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Airborne Algae: Their Abundance and Heterogeneity

Abstract. *The literature on the occurrence of airborne algae is reviewed briefly. Airborne algae were isolated into culture in both quantity and diversity. Qualitative experiments and culture techniques are discussed, as are quantitative sampling techniques and preliminary correlations of the occurrence of algae, fungi, and pollen in the air. The data disclose an important pathway for the dispersion of soil algae and support an observation that algae may be important as causal agents in inhalant allergies.*

It is well known that algae occur in and have been recovered from the atmosphere (*1*). However, these reports have not emphasized the potential abundance and variety of airborne algae because of the lack of prolonged cultivation after exposure to air. Accurate taxonomic determination of airborne algae has lagged because of failure of many investigators to realize the need for continuing studies of the morphology and physiology of these organisms in culture.

The investigations by Van Overeem (*2*) in 1937 represent pioneering efforts to recover and cultivate airborne algae. Van Overeem collected 24 air samples on six different occasions from an airplane at various altitudes over the Netherlands. Of nine different algal isolates recovered, *Chlorococcum* appeared most frequently. Samples from an altitude of 500 m provided the most abundant algal flora. Further collections at ground level, by means of an air pump, and rainwater samples revealed at least eight different species, *Chlorococcum* again being the most abundant. A Myxomycete plasmodium, moss protonemata, and fern prothallia (apparently developed in culture from spores) were also obtained by these collection methods.

Others have cultivated algae obtained from the air (*3*), but no investigators, so far as we are aware, seem to have obtained adequate data on airborne algae with respect to: (i) the heterogeneity of airborne algal genera and species; (ii) the flora of diverse geographical locations; and (iii) aerodynamically sound quantitative information. The purpose of this report is to summarize some of our recent investigations in these connections and to discuss briefly certain of their implications.

Preliminary investigations were begun in the summer of 1959 by exposing petri dishes containing sterile, solidified, inorganic nutrient medium known as "Bold's Basal Medium" (*4*). This inorganic medium did not encourage luxurious growth of heterotrophic contaminants (fungi and bacteria). Vitamin or other nutrient deficiencies later displayed by certain algal isolates in axenic culture (*5*) were apparently provided for by heterotrophs or other algae present. Table 1 lists 62 genera of algae found in the present investigation to be airborne.

To obtain qualitative information on airborne algae, we have studied collections made by the following methods: (i) sterile petri dishes (15 × 100 mm) containing agar medium were exposed to the air from 5 minutes to 12 hours at a number of stationary locations in Texas; (ii) hand-held petri dishes were exposed (3 to 5 minutes) from a moving (approximately 100 km/hr) automobile, in 14 states, and dishes were also exposed from an airplane for approximately 1 minute; and (iii) filter samples of air from 21 states supplied by stations of the National Air Sampling Network (*6*). After exposure to air, these samples were cultured under standard conditions (*7*) for 2 to 5 weeks, during which the impactions developed into macroscopically visible colonies.

Of these qualitative sampling methods, the exposures from automobiles and airplanes generally yielded the greatest quantity and diversity of algae. High-velocity winds, exerting sufficient force for effective impaction upon moist agar and immediate capture on an environment favorable for algal growth, were instrumental in the success of this method. For example, an automobile exposure in Pennsylvania on a clear, windless day yielded more than 140 algal impactions, including approximately 25 different genera. Another such exposure (10 seconds) was made recently near Austin, Texas, from an automobile moving at 60 miles per hour (96 km/hr) through a local dust cloud blowing across the highway from a plowed field. Immediate examination revealed, by direct count, less than 30 algae, but after 2 weeks' cultivation under standard conditions, more than 5000 algal impactions were recorded. Of these, 4500 were chlorophycean or xanthophycean, while approximately 500 impactions were cyanophycean. Mosaic sampling of this plate revealed a marked heterogeneity of algal genera and species, probably equal in range to what might have been obtained from an equivalent soil sample from the same site.

Exposures of petri dishes for 1 minute from a plane at 1100 m above the ground over central Texas have indi-

Table 1. Algae recovered from air and cultivated in or on "Bold's Basal Medium."

		Chlorophyta	
Borodinella	*Cylindrocystis*	*Palmellococcus*	*Scenedesmus*
Bracteacoccus	*Dictyochloris*	*Planktosphaeria*	*Spongiochloris*
*Chlamydomonas**	*Friedmannia*	*Pleurastrum*	*Spongiococcum*
Chlorella†*	*Hormidium**	*Protococcus*-like†	*Stichococcus*
Chlorococcum†*	*Hormotilopsis*	*Protosiphon*	*Tetracystis*‡*
Chlorosarcina	*Nannochloris*†*	*Psuedoulvella*-like	*Tetraspora*
Chlorosarcinopsis†*	*Neochloris†*	*Radiococcus*	*Trebouxia†*
Chlorosphaeropsis	*Oocystis*	*Radiosphaera*	*Ulothrix*
Coelastrum	*Ourococcus*	*Roya*	*Westella*
Cosmarium	*Palmella*		
		Cyanophyta	
Anabaena†	*Gloeocapsa†*	*Myxosarcina*	*Schizothrix*
Anacystis†	*Lyngbya*	*Nostoc†*	*Synechococcus*
Arthrospira	*Merismopedia*	*Oscillatoria†*	*Scytonema†*
Chroococcus-like	*Microcoleus*	*Phormidium*†*	*Tolypothrix*
Fremyella			
		Chrysophyta	
Hantschia	*Navicula*†*	*Heterococcus*†*	*Tribonema**
Melosira-like	*Botrydiopsis*†*	*Monocilia*	

* Indicates samples from airplanes in addition to ground samples. † Indicates those genera most frequently encountered. ‡ New genus, unpublished.

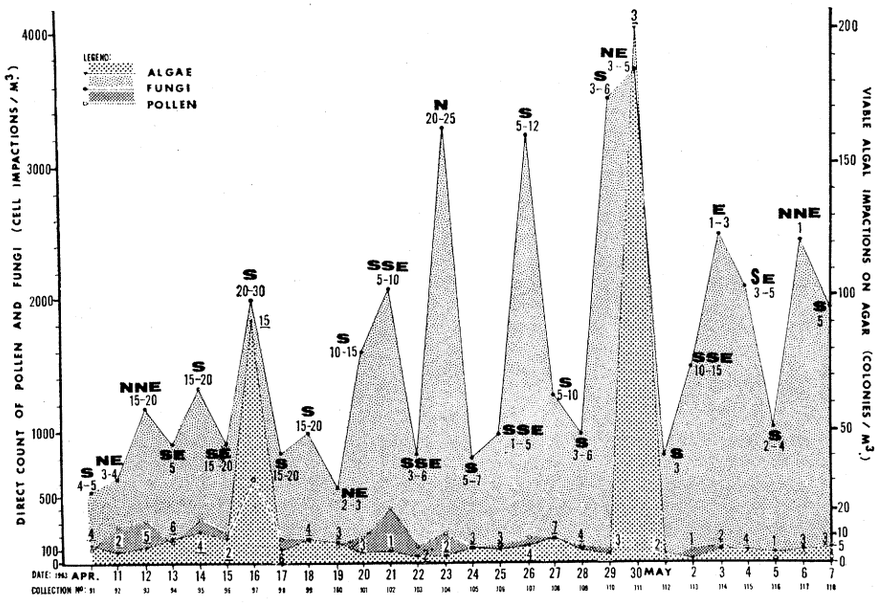

Fig. 1. Summary of 28 consecutive days of quantitative sampling of fungi, pollen, and algae obtained 25 m above the ground at the University of Texas, Austin. The numbers below the collection dates refer to the specific collection numbers. Quantitative counts for fungi and pollen are expressed as direct observations of cell impactions per cubic meter (left axis), while algae are expressed as viable impactions, counted as colonies per cubic meter, on agar (right axis). The data near the markers for fungi indicate the wind velocity and direction for all collections made on that date. Numbers adjacent to the markers for algae indicate the number of different algal genera obtained from a given collection.

cated a remarkable heterogeneity of airborne algae, often equivalent to exposures from the automobile (Table 1). One such exposure contained 10 different algal genera from 16 total impactions.

Stationary exposures of petri dishes often gave results comparable to mobile exposures either when wind velocities were high (above 25 km/hr, generally), or during long periods (10 to 12 hours) of sedimentation in still air, beginning at dusk, after a windy day.

Filter samples did not yield as great a quantity of airborne algae as the petri-dish exposures, possibly because of the prolonged interval of desiccation between impact and cultivation. However, a diverse flora of unusual genera and species (over 14 different genera) was obtained, which in itself makes this method useful.

The following are indicated by our qualitative studies of airborne algae: (i) a surprisingly large number of different genera and species of soil algae is constantly present in and can survive during transport in the air; (ii) the origin of airborne algae is primarily from the soil; (iii) cultivated soils, when blown as dusts, yield a greater quantity and diversity of algae than do undisturbed soils; (iv) 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; and (v) the Chlorophyceae and Myxophyceae seem to be most abundant and diverse in the air samped, but Bacillariophyceae and Xanthophyceae are also present.

Quantitative sampling for airborne algae has been conducted primarily with Rotorod samplers modified for algal cultivation. The Rotorod was chosen for the quantitative investigations because it samples with great retentive efficiency (0 to 60 km/hr wind velocity) those particles which fall within the size range of most algal cells or clumps (above 5 μ) and of pollen and fungi. Also, the instrument's response to shift of wind direction is extremely rapid.

As modified in our laboratory, the Rotorod sampler consists of a pair of Lucite rods (1.59 mm^2 × 60.0 mm). These Lucite rods are attached to a U-shaped brass rod by small plastic washers and then coated with silicon grease just before use. Our quantitative studies have been conducted at 25 m above ground at the University of Texas campus in Austin. Daily collections were made, each with an exposure time of 20 minutes, representing 2280 liters of sampled air. After a given exposure, one rod is retained for a direct fungal and pollen analysis (also algal analysis for those few genera that can be identfied by inspection). The remaining rod is streaked across the surface of a petri dish containing agar medium. After streaking, the rod is left on the agar surface, with the impaction surface facedown, so that algae not removed by streaking will develop on the rod. Culture from 3 to 4 weeks under standard conditions reveals the presence of most algae that will ever develop. In addition to algae, bacteria, and fungi, more than 16 different species of Myxomycetes, all of the Physarales, have been recovered, cultured, and grown to maturity on the basal inorganic medium (*8*). For purposes of comparison with Rotorod sampling, petri dishes of agarized medium, although not nearly so efficient as the Rotorod, were exposed directly to the air at the same time.

We do not believe that quantitative data obtained by this or any method represent the exact number of algae that existed in the air at the time of collection. Nonviable algae may make impact upon the rod and never be discerned. In addition, direct observations of the Lucite rods often reveal the presence of clumps of algae (containing up to 50 cells). Filamentous fragments, hormogonia, tetrads of cells, and other groupings tend to cause deviation of the impaction number from the exact cell number. Furthermore, the environment for germination and continued growth of algae removed from the air must be carefully considered. No single medium would necessarily support the growth of all viable algae impacted. However, "Bold's Basal Medium" is considerably more favorable than other media commonly employed in the cultivation and maintenance of algae (see *9*).

Figure 1 summarizes quantitative data obtained on the 28 consecutive days from 10 April 1963. Comparisons are made between direct counts of fungal spores and pollen grains, and impactions of viable algae as colonies on agar. Samples collected on 16 and 30 April are particularly significant. On 16 April, 102 algal impactions per cubic meter, consisting of more than 15 different genera, were recorded. However, 228 algal impactions per cubic meter from the collection

of 30 April were represented by only three different genera (226 impactions were *Chlorella*). Two explanations for the high density of *Chlorella* exist. Either one to several large clumps of *Chlorella* were impacted and dispersed in the streaking process, or a homogeneous suspension was impacted with minimum dispersion during streaking.

The following information has emerged from our quantitative studies of airborne algae: (i) the algal content of dust can be extremely high (some of our highest counts have revealed no less than 3000 algae per cubic meter); (ii) counts of this nature indicate that algae may exist in sufficient quantity in the air to be a possible cause of inhalant allergy as McElhenney *et al.* (*10*) had previously indicated; (iii) the algal population in blowing dust frequently exceeds that of fungi which formerly have been classified as primary agents for dust allergies; (iv) the algal content (as measured by impaction number) of nondusty air is generally much lower than the fungal or pollen content; however, algae are present at all times except after washout by rain, and so forth; (v) the algal content of air frequently exceeds that of pollen, particularly during seasons when the production is low; (vi) algal diversity does not always accompany an increase in quantity; and (vii) seasonal periodicity in quantity or diversity, has not, as yet, been demonstrated to exist in the Austin area.

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Lateral Hypothalamic Lesions: Effects on Drinking Elicited by Carbachol in Preoptic Area and Posterior Hypothalamus

Abstract. *Lateral hypothalamic lesions in rats caused a pronounced depression of drinking in response to injections of carbachol into the preoptic area or the posterior hypothalamus. After the lesions were induced, daily free water consumption recovered 70 to 80 percent of that of control animals, but there was little, if any, recovery of drinking induced by carbachol.*

Drinking is elicited in satiated rats by minute injections of cholinergic substances into the hypothalamus (*1*, *2*) and also into diverse portions of the limbic system (*3*). Lateral hypothalamic lesions produce a primary adipsia and aphagia (*4*, *5*), but lesions in most parts of the limbic system have not been reported to have these effects (*6*). This study concerns the effects of lesions in the lateral hypothalamic "feeding-drinking" area on the elicitation of drinking by intracerebral injections of the cholinergic substance, carbachol.

Electrodes were implanted bilaterally and a double-walled cannula (*1*) was implanted unilaterally in the brains of 18 male Sprague-Dawley rats about 100 days old and weighing 300 g. The operation was performed under pentathol anesthesia. In six of the rats the electrodes were aimed at the lateral area of the hypothalamus and the cannula at the preoptic area. In another four, with electrodes aimed at the lateral hypothalamus, the cannula was aimed at the region of the posterior hypothalamus. A third group (four rats) had electrodes aimed at the preoptic area and cannulas at the lateral hypothalamus, and a final group of four had electrodes aimed at the posterior hypothalamus and cannulas at the lateral hypothalamus. Pairs of rats with similar implants were yoked and treated similarly throughout the experiment. One rat of each pair was randomly chosen to receive lesions after baseline data were obtained. Bilateral lesions were made by passing 1.0 ma of anodal direct current for 10 seconds through each implanted electrode with the cathode in the rectum.

After implantation all rats were tested for drinking elicited by two doses of carbachol dissolved in isotonic saline (24×10^{-10} mole and 72×10^{-10} mole in 1 μl of solution) according to the technique of Miller *et al.* (*2*) and with crystalline carbachol (*1*). The experiment was carried out with the injection procedure that produced the most drinking for each pair of animals. The rats with cannulas in the posterior hypothalamus seemed to respond best to the crystals, while the others responded best to one of the two solutions, usually the weaker one.

One mock (insertion of needle or stylus, but no injection) and two carbachol injections per week, spaced at least 2 days apart, were given to all rats for 1 week prior to production of the lesions and for 4 weeks afterward. From the 5th week after production of lesions, one carbachol injection and one mock injection were given per week. Food was removed from the home cages 1 hour before the injection and was not replaced until 1 hour after the injection. Water was always available in inverted graduates with standard drinking nozzles. Carbachol-induced water consumption was measured for 1 hour after injection and normal water intake during the rest of the day. The rats were weighed once each week, and all analyses were based on intake per 100 g of body weight. Carbachol effects were measured by subtracting the water intake during the weekly mock test from the average intake during the weekly carbachol tests.

During the aphagic-adipsic period after the production of lesions, water and wet mash (50 percent ground Purina chow and 50 percent water) were continuously available to the animals with lesions until they started drinking water and eating regular chow. They were maintained by tube feeding of Metrecal until wet mash was accepted. Control rats were maintained on Purina chow and water.