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A quantitative investigation of airborne algae and lichen soredia obtained from pollen traps in south-west Spain

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Over the course of nearly a year, counts were made of the algae present in the samples from a Burkard-type volumetric aerobiological trap which sampled continuously the atmosphere of the city of Badajoz (SW Spain), yielding hourly and daily results. The method permits only the major taxa of algae to be distinguished. Most of the algae found belong to the Chlorococcales (Chlorophyceae) and small centric diatoms (most of them *Cyclotella*). Some filamentous algae were also found and even *Pediastrum* coenobia as well as lichen soredia. Up to 12 Chlorococcales coenobia per cubic metre were found. The mean number of cells in the coenobia was more than 10, although some coenobia with more than 100 cells were observed. Their greatest concentration was during the months of May and June. The mean daily concentration of diatoms was highly variable, with maximal concentrations of more than 70 cells m^{-3} in June. The maximum daily concentration of lichen soredia was 5 m^{-3}, with the concentration being fairly constant throughout the year but particularly high in spring and early summer. The hourly distribution showed maxima during the day and minima during the night. Significant positive correlations were found with temperature, and negative correlations with relative humidity in the three groups studied. The wind speed seemed to have a positive influence on the concentration of diatoms and Chlorococcales coenobia, although the direction of the wind also had an effect.

Key words: aerobiology, airborne algae, Chlorococcales, diatoms, lichen soredia

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Introduction

Because of their abundance in the atmosphere, pollen grains and spores have been the almost exclusive focus of aerobiological studies. Nevertheless, the air transports a great number of other particles of biological origin which may have considerable significance. The airborne algae are an example. The broad cosmopolitanism of the great majority of freshwater algae probably results from their microscopic dimensions as well as the vectors that contribute to their dispersion. One such vector is the air, and others are of biological origin (Kristiansen, 1996).

Airborne algae are the cause of economically important effects, such as the contamination of drinking water, and the deterioration of architectural structures or works of art (Ariño & Saiz-Jiménez, 1996*a*, *b*; Wakefield *et al*., 1996). They are also capable of producing allergic reactions in humans (Bernstein *et al*., 1979). Algae in the atmosphere may even be used as bioindicators of atmospheric contamination, and of ozone in particular (Roy-Ocotla & Carrera, 1993).

Among studies of the qualitative composition of algae in the atmosphere which have used culturing for correct identification, few have been able to attain a reasonable level of identification at the same time as providing a quantitative evaluation. Brown *et al*. (1964) used Petri dishes mounted in moving settings (in cars or aeroplanes) and a Rotorod (two vertical rods, coated with an adhesive, fixed to a forked arm rotating at 2500 rpm) which transferred trapped algae into a culture medium. Schlichting (1964), as well as using Millipore filters, passed the air current through a flask containing culture medium. The even earlier work of Gregory *et al*. (1955) was the first to use Hirst-type devices (Hirst, 1952) to count airborne algae.

The most recent review of the subject of algae in the atmosphere is that of Kristiansen (1996), who surveyed the mechanisms of dispersion, whether by organisms (mainly birds), water currents, man, or the air. His conclusion was that knowledge of how algae disperse is incomplete and that further investi-

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gation is required. This situation contrasts with the depth of the more recent studies carried out in the Antarctic (Harmata & Olech, 1991; Broady, 1996; Marshall & Chalmers, 1997), including lichens (Marshall, 1996), despite the paucity of algae in this zone of the Earth (Broady, 1998).

The present work was developed as a complement to the aerobiological investigations carried out in Extremadura (SW Spain), which are basically centered on the study of pollen grains and spores. We were led to perform the study by the almost year-long low but constant presence of algae in our aerobiological preparations, together with the relative lack of studies that have dealt with this subject.

Materials and methods

Atmospheric sampling was carried out using a Burkard-model volumetric trap that is based on the technique of Hirst (1952). Air was pumped in at 10 l min^{-1} and impacted on a surface coated with an adhesive which trapped all particles. Petrolatum white was used as the adhesive. Preparations were mounted in glycerinated gelatine containing phenol to prevent both fungal and algal growth. The particles collected during one sampling day were trapped on a rectangular area of 14×48 mm (2 mm h^{-1} velocity, 14 mm sporetrap slit length). The particles were counted under $\times 400$ optical magnification using four longitudinal scans (each covering the 24 h period). Since the concentration of particles diminished away from the centre line of the rectangle towards each of the longer sides (corresponding to the extremes of the slit), the scans were made in the central part of the rectangle with a 1 mm separation (for details see Tormo *et al.*, 1996). Results are expressed as the number of particles per cubic metre of air sampled, averaged over periods of 1 h or 1 day. Sampling was begun on 14 February and ended on 31 December 1996. One week's data were lost due to apparatus malfunction, so that the total number of daily samples analysed was 315.

The trap was placed 6 m above the ground near the outskirts of the city of Badajoz (SW Spain, 38° 52′ 55″ N, 6° 58′ 04″ W). The surroundings are free of any high buildings which might have been an obstacle to the unhindered circulation of air. The River Gevora, a tributary of the River Guadiana, runs about 100 m away. The surrounding vegetation is dominated by herbaceous crops under a dryland or irrigation regime (62%), orchard or forestry repopulation tree cultivation (12%), and seminatural formations of pastures or holm oak stands (26%), according to MAPA (1985).

For identification we used Bourrelly (1970, 1972) and Cambra & Hernández-Mariné (1989) as well as aid from various specialists. For the lichen soredia, we made microscope preparations of the most frequent lichens of the zone for comparison. It is difficult to identify algae to the species level, since, for most of them, it is necessary to establish cultures where the complete life cycle may be observed (Broady, 1998). Since this is impossible with the method used here (Brown *et al.*, 1964), we divided the observed algae into three large groups: coenobial chlorophytes (mostly Chlorococcales), diatoms, and algae in lichen soredia.

Spearman (non parametric) correlation coefficients were calculated between the daily concentration values and the daily records for rainfall (mm), relative humidity (%), mean temperature (°C), mean wind speed (km h^{-1}) and the number of hours per day of still air (calms) and of wind from each quadrant.

Results

In 1996, the mean daily temperatures in Badajoz varied between 4·8 °C in February and 29·8 °C in July. The total annual rainfall was 599·6 mm, greater than the average value (483·4 mm, normal reference period 1961–90). The rainfall was concentrated in the months of January (181·7 mm) and December (139·7 mm). The dominant winds were those from the west (quadrants 3 and 4, Fig. 1*C*). Mean wind speed ranged between 20 and 40 km h^{-1}, with few variations over the course of the year. Relative humidity varied between 40–60% in summer, 60–80% in spring and autumn, and 80–95·5% in December (Fig. 1*B*).

The main daily concentration of pollen grains during the study period was highest from April to June, with a maximum at the end of May of around 1500 grains m^{-3}, followed by a sharp fall (Fig. 1*A*).

The Chlorococcalean algae occurred as coenobia 5–10 μm in diameter, generally flat, occasionally irregular, with a cell number of 2, 4, or most frequently 8, although at times isolated cells were found or larger coenobia with up to 100 cells. In total, 677 coenobia were counted over a period of 215 days. Some coenobia were observed on most days but their concentration reached maximal values during May and June, with up to 12 coenobia m^{-3} (Fig. 2*A*). Their daily concentrations were positively correlated with temperature and wind speed, and negatively correlated with rain and relative humidity (Table 1). With respect to the direction of the wind, there was a positive correlation with winds from the NW and a negative correlation with winds in the opposite direction. Most coenobia have 2, 4 or 8 cells and this is evidence for growth by bipartition (Fig. 2*D*). There was no seasonal pattern in the number of cells per coenobia, i.e. coenobia with many or with few cells could be found at any time of the year (Fig. 2*C*). The hourly distribution of the coenobia showed lowest concentrations at dawn and in the early morning (mean, 1·03 coenobia m^{-3} between 06:00 and 11:00 h) and maximal concentrations during the late afternoon, between 17:00 and 19:00 h (mean, 2·03 coenobia m^{-3}; difference between means significant at $p = 0{\cdot}001$, *t*-test; Fig. 2*B*).

The majority of diatoms found were small centric forms belonging to the genus *Cyclotella*. Their size ranged between 8 and 12 μm in diameter, often with

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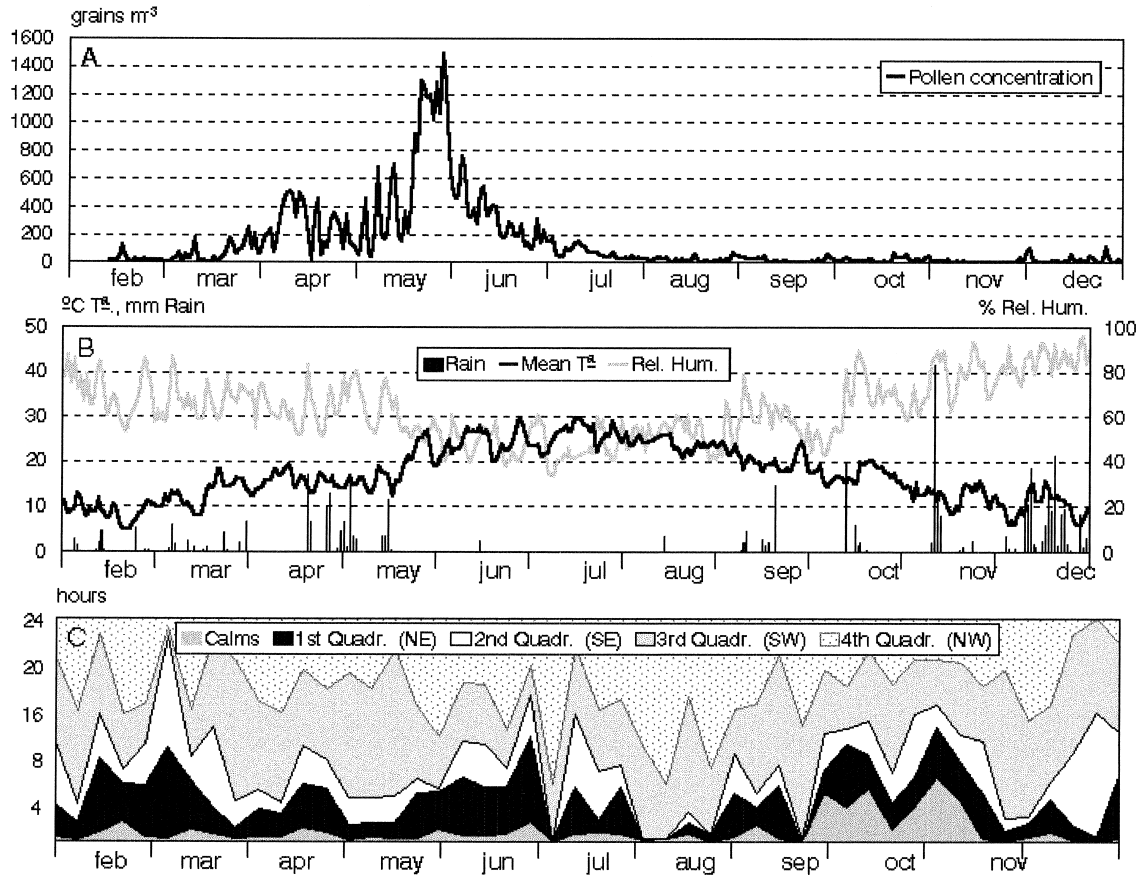

Fig. 1. (*A*) Pollen concentration in grains m^{-3} found per day. (*B*) Mean daily temperatures in °C, total daily rainfall in mm, and daily relative humidity in %. (*C*) Number of hours per day of still air (calms) and of wind from the quadrants 1 (NE), 2 (SE), 3 (SW) and 4 (NW).

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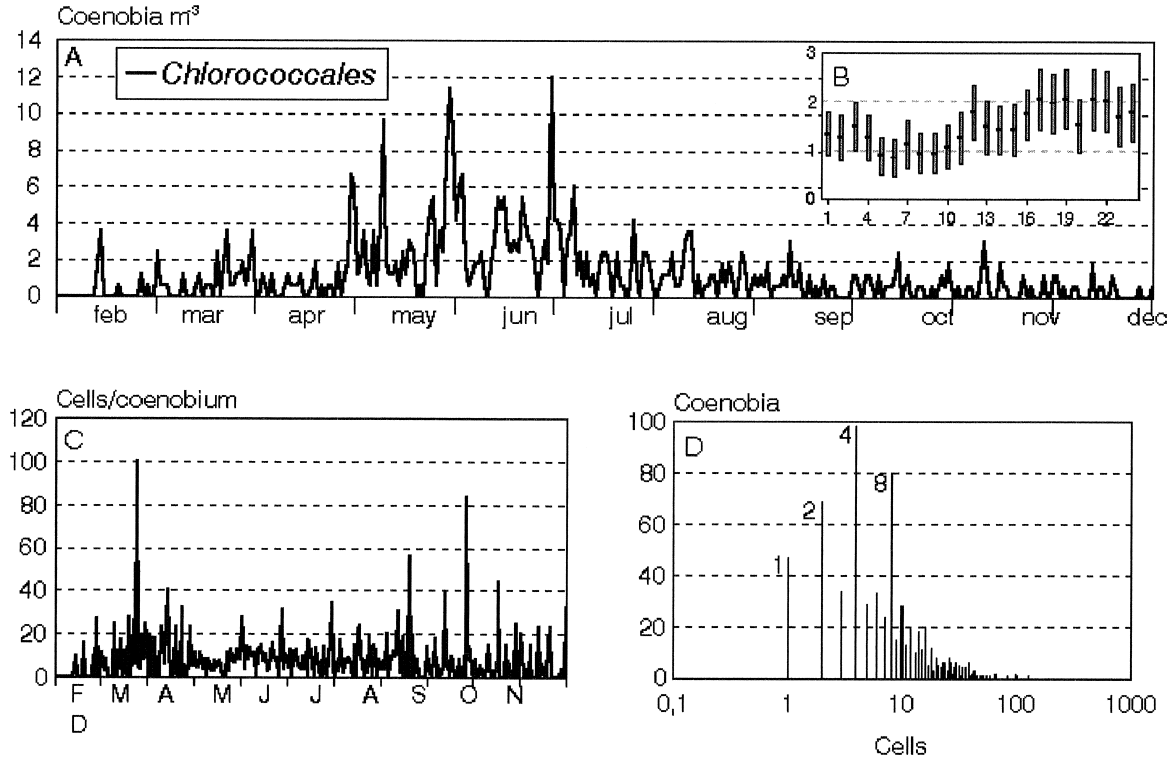

Fig. 2. (*A*) Mean daily concentration of Chlorococcales coenobia m^{-3}. (*B*) Mean hourly concentration of Chlorococcales coenobia m^{-3} with 95% confidence intervals. (*C*) Absolute number of cells per coenobium over the course of the study period. (*D*) Distribution of the number of coenobia as a function of the number of cells per coenobium.

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Table 1. Spearman rank correlation coefficients between the daily concentrations of Chlorococcales coenobia, diatom cells, or lichen soredia and the daily meteorological parameters of rainfall, temperature (maximum, minimum, and mean), relative humidity (RH), wind speed, hours of still air, or of wind from each of the four quadrants (NE, SE, SW, NW)

	Rain	T_{min}	T_{max}	T_{mean}	RH	Wind	Calms	NE	SE	SW	NW
Chlorococcales	$r = -0{\cdot}138$**	0·380***	0·396***	0·405***	−0·379***	$r = 0{\cdot}187$**	−0·163**	−0·097	−0·206***	0·043	0·187**
Diatoms	$r = -0{\cdot}250$***	0·483***	0·464***	0·484***	−0·414***	$r = 0{\cdot}148$**	−0·130**	−0·158**	−0·204***	−0·062	0·344***
Soredia	$r = -0{\cdot}037$	0·127*	0·126*	0·128*	−0·149**	$r = 0{\cdot}047$	0·023	0·051	−0·017	0·060	0·074

Number of replicates (days), 315; significance levels, * ($p < 0.05$), ** ($p < 0.01$) and *** ($p < 0.001$).

a peripheral band of radial striae and foramina of diverse sizes in the centre. On very few occasions, pennate diatoms were also found. A total of 782 diatoms was counted on 78 days of the study. Diatoms appeared in the atmosphere suddenly towards the end of May with a maximum of 72 cells m^{-3}, with only a few sporadic cells previously. From that time onwards there was a decline, but with occasional peaks in the concentration up to the end of August, after which the diatoms again appeared erratically (Fig. 3*A*). Positive correlations were again found with temperature and wind speed, and negative correlations with rain and relative humidity (Table 1). Wind direction showed the same influence as in Chlorococcales, but a negative correlation with NE winds was also found. The hourly distribution showed minimal values during the night (0·18 cells m^{-3}, 01:00 to 07:00 h) and a progressive increase from mid-morning onwards to a maximum in late afternoon (3·85 cells m^{-3}, 17:00 to 20:00 h; difference between means significant at $p = 0{\cdot}001$, *t*-test; Fig. 3*B*).

The lichen soredia were between 10 and 20 (−30) μm in diameter. They contain algal cells assembled in various groups, each group with 6–14 cells and surrounded by a mucilaginous pod, and the whole surrounded by a great quantity of linked or free hyaline hyphal cells. A total of 213 soredia were counted over 137 days (Fig. 3*C*). There were few variations in the distribution of soredia over the course of the year. The highest values appeared between May and July, with up to 4 soredia m^{-3} at the end of May, and minima in February–April and September–November, and a second peak in December with 1 soredium m^{-3} as the mean weekly value. There was a positive correlation between soredia concentration and temperature and a negative correlation with relative humidity (Table 1). The hourly distribution showed a more or less constant distribution during daylight hours up to and including nightfall (0·42 soredia m^{-3}, 10:00 to 21:00 h), and minimum values during the night (0·19 soredia m^{-3}, 01:00 to 06:00 h; difference between means significant at $p = 0{\cdot}001$, *t*-test; Fig. 3*D*).

We also found other types of algae – filamentous algae, *Pediastrum*, *Scenedesmus*, cyanophytes such as *Stigonema*, but all were found only sporadically. Care was taken not to include other green-coloured aerobiological particles, such as bryophyte or *Equisetum* spores, or even bryophyte propagules.

Discussion

There are many difficulties involved in quantifying the algae that appear in the atmosphere. Firstly there is the problem of choosing a sampling method that allows one to know how much air has been

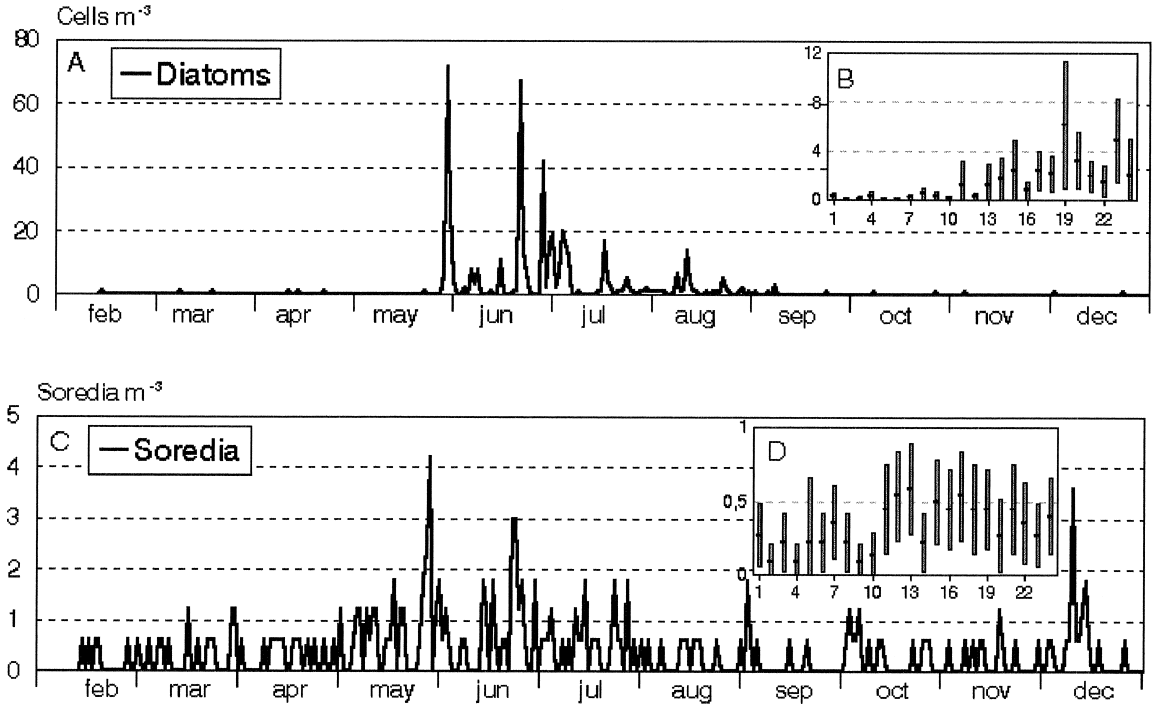

Fig. 3. (*A*) Mean daily concentration of diatom cells m^{-3}. (*B*) Mean hourly concentration of diatoms cells m^{-3} with 95% confidence intervals. (*C*) Mean daily concentration of soredia m^{-3}. (*D*) Mean hourly concentration of soredia m^{-3} with 95% confidence intervals.

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sampled and also provides a way to isolate the trapped algae for culture and correct identification (Cambra & Hernández-Mariné, 1989). It is very difficult to obtain high precision and reliability in these two requirements at the same time. Secondly there is the fact that many algae do not appear as isolated cells, but form coenobia or colonies with a highly variable number of cells (Brown *et al.* 1964). Thirdly, there exists no culture medium which guarantees the growth of whatever algae may be present, so that the choice of any one medium involves eliminating some algae that cannot grow in it.

These problems often lead to inconsistent quantitative results. Thus, for instance, Brown *et al.* (1964) found up to 200 viable colonies m^{-3}, including Chlorophyta, Cyanophyta and Chrysophyta, with mean values of 5–10 cells per colony, although they calculated that the air may contain up to 3000 algae m^{-3}. Our results, however, are closer to those reported by Schlichting (1964), who found maximum values of around 35 algae m^{-3}, and those of Gregory *et al.* (1955), who reported mean values of 110 coenobia m^{-3} for *Gloeocapsa*.

Generally, it is found that by far the most frequent algae are the chlorophytes, especially the Chlorococcales (Schlichting, 1964), and the cyanophytes and diatoms are usually less frequent. Thus, for instance, Brown *et al.* (1964) found that the cyanophytes represented a tenth of the predominantly chlorophyte total.

With respect to meteorological factors that affect the algal concentrations, Schlichting (1964) noted that the direction of the wind and especially its angle of elevation are more important than its speed, with ascending thermal currents providing the highest concentrations, since the soil, trees and stone walls are the principal sources of airborne algae. Our results seem to confirm these observations, since there did not appear to be more algae when the wind blew from the direction of the river (NE), than when it was from the opposite direction (NW). The sudden appearance of diatoms towards the end of May, which coincided with the end of the spring rains, could be interpreted as a result of favourable conditions for their development during the preceding days. However, it is also possible that the rain had cleansing effect on the atmosphere during this period and reduced the abundance of diatoms (see negative correlation in Table 1), as was also seen with airborne pollen grains (Fig. 1*A*). It is also worth noting that both rainfall and relative humidity were negatively correlated with the concentrations of algae. This suggests that dry ambient conditions favour the resuspension of algae from the soil by ascending currents, which is supported by the positive correlations with temperature and wind speed.

It is important to know how significant algae are in the atmosphere relative to other aerobiological particles. Over the same period (315 days) and using the identical assay technique, we counted a total of 74892 grains of pollen, with a mean concentration

of 142·3 grains m^{-3}. The mean concentration of Chlorococcales coenobia was only 1·3 coenobia m^{-3}, that of diatoms 1·5 cells m^{-3}, and of lichen soredia 0·4 soredia m^{-3}. Using the same technique in different years (1993–95), the concentration of fungal spores and propagules varied between 1841·5 and 2522·1 spores m^{-3}. The algae can be seen, therefore, to make only a very minor contribution to the total population of aerobiological particles.

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