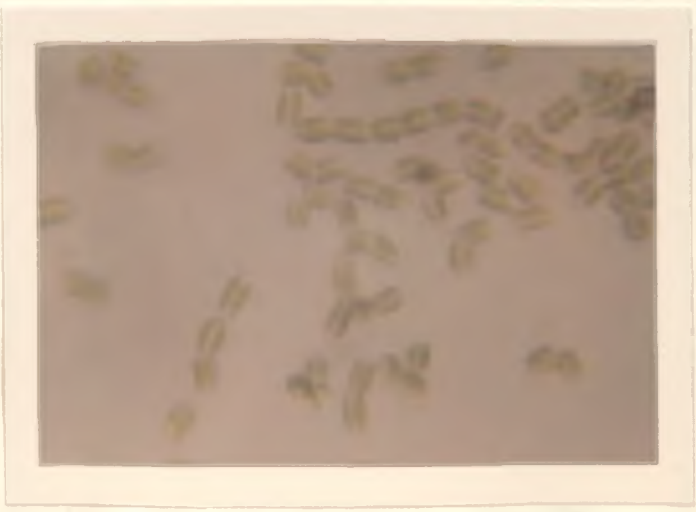
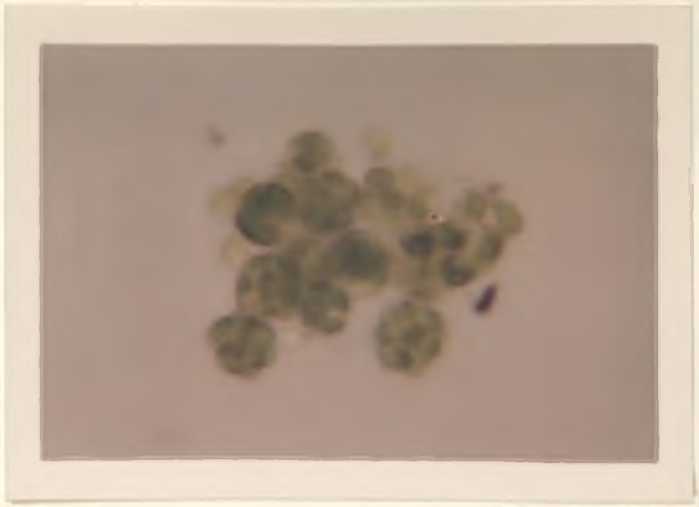


Fig. 4. Stichococcus subtilis (Kuetz.) Klerek. Approximately X 420.
Date Collected: May 3, 1964
Place Collected: Mt. Lemmon Highway, Windy Point,
Elevation 6, 620 ft.
Morphological variant.

Fig. 5. Bracteococcus Tereg, 1923. Approximately X 420.
Date Collected: February 16, 1964
Place Collected: Mt. Lemmon Highway, Elevation 6, 750 ft.

Fig. 6. Chlorosarcinopsis Gerneck, 1907. Approximately X 420.
Date Collected: February 2, 1964
Place Collected: Mt. Lemmon Highway, Windy Point,
Elevation 6, 620 ft.



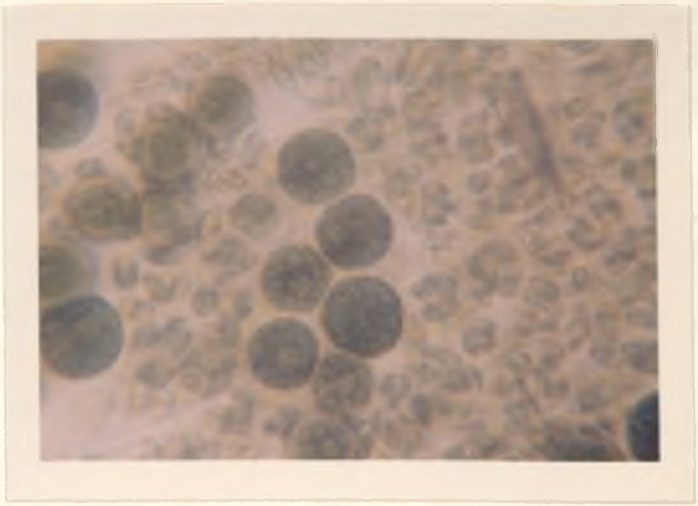
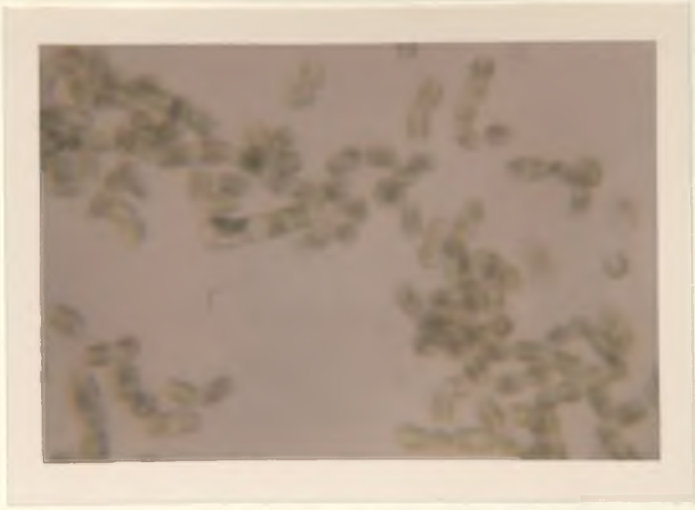
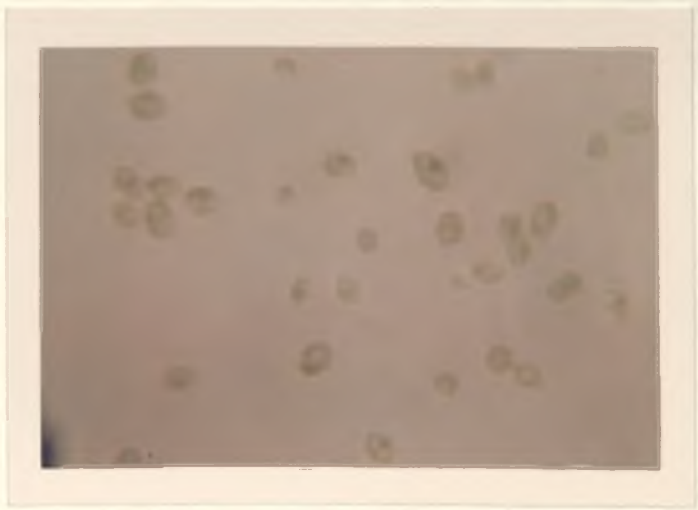


Fig. 7. Hormidium Kuetzing, 1843. Approximately X 420.
Date Collected: December 22, 1963
Place Collected: Mt. Lemmon Highway, Elevation 5, 550 ft.

Fig. 8. Oocystis Nageli, 1855. Approximately X 420.
Date Collected: February 16, 1964
Place Collected: Mt. Lemmon Highway, Elevation 6, 750 ft.

Fig. 9. Planktosphaera G. M. Smith, 1918. Approximately X 420.
Date Collected: January 26, 1964
Place Collected: Mt. Lemmon Highway, Elevation 4, 750 ft.





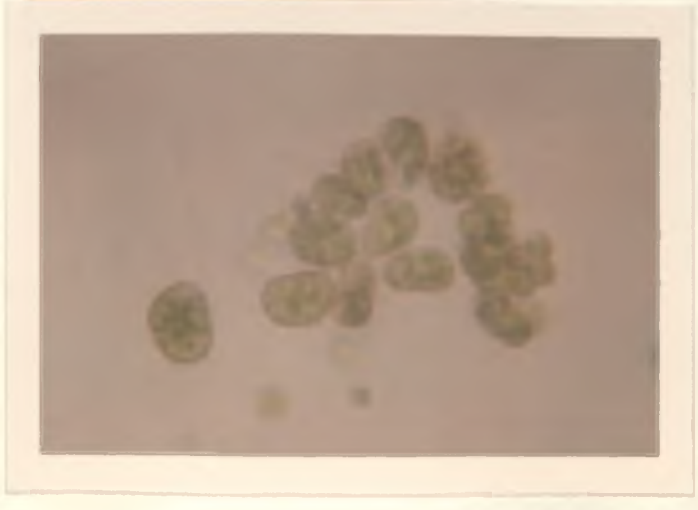


Fig. 10. Spongiochloris Starr, 1955. Approximately X 420.
Date Collected: February 2, 1964
Place Collected: Mt. Lemmon Highway, Elevation 5, 550 ft.

Fig. 11. Hantzschia amphioxys (Ehrenb.) Grun. Approximately X 420.
Date Collected: May 5, 1964
Place Collected: Mt. Lemmon Highway, Windy Point,
Elevation 6, 620 ft.

Fig. 12. Oscillatoria Vaucher, 1803. Approximately X 420.
Date Collected: January 26, 1964
Place Collected: Mt. Lemmon Highway, Elevation 3, 600 ft.

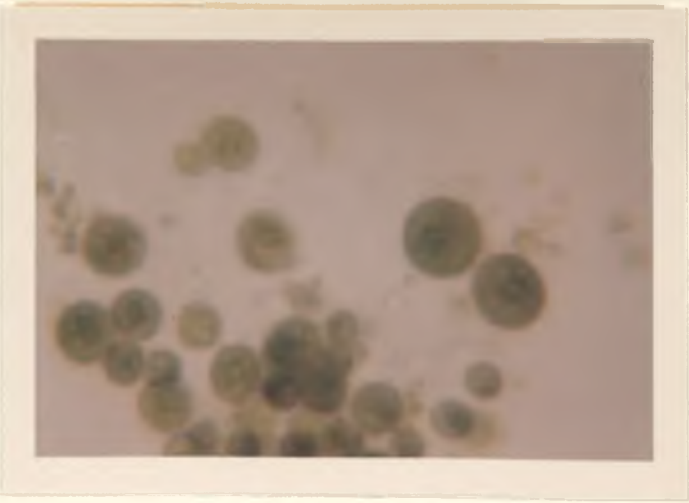
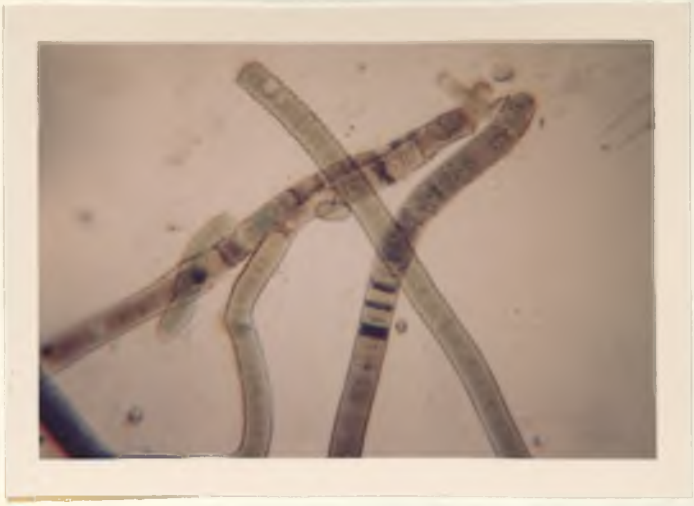


Fig. 13. Scytonema Hofmannii Ag. Approximately X 420.
Date Collected: January 26, 1964
Place Collected: Mt. Lemmon Highway, Elevation 3, 600 ft.

Fig. 14. Nostoc Vaucher, 1803. Approximately X 420.
Date Collected: January 12, 1964
Place Collected: Mt. Lemmon Highway, Elevation 5, 550 ft.
Juvenile growth.

Fig. 15. An unidentified fern prothallus.
Date Collected: December 22, 1963
Place Collected: Mt. Lemmon Highway, Elevation 3, 600 ft.



DISCUSSION

Of the two sampling methods employed, the qualitative sampling technique yielded by far the greater quantity and diversity of algae. The success of this method, when compared with the quantitative method, was due, probably, to the far greater volume of air passing over the agar surface as well as greater velocity of air exerting sufficient force for effective impaction directly upon an environment favorable for algal growth, i.e., a moist agar surface.

Fourteen algal colonies were obtained using the quantitative sampling technique, but, in general, the yield of algal isolates was very low when compared with the 90 colonies collected by the qualitative sampling technique. Particles were obtained under more uniform conditions by using short sampling periods (30 min for the quantitative sampling technique). In such a relatively short period of time, variations in air temperature, relative humidity and the other environmental conditions would be minimal.

A modification of procedure that might lead to more positive correlations would be to determine the environmental conditions on days preceding the sampling of airborne algae. The environmental conditions, e.g., wind velocity, are largely responsible for spore dispersal and therefore data indicating the extent of the environmental

disturbance would be of greater value in determining the extent of the algal spora than the environmental conditions prevailing during the actual sampling.

The taxonomic criteria of the algae collected were based upon morphological characteristics. A number of cells of different forms, however, appeared to be Stichococcus. It is entirely possible that the genus Stichococcus may assume radical morphological changes under different environmental conditions. Since it is possible that Stichococcus is an ecophene-producing organism and has not to date been thoroughly studied in the field and/or laboratory, identification of this organism was difficult. Consideration of such possible variation was made in identification by assuming the stability of the chloroplast and the pyrenoid.

What accounts for the seasonal variation in number of colonies as expressed in Table 3 is not known. There exists the possibility that the physiological state of the algal spores may vary at different times of the year and hence spore production would also vary. There exists the possibility that some of the vast numbers of fungi and bacteria also collected were producing substances, e.g., antibiotics, which were detrimental to algal growth. No observations were made to substantiate this point, however. Further, what might appear to be seasonal variations in growth may be the variations in the germination of the spore or spore-like body.

SUMMARY

1. Sixteen species of algae were found to be airborne during the period of the study.

2. The genus Stichococcus exhibited wide morphological variation.

3. The species of algae collected, in general, were soil algae.

4. The interaction of the atmospheric conditions under which airborne algae were found makes it difficult to predict the specific conditions under which airborne algae occur.

5. The greatest numbers and diversity of algae were found during the winter months of December, January and February.

6. It was noted that certain algae, e.g., Oscillatoria, are found only at certain times of the year.

7. It would be difficult to predict algal succession in the atmosphere from the data found in this investigation.

LITERATURE CITED

Bold, H. C. 1949. The morphology of Chlamydomonas chlamydogama, sp. nov. Bull. Torrey Botan. Club 76: 101-108.

Brown, M. R., D. Larson and H. Bold. 1964. Airborne algae: their abundance and heterogeneity. Science 143: 583-585.

Cunningham, D. D. 1874. Microscopic examinations of air. Nature 9: 330-331.

Deason, T. R. and H. Bold. 1960. Phycological studies. University of Texas Press, Austin. 72 pp.

Gislen, T. 1948. Aerial plankton and its conditions of life. Biol. Rev. 23: 109-126.

Gregory, P. H. 1961. The microbiology of the atmosphere. Leonard Hill Books Limited, London. 251 pp.

Gregory, P. H. and O. J. Stedman. 1953. Deposition of air-borne Lycopodium spores on plane surfaces. Ann. Appl. Biol. 40: 651-674.

Herndon, W. 1958. Studies on chlorosphaeracean algae from soil. Am. J. Botany 45: 298-308.

Lackey, J. B. 1939. The microscopic flora and fauna of tree holes. Ohio J. Sci. 40: 186-192.

Prescott, G. W. 1954. How to know the fresh-water algae. Wm. C. Brown Co., Dubuque, Iowa. 211 pp.

Pringsheim, E. G. 1946. Pure cultures of algae. Cambridge Univ. Press, London. 119 pp.

Proctor, B. E. 1935. The microbiology of the upper air, II. J. Bacteriol. 30: 363-375.

Schlichting, H. E. 1961. Viable species of algae and protozoa in the atmosphere. Lloydia 24: 81-88.

Smith, G. M. 1950. Fresh-water algae of the United States. 2nd ed. McGraw-Hill Book Co., New York. 719 pp.

Starr, R. C. 1955. A comparative study of Chlorococcum meneghini and other spherical, zoospore-producing genera of the Chlorococcales. Indiana University Press, Bloomington. 111 pp.

_____. 1960. The culture collection of algae at Indiana University. Am. J. Botany 47: 67-86.

Tiffany, L. H. 1938. Algae, the grass of many waters. Charles C. Thomas, Publisher, Springfield, Illinois. 171 pp.

U. S. Department of Health, Education, and Welfare. 1959. Technical Development Laboratories and U. S. Army Chemical Corps. Sampling microbiological aerosols. Public Health Monograph No. 60. U. S. Government Printing Office. 53 pp.

Van Overeem, M. A. 1936. A sampling apparatus for aeroplankton. Proc. Acad. Sci. Amst. 39: 981-990.

Watson, H. H. 1954. Errors due to anisokinetic sampling of aerosols. Am. Ind. Hyg. Assoc. Quart. 15: 21-25.

SELECTED BIBLIOGRAPHY

Bold, H. C. 1942. The cultivation of algae. Botan. Rev. 8: 70-138.

Gregory, P. H. 1951. Deposition of air-borne Lycopodium spores on cylinders. Ann. Appl. Biol. 38: 357-376.

_____. 1960. Outdoor aerobiology. Endeavour 19: 223-228.

National Research Council. 1941. The Committee on Apparatus in Aerobiology. Techniques for appraising air-borne populations of micro-organisms, pollen, and insects. Phytopath. 31: 201-225.