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Monitoring of airborne biological particles in outdoor atmosphere. Part 1: Importance, variability and ratios

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Summary. The first part of this review ("Monitoring of airborne biological particles in outdoor atmosphere. Part 1: Importance, variability and ratios") describes the current knowledge on the major biological particles present in the air regarding their global distribution, concentrations, ratios and influence of meteorological factors in an attempt to provide a framework for monitoring their biodiversity and variability in such a singular environment as the atmosphere. Viruses, bacteria, fungi, pollen and fragments thereof are the most abundant microscopic biological particles in the air outdoors. Some of them can cause allergy and severe diseases in humans, other animals and plants, with the subsequent economic impact. Despite the harsh conditions, they can be found from land and sea surfaces to beyond the troposphere and have been proposed to play a role also in weather conditions and climate change by acting as nucleation particles and inducing water vapour condensation. In regards to their global distribution, marine environments act mostly as a source for bacteria while continents additionally provide fungal and pollen elements. Within terrestrial environments, their abundances and diversity seem to be influenced by the land-use type (rural, urban, coastal) and their particularities. Temporal variability has been observed for all these organisms, mostly triggered by global changes in temperature, relative humidity, et cetera. Local fluctuations in meteorological factors may also result in pronounced changes in the airbiota. Although biological particles can be transported several hundreds of meters from the original source, and even intercontinentally, the time and final distance travelled are strongly influenced by factors such as wind speed and direction. [**Int Microbiol** 2016; 19(1):1-13]

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Airborne biological particles and their importance in the atmosphere

According to Després et al. [26], bioaerosol particles can include viruses, bacteria, fungi and their spores, pollen, fragments of lichen, plants or invertebrates. In a recent study, Fröhlich-Nowoisky et al. [38] described also the diversity of airborne archaea, adding up new organisms to the list. The attention and impact of research on airborne microbiota have diversified and grown as the knowledge has improved. Initially focused on global human airborne diseases such as viral infections (influenza or meningitis), bacterial diseases (Legionnaires' disease) and fungal infections (aspergillosis) [32], current studies have extended the importance of the biological aerosols to more particular situations. For instance, respiratory diseases developed by farmers and livestock workers have been related to inhalation of organic dust carrying particle-associated bacteria, archaea, fungi and viruses [7], with subsequent exposure to bioaerosols containing antibiotic re-

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sistant bacteria [3]. It has been also demonstrated that the exposure to high concentrations of certain species of airborne pollen and fungal propagules can cause allergy-related diseases including hay fever, asthma, rhinitis and atopic eczema to a significant percentage of citizens [4,92], affecting 18–20% of the European population [9]. Thus, monitoring pollen and fungal spores in the air, as aerobiological networks of different countries around the world do (Fig. 1), is relevant for the diagnosis, treatment and prevention of these allergic diseases, whose prevalence is now three times higher than 30 years ago [105]. More recently, research has focused on health risks to pets and other animals inhabiting human environments that could potentially act as disease vectors [29].

In addition to her role in human health, bioaerosols have relevance in global economy too. The concern about cross-pollination between genetically modified crops and natural plant varieties has led to develop procedures to track pollen particles [35]. Many airbone fungal species can cause severe plant and animal diseases resulting in large losses in agriculture and cattle raising; and porcine epidemic diarrhea and foot-and-mouth disease viruses, also transmitted by aerosols, are a major concern for animal farming [1,34]. Another interesting aspect related to the study of airborne microbiota is the biological deterioration or biodeterioration, triggered when certain microorganisms colonize the surfaces of materials such as those in monuments and buildings of important cultural heritage. The risks of biodeterioration are not restricted only to external stone surfaces in buildings such as cathedrals [85], but also to cave paintings [83], paper-based historical archives [8] and cinematographic films stock [106].

Recent studies have suggested that air is not merely a dissemination path for the biota (bacteria, viruses, fungi, spores and pollen) coming from other environments (soil, water or plant/animal microenvironments) but that it also has its particular communities of organisms (airbiota), so it should be considered as an ecosystem in itself [40,110]. The atmosphere can be seen as an extreme environment due to its chemical and physical characteristics, such as extremes of temperature (both low and high), solar irradiation (especially the UV part of the spectrum), desiccation, and the presence of strong chemical oxidants (e.g., ozone, hydroxyl and nitrate radicals), low nutrient availability and its large dispersing potential [39,40]. Under such conditions, the main activity in the airbiota ecosystem is expected to be bacteria-related. Because of their small sizes and ease of reproduction, bacteria can remain in the airspace for days or weeks, long enough to complete reproductive cycles. Accordingly, fog and cloud droplets have been proposed as an atmospheric niche for bacterial growth, providing nutrients as well as protection against desiccation and UV radiation [104]. However, the knowledge on the distribution of bacteria in the atmosphere is still limited in contrast to other environments to confirm this theory. For instance, Tamames et al. [101] studied the environmental distribution of prokaryotic taxa using 16S rDNA sequences from 3,502 sampling experiments in natural and artificial sources. They identified selective environmental factors that could explain bacterial distribution in most cases: particular conditions in animal tissues and in thermal locations, or salinity were found to be major constraints on prokaryotic diversity, while soil and freshwater habitats would be far less restrictive environments. Unfortunately, data for air habitats in that study were inadequate due to insufficient sampling, and more studies are needed to draw any conclusion. Thus, so far, it is still unclear whether airborne bacteria are an actual ecological community or only a pool of organisms passively gathered together from different sources.

Last but not least is the ability of biological particles as agents to cloud condensation nuclei (CNN) and/or ice nuclei (IN). Nucleating particles are necessary to induce water vapour condensation to form liquid water (CCN) or to trigger crystallization of supercooled water (IN). For a long time, it was assumed that only inorganic particles such as those making up dust were responsible for CCN and IN in the atmosphere. However, in the 1970s the presence of biological IN was discovered. Maki et al. [61] identified *Pseudomonas syringae* as a responsible agent for IN, and it has been used as model organism of atmospherically relevant IN active bacteria. The contribution of pollen, fungi, and bacteria to atmospheric CCN and IN has been studied in more detail over the last few decades. Some works have characterized bacteria isolated from rainwater using specialized and sterile devices. Joly et al. [53] found that 2.7% of cultivable strains were ice-active at ≤ –8 ºC, and Šantl-Temkiv et al. [86] observed that approximately 12% of cultivable bacteria caused ice formation at ≤ –7 ºC. These bacteria had probably been emitted to the atmosphere from vegetation or terrestrial surfaces, e.g., by convective transport. In both studies the main bacteria isolated were *Pseudomonas* spp., which is consistent with other reports from aerosols, clouds and fog [2,11]. Steiner et al. [100] and O'Sullivan et al. [70] have shown that small pollen particles (SPP) can contribute to CCN and IN, influenced by pollen concentrations and the number of SPP generated from a single pollen grain. With regards to fungi, Spracklen and Heald [99] found that fungal spores and bacteria contributed very little (0.003%) to global average immersion freezing IN rates, which were dominated by soot and dust. This findings concur with global modeling studies that found bio-

Fig. 1. Air collector placed on the roof of the Higher Technical School of Industrial Engineering, Madrid. The octagonal dome, 42 m high, was built in 1881–1887, originally to host the Palace of Arts and Industry of Madrid. The School was located in the building in 1907. (See cover and page A2 of this issue.)

logical particles to be non-significant as a source of IN at global scale [51]. Even so, biological particles may be important at altitudes between 4 and 7 km, where the contribution of IN of biological origin becomes dominant at temperatures warmer than –15 ºC whereas mineral dust particles are typically considered to be the major contributor below this temperature [24,66].

Huffman et al. [52] and Schumacher et al. [89] suggested that bioaerosols might also play a major role in midlatitude semi-arid forest ecosystems, which is consistent with the observation that biogenic emissions significantly impact CCN in the region [58]. Accordingly, deforestation and changes in land use and biodiversity might have a significant influence on the abundance of IN, the microphysics and dynamic of clouds and precipitation in these regions, and thus on regional and global climate [25].

Thus, far from being airborne inert matter, biological particles have a relevant role in the atmosphere with consequences for health, economy and meteorology, although further studies are necessary to clarify their role.

Variability of biological communities in atmospheric aerosols

Geographic patterns, global dispersion and spatial variability. We have a limited understanding of both how airborne communities vary across different geographical regions and what are the factors that determine their patterns across large scales [110]. The nature of the biological particles found above terrestrial areas is likely to be different from that of biological particles found above oceans. For example, bioaerosols in marine environments can be expected to be rich in bacteria while, over land, they should be rich in pollen and fungi, in addition to bacteria. Oceans cover more than 70% of the global surface and have a bacterial concentration of 10^4 ml^{-1} in surface waters [16]. It is assumed that bacteria coming from this source, where sea spray aerosols are primarily formed at the air/sea interface through bubble-mediated processes, may have a major contribution [6]. The spatial distribution of bacterial populations in marine bioaerosol samples has been investigated using culture-independent techniques by Seifried et al. [90] and Xia et al. [112]. They have reported that the bacterial community in marine aerosols is more diverse than previously thought and the overall biological community is markedly different. Seifried et al. [90] have detected a strikingly abundance of Proteobacteria, which represent ca. 50%, with the genus *Sphingomonas* being the major representative. Xia et al. [112] have also suggested that the composition of the bacterial community would depend on whether the air masses came from the adjacent terrestrial areas or from marine environments. In agreement to this, different studies have concluded that terrestrial bioaerosol samples mainly contain Gram-positive and spore-forming bacteria, whereas marine bioaerosols comprise high abundances of Gram-negative bacteria and lower concentrations of fungal spores than land samples [37,44]. Continental environments

have also their own particularities. According to Shaffer and Lighthart [91], bacterial concentrations in air samples from terrestrial regions are under the influence of the land-use, they having observed differences between forests, rural and coastal areas. In contrast, Bowers et al. [13] have found similar levels of bacteria independently of the land-use types when analyzing samples from agriculture fields, suburban areas and forest. However, their results indicate a wider diversity in rural areas than in cities.

Within metropolitan areas, bacterial concentrations have particularly high spatial variation because they are released form strong points sources in contrast to the more spatially homogeneous release in agriculture areas [26]. We will discuss the state-of-the-art of the knowledge about airborne biological particles in urban areas more extensively in the Part 2 of this review [69].

Some studies have been performed in remote places including Antarctica, such as the one carried out by Pearce et al. [73] using culture-independent techniques. In accordance with the harsh conditions there, the results showed low bacterial biodiversity and many of the DNA sequences belonged to uncultivated microorganisms. Those that could be identified were associated to local origin (research station), but they also found bacteria from distant sources (either marine or terrestrial). These results highlight the strong influence of the meteorology to spread biological communities from their real source.

While airborne bioaerosols have been sampled from diverse locations around the world for many years, many questions remain about the nature of biological "pollution," particularly on the topic of global dispersion and how far bioaerosols can travel from the points of origin [97]. Some aerobiological studies indicate that pollen can be transported up to 100–1000 km [88,98], and evidences for long-range transport of pollen and fungal spore bioaerosols have been also described by Mandrioli et al. [63].

A recent work of Prussin et al. [80] simulated the global transport of atmospheric particles, considering a scenario in which a small virus and a large fungal spore were released at the same time from the top of a 10-m tall building. According to their model, they concluded that the spore would be transported a horizontal distance of less than 150 m before settling to the ground, while the viral particle would be transported nearly 200,000 km. Although too simple, the study leads to remark two important ideas regarding biological particles in the atmosphere: (i) the potential for long-distance transport of very small particles, especially viruses; (ii) the distance transport can be significantly modified by different factors such as aggregation between different biological particles (and also with non-biological matter), adaptations for aerial transport (as some fungal spores or pollen have), and the influence of meteorological factors (wind speed, precipitation). Thus, it is difficult to interpret whether a particular airborne community is representative of the local area where it has been found or a combination of more distant sources.

Additional information can be found in a review of bacterial distribution in the global atmosphere published by Burrows et al. [16], revealing the complexity of the matter.

Abundance vs. altitude. Of all the different layers that form the atmosphere, it is in the troposphere (the lowest layer, reaching a maximum height of around 18–20 km above ground) where biological particles are to be found and where studies on aerobiology are carried out. Nonetheless, some microorganisms have been found also in the stratosphere (the second layer of Earth's atmosphere, between 20 and 50 km above ground).

Within the troposphere, there is a first layer, known as the near-surface or lower atmosphere in which all of the aforementioned biological particles are found (i.e., viruses, fungi, bacteria, pollen, et cetera). Physics suggests that the concentration of these particles will gradually decrease towards zero as altitude increases, the largest ones, such as pollen grains, being the first to disappear. At higher altitudes, in the free troposphere, only smaller biological particles (such as fungi and bacteria and probably viruses) are expected to be found. However, there is no information about the point at which they shall no longer be found and note that pollen grains have been observed up to 2 km [68], supporting the influence of many factors, including atmospheric turbulences promoting vertical transport.

Human exposure to aeroallergens usually occurs at ground level. However, the stations of the aerobiological networks usually operate with samplers placed on the roof of buildings, around 10–30 m over ground level. Several studies, including those performed by Fernández-Rodríguez et al. [31] for pollen and Khattab and Levetin [57] for fungi have examined the abundances of these particles using spore traps set at ground level and rooftops at 12–16 m. Both authors conclude, and it is widely accepted, that pollen and fungal spore counts do not significantly differ between the two locations but some divergences have been observed when different pollen types or fungal spores are separately analyzed. In this regard, Hart et al. [50] analyzed three different heights (12, 24 and 34 m) and reported that the differences in the pollen abundance between the different levels were highly correlated with weather condi-

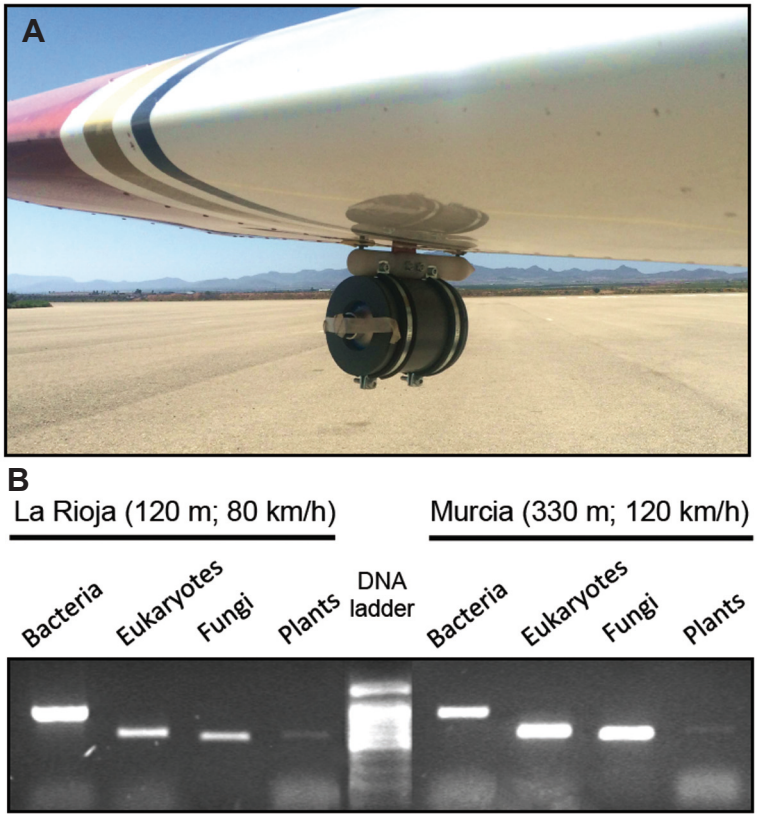

Fig 2. (**A**) Airborne particle collector designed for the Program AIRBIOTA-CM to sample high volumes of air for metagenomic studies. This prototype can be attached to UAVs or any other vehicle as shown in the picture (wing of an airplane). (**B**) Agarose gel showing the results of the PCR to detect the DNA from different groups of organisms obtained with such collector after sampling in the specified locations during 1 h in 2015 summer session (unpublished data). Flights were conducted by Airestudio Geoinformation Technologies S.Coop and Ingeniería Medio Ambiental S.L. (IMA) in La Rioja and Murcia (Spain), respectively.

tions, especially wind speed and rainfall. These results corroborate the hypothesis that recurring meteorological factors favor vertical exchange and long-range transport of pollen grains suggested by Mandrioli et al. [63]. Unfortunately, there is not enough information correlating pollen distribution in height and weather variables to set a conclusion.

For altitudes above towers, the use of light aircrafts (Fig. 2), air balloons and more recently unmanned aerial vehicles (UAVs), have been proposed. UAVs can fly below 1000 feet, altitude at which light aircrafts can be operated; hot air balloons have been used for some studies at higher altitudes. Fungal spores have been detected by culture and microscopy in air samples collected using UAVs at 25–45 m [103] and 320 m [87]. West and Kimber [107] recently reviewed the use of UAVs for agronomic studies for plant pathogens monitoring.

At altitudes of 10 km, DeLeon-Rodriguez et al. [22] have found, by quantitative PCR (qPCR), that bacterial abundance at this height (5.1×10^3 cells/m^3) is at least two orders of magnitude greater than that of fungi, which suggests that particles with sizes similar to those of bacterial cells (0.1–3 µm) tend to stay longer in the atmosphere than larger particles, such as fungal cells and spores (typically >3 µm in diameter). These conclusions have been intensely questioned by Smith and Griffin [94], and replied by DeLeon-Rodriguez et al. [23].

At altitudes of 20 km, at the interface between the troposphere and the stratosphere, Griffin [42] and Smith et al. [96] identified the presence of viable microorganisms by using culture techniques. They found bacteria of the genus *Bacillus* and the fungal genus *Penicillium*, which are common in terrestrial and aquatic environments. All of the isolates identified were spore-forming pigmented fungi or bacteria of terrestrial origin, which suggested that the presence of viable microorganisms in the Earth's upper atmosphere might not be uncommon. In fact, spores can protect microorganisms from physical stresses such as UV radiation induced DNA damage, dryness, and extreme temperatures. Although both studies provide evidence for the long-distance, stratospheric level transport of microbes across the open oceans, this does not prove the existence of an independent airborne microbial ecosystem.

Lastly, Smith [93] reviewed the presence of microorganisms in the upper atmosphere (from 21 km to 77 km), noting the lack of publications about stratospheric microbiology missions, mainly due to the difficulties in obtaining sufficient biomass to study (morphology, culture or DNA analysis). He remarked that while astrobiology experiments in space are expensive and occasional, many fundamental questions about life in the uni-

verse could be studied in our own atmosphere. At the very least, studying life in the limits of our atmosphere can provide a better comprehension about the diversity, distribution, and evolution of life on Earth. Furthermore, radiation-resistant cells found in the upper atmosphere could help us to identify genes or enzymes that provide such great endurance [93].

So far, most studies at different altitudes have been conducted using culture methods. The biases introduced by culturing have been well documented for other environments and ecosystems, but the limitations are even more pronounced with air samples since microbes can be damaged or inactivated by desiccation and irradiation during atmospheric transport [95]. In the next few years, next-generation sequencing will confirm the biodiversity and relative abundances of the different biological particles as a function of altitude, although new devices and procedures must be developed to face this challenge.

Meteorological factors

As exposed above, air samples collected from different locations may differ with respect to the relative abundances of specific biological particles. Moreover, samples from a same location may vary significantly in abundances and biodiversity throughout a given season but also in a short time (days or even hours). These differences are probably caused by changes in regional and local meteorological factors such as temperature, wind speed, and rainfall. Temperature, set by global location and time of the year, is probably one of the most influential factors. An extensive review about the influence of meteorological factors on atmospheric bioaerosols was published by Jones and Harrison in 2004 [54]. Here, we review more recent publications, mostly based on DNA-sequence analyses, to extract some common conclusions.

Temporal variability. Many works have been conducted that studied the seasonal changes of bioaerosols in different locations, especially by analyzing bacterial diversity. In general, microorganisms are less abundant in the air during winter [5,12,14], which can be explained by the unfavorable weather conditions for bacterial growth and changes in the emission sources (plants, soil and water), as suggested by Bowers et al. [12,14]. Nevertheless, not only changes in bacterial abundances but also in diversity between seasons have been observed by Tanaka et al. [102] in a survey over one-year period. Franzetti et al. [36] studied the bacterial communities associated with airborne particulate matter, finding large seasonal variations, with plant-associated bacteria dominating in summer and spore-forming bacteria in winter. Those findings were supported by other authors including Brodie et al. [15], who suggested that warm temperatures might increase dehydration in soil/plant-related bacteria, inducing spore formation and their aerosolization.

Regarding fungal variability, Pashley et al. [72] studied by restriction fragment length polymorfism (RFLP) analyses the fungal variation in the atmosphere in three selected days representing dry and wet summer periods. They found differences not only in the fungal composition but also in the abundances of clones, which suggested a clear influence of the meteorology.

Oliveira et al. [71] conducted a 2-year study to analyze the influence of several environmental factors on fungal spores composition in the air in two cities of Portugal. They found higher abundances of spores in summer and autumn, with the lowest peak during winter months. Their results classified the fungal spores in three types based on their peak of abundance: summer (with *Alternaria* and *Cladosporium* as main representatives), spring-autumn, and late spring-early summer spores. Within the selected spore types under study, they concluded that summer spores correlated positively with temperature and negatively with relative humidity and rainfall. The opposite was determined for spring-autumn spores, and no correlations with meteorological factors were found for *Aspergillus/Penicillum* spore type. These results agree with the study performed by Grinn-Gofroń and Bosiacka [45], in Szczecin, Poland. They reported that air temperature was the most influential variable to explain the variation in airborne spore composition, the allergens *Alternaria* and *Cladosporium* being the most abundant at high temperatures, while there was a negative correlation with relative humidity. These examples show the complexity to study the influence of meteorological factors, since different fungi react inversely to variations of the same factor. Nonetheless, they clearly show the influence of environmental factors on fungal spore dispersion. The most seasonal variation in biological particles, however, is probably represented by plants. Pollen spectra change dramatically over the year: while some pollen types appear, some others disappear completely because of different phenological stages. For instance, in Madrid (Spain), poplar and elm trees (genera *Populus* and *Ulmus*, respectively) have a peak of pollination in winter, and pollen grains of these two genera are not present in the regional atmosphere at a different times of the year [47]. Current studies about the influence of meteorological factors in pollen spreading usually focus on one particular pollen type, specially those with allergenic effects,

concluding that the pollination calendar can extend or shorten depending on factors such as temperature and rainfall, and the abundances of pollen in a particular area are highly influenced by wind speed and direction [81,84].Changes in meteorological factors over the seasons correlate with fluctuations of several concomitant factors such as temperature, relative humidity, and solar radiation [54]. Consequently, the single influence of any of these factors on the airborne biological particle is difficult to analyze.

In addition to seasonal changes and fluctuations in shorter periods of time have been observed. Lighthart and Shaffer [60] suggested that the bacterial abundance could be split into five spans during the day, the lowest concentrations occurring before dawn and the highest during the morning. Diurnal variations were also observed by Fang et al. [30] in China, but with the opposite pattern, and Fierer et al. [33] described significant changes in bacterial and fungal relative abundances in a 10-day span. However, Polymenakou [76] and Womack et al. [110] concluded that, despite short-term fluctuations, the microbial composition tended to remain steady throughout the year.

The relationships between the different biological particles have been scarcely explored. Whon et al. [108] studied the presence of airborne viruses and bacteria over several months, always observing higher abundances of viruses. Bowers et al. [10] studied, by culture-independent analyses, the relative abundance of bacteria, fungi and pollen associated to particulate matter over a year. They found that bacteria were always the dominant organisms in two different locations, with fungal relative abundances peaking during spring and summer months. In addition, Fierer et al. [33] detected significant daily changes in bacterial and fungal abundances, inverting their relative importances. All these results were probably influenced by weather conditions and local sources of organisms. Therefore, additional studies are needed to reach a conclusion.

Wind as responsible for transport of biological particles and dust. Wind is the main factor responsible for releasing and transporting biological particles from terrestrial environments and also promotes the formation and spread of marine bioaerosols. Wind speed initially contributes to the release of spores and bacteria from the surfaces of plants and soil, and to their dispersion. The typical threshold wind speed required to remove biological particles from the ground (3.0–5.4 m/s) is greater than that necessary to remove them from plants (0.5–2.0 m/s) [21]. However, for the vast majority of fungi, there is no information about the strength of the spore attachment and the values of the threshold wind speed required [65].

Local wind direction was largely responsible for changes in the concentrations of different airborne biological particles. Di Giorgio et al. [27] described that bacterial concentrations increased with wind speed, and Rojo et al. [84] found that pollen spectra were governed by the location of pollen source and wind direction.

High wind speeds favor that marine bacteria are ejected into the air along with sea spray aerosol particles [67]. Moreover, it has been observed that the wind speed is related with the wind direction. Polymenakou and Mandalakis [77] identified higher numbers of marine bacteria-associated sequences when south winds crossed the inland of Crete, while the opposite was observed when north winds passed over the Aegean Sea. This discrepancy could be partly explained by the fact that north winds were blowing at very low speed (11 ± 6 km/h), which constrained the formation of sea-spray aerosol and the ejection of marine microbes from sea surface to the atmosphere. In a similar way, Fahlgren et al. [28] described, in a sea coast of Sweden, higher abundances of airborne bacteria in winter, contrary to other authors, who observed the opposite [5,12,14]. However, they explained that this controversial results might be due to the wind speed over the sea source regions (upwind of the sampling site), which was higher in winter than in summer in that particular location.

Consequently, aerobiological studies must take into account variables such as wind speed and direction, which can change the results and conclusions dramatically.

Strong winds over arid lands can lift dust above the boundary layer and transport it several kilometers or even more than 5000 km before settling it, depending on the particle characteristics (size, chemical composition) and the air-mass properties (e.g., velocity, density, height). The intercontinental transport of millions of tons of desert dust per year has been studied for decades, but research on the biological particles traveling between continents with the dust started recently (reviewed by Kellogg and Griffin [56]). Along with organic and inorganic nutrients bound to dust minerogenic particles, biological particles are mobilized from the arid soil and transported over long distances [44,78], and might include allergens and pathogens as well as pollutants. Therefore, transport of desert dust is believed to play a major role in many geochemical, climatological, environmental and health processes.

In general, most studies comparing dust events with non-dusty events describe higher abundances of the biological particles and a greater diversity of the microbial community structure. Thus, Griffin et al. [43] found that the abundances of airborne microorganisms can be 2–3 times those found during “African dust-events” in the Caribbean, observing an

order of magnitude increase in dust-associated viruses versus background. Correspondingly, concentrations of bacterial cells and mineral particles were ten-fold higher during Kosa (Asian dust) event than in non-Kosa event days [62]. Even Katra et al. [55] found higher numbers of bacterial and eukaryotic OTUs that were unique when the organismal diversities of dust from two storm events separated by 18 days in southern Israel were analyzed, also implying different origins of the biota.

Besides viruses and bacteria, dust storms can contribute with foreign fungal and pollen diversity. Because of their sizes, it is improbable that they are carried attached to dust particles; they must be dragged by the air mass and transported along with the storm. Similar to the results for bacteria, it is widely accepted that these events lead to an increase in fungal concentration and diversity [46,111]. Cariñanos et al. [18] also detected pollen from five non-native plants exclusively during dust events from North Africa to Spain via Saharan dust, which supported that not only microorganisms would be submitted to dust storm transportation.

The study conducted by Polymenakou et al. [75] found a correlation between particle sizes and microbial community structure during a dust storm in Crete, Greece, which suggests the existence of a preferable particle-selection by microorganisms to travel. Additionally, they observed that a large fraction of microorganisms at respiratory particle sizes (<3.3 μm) were phylogenetic neighbors to human pathogens.

Because of the character extremophile of the atmosphere, most bacteria may not survive during their long-range transports. In agreement, Hara and Zhang [49] found that the bacterial viability in long-range transported dust was less than 40%, whereas in non-dusty air it was more than 76%. Despite such differences, the final viable bacterial concentrations were comparable or even higher in long-range transported dust than in non-dusty air because of the great input. Note that they found a quantitative relation between coarse particles (diameter >1.0 μm) and viable bacterial cells for dust samples, which suggested a protective effect of the large particles towards the microbial community.

In parallel, Cao et al. [17] studied the microbiota associated to air pollutants $PM_{2.5}$ and PM_{10} (particulate matter with diameters less than 2.5 and 10 μm, respectively) during a severe smog event in Beijing, China. They found that the relative abundances of bacteria, archaea, fungi and viruses seemed to rise with the increase in PM concentration. The authors suggested that this could represent a recently evolved transfer-mechanism supporting and increasing their natural dust-mediated dispersion.

Ratios of airborne biological particles and air genome

Ratios of airborne biological particles. Ratios or proportions between different microorganisms are frequently used in environmental studies. The most common are the bacterial/fungal ratio (BFR) and the virus/bacterial ratio (VBR). The BFR for air samples has been estimated based on the microbial abundances of several works. Di Giorgio et al. [27] observed that BFRs were different in urban and natural areas. In the metropolitan spaces, BFR held steady at 4 during the whole year, while in the rural areas this value was around 2 from October to March (fall and winter) and ranged from 1 to 0.4 from March to October (spring and summer). However, Haas et al. [48] found that the levels of fungi in an urban environment (the city of Graz, Austria) changed from equal to higher than those of bacterial levels (BFR ranged from 1 to 0.06). Even a higher variation was described by Fierer et al. [33], with bacterial/fungal ratios that ranged from 8 to 0.08 throughout the five sampling days in the University of Colorado.

The BFR also varies with altitude and the studies performed at a height of 10 km by DeLeon-Rodriguez et al. [22] yielded results that implied that this altitude was much more favorable for bacteria (BFR = 29). Overall, BFRs for airborne samples seems to vary between 0.06 and 29, clearly influenced by location, altitude and seasonal factors. We calculated a fungi/pollen ratio (FPR) estimation of 100, indicating higher concentrations of fungal spores than of pollen grains in a boreal forest in Finland, based on data from Manninen et al. [64] .

The VBR is usually employed to describe the relative abundance between viruses and bacteria. By using fluorescence microscopy, Prussin et al. [79] determined a VBR value of 1.4 from outdoor air samples collected during September and October in (Blacksburg) Virginia (USA), indicating that ca. 40% more viruses than bacteria were present in the air. Whon et al. [108] examined outdoor air in Korea and found an average VBR of 2.2 while Griffin et al. [43] observed a VBR of 1.3 in the Caribbean air. Considering that these values are not directly comparable because different methodologies were applied, and that the VBR in other environments can range dramatically from 0.2 (in the human gut) [82] to 2750 (in agricultural soil) [109], the VBRs obtained from air samples could be a very good approximation to the real value, although further research is required to confirm it.

DNA in the atmosphere, or air genome. Studies on the biodiversity and abundances of biological particles in the atmosphere have been hampered by the low concentra-

Table 1. Estimation of air volumes required for air genome studies

	Biological abundance in the outdoor atmosphere	Genome size (Mb)	DNA concentration (pg/m^3)	Air required (m^3) to sample for 1 ng DNA
Pollen	$10–10^4$ grains/m^3 (Gutiérrez-Bustillo et al., 2001) [47]	$5.8 \times 10^2–1.2 \times 10^5$ (Gregory et al., 2007) [41]	$5.8–1.2 \times 10^6$	$8.3 \times 10^{-4}–1.7 \times 10^2$
Fungi	$10^3–10^4$ spores/m^3 (Codina et al., 2008) [20]	$9.0–8.1 \times 10^2$ (Gregory et al., 2007) [41]	$9.0–8.1 \times 10^3$	$0.12–1.1 \times 10^2$
Bacteria	$10^4–10^6$ cells/m^3 (Lighthart, 2000) [59]	0.58–10 (Claverie et al., 2006) [19]	$5.8–1.0 \times 10^4$	$0.10–1.7 \times 10^2$
Viruses	$1.7 \times 10^6–4.0 \times 10^7$ VPLs/m^3 (Whon et al., 2012) [108]	0.002–2.5 (Whon et al., 2012) [108] (Philippe et al., 2013) [74]	$3.4–1.0 \times 10^5$	$0.01–2.9 \times 10^2$

tions of biological particles per m^3 of air, especially for culture-dependent methods. Among the main airborne biological particles, i.e. pollen, fungi, bacteria and viruses, the latter are the most difficult to study because (a) their sizes are orders of magnitude smaller than those of the other airborne biological particles, and (b) they are obligate intracellular parasites and therefore the use of culture-dependent techniques are limited by our knowledge about the host of each specific virus—the viability of the viruses during collection turns a major issue.

In the case of a metagenomic approach, viruses do not share a common marker gene, such as 16S ribosomal DNA in bacteria, or internal transcribed spacer 2 (ITS2) in fungi. So, a "shotgun" sequencing procedure is required to study viral community structure. Additionally, the extraction efficiency of nucleic acid from viral particles is usually low, making it even more difficult to obtain enough DNA. Former NGS technologies required 1–5 μg of DNA to prepare a shotgun-sequencing library, while current kits have reduced remarkedly this amount to ca. 1 ng of viral genomic material [80].

Assuming high concentrations of airborne viruses such as those found by Whon et al. [108] in Korea, ($1.7 \times 10^6–4.0 \times 10^7$ viruses/m^3), and according to our estimations (Table 1), it would be necessary to sample 290 m^3 of air to recover 1 ng of DNA to perform a viral metagenomic study.

Obviously, it is possible to find lower concentration values in air than those listed in Table 1 due to meteorological factors, seasonal variations, height, location, et cetera. In these cases, sampling larger volumes of air would be required to obtain enough DNA but fast advances in sequencing platforms lead to require less and less genetic material for the analyses.

Conclusions

Outdoor air contains plenty of biological particles from different sources, mostly bacteria, fungi and spores thereof, pollen and viruses. Some of these organisms, or just parts of them, can cause allergies, disorders and diseases in humans and other animals, with important health and economical consequences. They have been also suggested to influence weather conditions and have a role in climate change. Therefore, monitoring and broadening our knowledge about their distribution and global and local patterns should be a priority. It is a complex matter and so are the challenges to face. Oceans, forests, urban spaces, et cetera have different point sources of bacteria, fungi, pollen and viruses, which vary substantially from one environment to another, not only in concentration but also in the diversity of each group of organisms. In addition, meteorological factors such as temperature or wind affect dramatically to their air transport, and they may undertake major temporal changes. As a consequence, sampling organization is crucial to obtain valid representative results.

Ideally, all the biological particles should be analyzed together. So far, most studies analyzing the diversity and/or distribution of airborne biological particles focus on one only type of them, e.g. bacteria, fungi or pollen, separately. Works considering two or more usually provide ratio information (VBR, BFR, FPR), which is a good advance to consider the atmosphere as a particular ecosystem as has been proposed by some authors. The limitation to confront an integral study of all the biological particles has been set mainly by methodology. Traditional methods in aerobiology and microbiology applied to the study of air samples are based on microscopy and culture-dependent techniques; they are time-consuming, require great expertise for visual identification, and the bias of culturing must be assumed. Moreover, the low concentrations of airborne biological particles require the analysis of high volumes of air, which is limited by the samplers, time and the collection surface. Nevertheless, new tools and devices, such as the above mentioned UAVs, offer new alternatives.

Thus, metagenomics emerges as a promising solution for

many of these difficulties. The development of DNA sequencing technologies has made it possible to detect all the organisms in a sample independently of their viability. The great sensitivity of such techniques allow to detect even minor representatives in the samples. The application of high-throughput DNA sequencing to study airborne biological particles will be addressed in Part 2 of this review [69].

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