[Frontiers in Bioscience E3, 772-787, January 1, 2011]

Airborne Algae and Cyanobacteria: Occurrence and Related Health Effects

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1. ABSTRACT

Published information on airborne algae and cyanobacteria worldwide and the related human health effects is scarce. Since 1844, a total of 353 morphological taxa (genera or species) have been identified in aerobiological studies. However, due to diverse methodologies and different microorganisms targeted in these studies, direct comparisons on the occurrences of airborne algae and cyanobacteria in various studies are rather dubious. Thirty-eight airborne algae and cyanobacteria were shown to induce allergy, skin irritation, hay fever, rhinitis, sclerosis and respiratory problems when aerosolized and inhaled. Another 14 airborne taxa are known toxin producers posing threat to human health. Most frequently associated with health effects are the genera *Chlorella*, *Scenedesmus, Chlorococcum*, *Klebsormidium* (*Hormidium*) and *Lyngbya.* In the air of the Mediterranean city of Thessaloniki, we found 63 algal and cyanobacterial taxa, with 21 reported for the first time in the air. Seven taxa were potentially harmful. Algae and cyanobacteria can contribute significantly in the total air particle load, rendering them as causative agents for health issues when inhaled.

2. INTRODUCTION

Back in 1997, a few days before Hurricane Nora hit the California coast, it was already known that marine plankton occurs in sea spray and, thus, it can be found in high altitudes in the atmosphere. Some time later, Sassen *et al.* in 2003 reported that they had observed marine plankton cells in the upper troposphere as “nuclei” of ice crystals in the cirrus clouds of Hurricane Nora (1). This extreme paradigm indicates the potential effect of the air-water interface on remote areas. Atmospheric motion can affect the dispersal of aquatic microorganisms away from their source, whether it is pelagic or benthic habitats. Wind can have severe impacts on the nearby areas of water bodies – according to its direction in relation to the coastline (2) or lakeshore (3) – if a harmful algal or cyanobacterial bloom occurs. Furthermore, in the land-water interface zones especially in shallow lakes (4), wind may also resuspend soil or sediment microorganisms including harmful algal cysts (5), releasing them into the air.

There is ample information on the temporal distribution of algae and cyanobacteria in source pool areas (freshwater and marine systems). However, whether there is a temporal pattern in the aerial movement of these microorganisms and which mechanisms are involved, still remain unknown. Who is taking off, who is flying and who eventually lands and establishes itself in the arrival area, is yet unidentified. Such basic ecological questions are of immediate interest to the health sector, since bioaerosols include potentially harmful algal and cyanobacterial cells. General hypotheses governing the atmospheric flow of biota have recently been proposed in the new focus of aerobiological research (6).

The hunt for airborne organisms that could cause disease initiated as early as during 1861 – 1882, the period known as the "Golden Age of Aerobiology" (6). Over the past years, the scale and significance of air quality impacts on human health have been stressed out by scientific research. Air pollution related health effects are one of the biggest environmental issues worldwide (7). Several studies and reviews on human health impacts by coarse and fine particulate matter (PM) and various aerosols have been published (e.g. 8 – 11) whereas the atmosphere has been recognized as a "spora" of microorganisms, including viruses, bacteria and microalgae, which can induce allergies and airborne diseases (12). However, relatively few studies have focused on bioaerosol associations with adverse human health effects (e.g. 13, 14). Even scarcer are the data on airborne algae and cyanobacteria and related health effects, although their ecological and economical importance has long been recognized (15). The limited information in the literature concerning airborne algae and cyanobacteria and the threat for air quality and human health is reviewed here. This paper serves a double purpose: (a) to present the current knowledge on airborne harmful algae and cyanobacteria and (b) to use original data from a case study in Greece emphasizing on airborne algal and cyanobacterial occurrence and the possible risks for air quality and human health.

3. SEARCH CRITERIA

In this review all references that could be found in the scientific literature until June 2010, involving studies on the occurrence of airborne algae and cyanobacteria and the adverse health effects certain taxa may cause in humans, are presented. Studies presented in this review were identified by the following strategy: PubMed (www.ncbi.nlm.nih.gov/pubmed/), Web of Science (http://apps.isiknowledge.com) and Scopus (www.scopus.com) electronic databases were searched with the MeSH and textword string "(air* AND alga*) OR (air* AND cyanobacteria*) OR (air* AND microorganism*) OR (airborne alga* AND health) OR (airborne cyanobacteria* AND health) OR (bioaerosol* AND health) OR (atmospher* AND microorganism* AND health) OR (air* AND microorganism* AND health) OR (air* AND toxin*) OR (aerosol* AND toxin* AND health) OR (allergy AND alga*) OR (allergy AND cyanobacteria)". An additional search in Google.Scholar (http://scholar.google.com) using the same key words was performed. Bibliography of collected primary papers and related review articles was examined for references not found in the above electronic sources. Books including taxonomic volumes and anecdotal reports were excluded from the search.

4. OCCURRENCE OF AIRBORNE ALGAE AND CYANOBACTERIA

The occurrence of airborne algae has long been acknowledged since the publication of Ehrenberg in 1844 (16), who identified 18 algae belonging to diatoms from air dust samples collected by Darwin when traveling in the Atlantic Ocean. Since then, only sporadically the scientific community has focused on airborne algae and cyanobacteria, in contrast with the respective studies on airborne (heterotrophic) bacteria (e.g. 17), viruses (e.g. 18, 19) and fungi (e.g. 20 – 22). Most of the available studies investigated the composition and seasonal variation of airborne algae and their relationship with meteorological factors (23).

Meier and Lindbergh in 1935 identified for the first time green algae and diatoms in high altitude, captured on slides after being exposed for at least half hour during an aircraft voyage over Greenland (24). Almost half a century later, Saxena investigating the role of nuclei from biological origin in the formation of clouds over Antarctica found the chlorophyte *Planctonema lauterbornii* (25). Broady and Smith (1994) during preliminary investigations on the diversity and dispersal of algae introduced into Antarctica by human activity, discovered low dispersal of algal propagules through the air (26). Elster *et al.* in 2007, only sporadically found colonies resembling the cyanobacterium *Merismopedia* sp. in Antarctic aerosol samples, while no culturable micro-photoautotrophs were present (27). However, Marshall and Chalmers (1997), in their long-term aerobiological study in Antarctica found chlorophyte propagules as well as cyanobacteria filaments (28), confirming the suggestions that particles can be transferred over long distances to Antarctica through air currents and wind (29, 30).

Van Overeem in 1937 was the first to attempt to recover cultivable airborne algae from samples of air at 2000 m and lower. He found in total nine different genera with the most abundant being the chlorophytes *Chlorococcum* and *Chlorella* (31). Gregory *et al.* in 1955 were the first to measure the concentration of an airborne cyanobacterium in the air. They calculated the abundance of the cyanobacterium *Gloeocapsa* sp. in air samples from three locations in England, revealing high numbers (32). In 1961, Schlichting identified 22 taxa in air samples from Michigan, Texas and North Carolina, belonging primarily to chlorophytes and cyanobacteria, while diatoms, chrysophytes and euglenes were rarely present (33). Brown *et al.* in 1964, by using different sampling methods, like exposed Petri dishes with culture medium and filtering, unveiled airborne algal and cyanobacterial diversity from different locations and different altitudes in Texas and 21 other states of the USA. They composed a taxa list of 62 genera with the most abundant and diverse group being chlorophytes. This study indicated that most airborne algae and cyanobacteria are derived mainly from soil (34). Furthermore, soil origin of most airborne chlorophytes and cyanobacteria recovered from selected agarized culture media was shown in Hawaii islands (35). Cyanobacteria were the dominant group in an aerobiological survey in Delhi area, India and consequently were considered the more resistant group to unfavorable conditions of the atmosphere (36). Folger in 1970 (37) recovered only freshwater diatoms, belonging to the genera *Cyclotella* and *Melosira*, in air samples collected during two crossings of the North Atlantic between Barbados-Gibraltar-

In 1987, the abundance and heterogeneity of algae in the Mexico City atmosphere was examined (38). The study indicated the importance of soil and subaerial chlorophyta and cyanobacteria as a source of the city's airborne photosynthetic microorganisms.

Maguire (1963) in an attempt to understand colonization processes of newly formed aquatic systems by small organisms placed beakers with sterile water in various distances from a primary source. He discovered, among other organisms, unicellular chlorophytes, which could not identify, proposing wind and animal transfer of algal cysts and propagules (39). Earlier, Messikommer (1943), examining the importance of wind on the transport of microscopic organisms, found various taxa growing in experimental containers exposed to the air, with most common being the chlorophytes *Chlorella* spp. and *Chlorococcum* spp., the diatom *Nitzschia palea* and the cyanobacterium *Nostoc sphaericum* (40).

The importance of meteorological conditions, such as wind velocity and direction, rainfall, hours of sunshine, air temperature and relative humidity, on the diversity and dispersal of airborne algae has been examined in different areas around the world. Gislén (1948) was the first to discuss on how the atmospheric conditions could affect the survival and dispersal of microorganisms, including algae (41). Schlichting composed lists of a total of 54 taxa including chlorophytes, diatoms, cyanobacteria and chrysophytes while studying the meteorological conditions affecting the dispersal (42) and the diversity (43) of airborne algae in Michigan and North Carolina. Smith in 1973 found mainly chlorophytes and a small number of cyanobacteria investigating the effects of meteorological conditions and various air pollutants on airborne algae. He concluded that meteorological conditions play an essential role in the survival and dispersal of airborne algae (44). Carson and Brown in 1976 noted that meteorological conditions are important for the release of algae from their natural environments, their survival in the atmosphere as well as their deposition and settlement to new habitats (45). Furthermore, the meteorological effects on variation of airborne algae in Mexico were examined in a study carried out by Rosas *et al.* in 1989. The results suggested that the meteorological conditions affecting most the aeroalgal community were rainfall, humidity, temperature and wind (46). However, Wee in 1982, while sampling in various locations in Singapore and Malaysia, concluded that no correlation was obvious between rainfall and airborne algae abundance, except negative effect of heavy rainfall. Cyanobacteria followed by chlorophyta were mainly recovered (47). Roy-Ocotla and Carrera in 1993 for the first time applied preprocessors of atmospheric parameters in order to assess the relationships between airborne algae and atmospheric conditions and to outline the key factors affecting the composition of the aeroalgal community. The combination of all parameters provides more comprehensive view for the presence and dispersion of algae in the atmosphere than each one of the meteorological parameters alone. They suggested that the atmosphere selects a distinct fraction of algae, which are present virtually everywhere, similar to those found in previous studies in different regions. They proposed that the ideal airborne alga, which can be dispersed independently of environmental conditions, is similar to a *Stichococcus*-like type (48).

Recent studies on airborne algae include the works done by Sharma and colleagues. They investigated the diversity and seasonal variation of viable algal particles in the atmosphere of Varanasi city in India (49) as well as the relationship between meteorological factors and airborne algal diversity (50). Hall in 1998 recovered a single colony of the chlorophyte *Pediastrum boryanum* in a pollen trap from Santa Fe, New Mexico. He proposed that the colony was transported from the nearby Cochiti Lake, suggesting that *P. boryanum* has the ability to tolerate desiccation and the severe atmospheric conditions (51). Tormo *et al.* (2001) in a quantitative investigation of airborne algae obtained from pollen traps in Badajoz City, Spain, found mainly chlorophytes and diatoms. However, the method of air sampling used was suitable only for the detection and identification of large-sized algae (52). García-Mojo *et al.* in 2004, while air sampling in Adirondack Park region, the largest public protected area in USA, found airborne algae, which could not identify (53). El-Gamal in 2008 in air samples from different areas of Cairo, Egypt, recovered 23 aerophytic cyanobacterial species (54). Air-dispersed phytoplankton diversity and colonization potential were investigated in a Mediterranean river-reservoir system (3), unveiling mostly taxa of the local phytoplankton community in addition with cosmopolitan ones, commonly mentioned in aerobiological research. Nanoplanktic algae and among them the known allergenic chlorophyte *Chlorella* were the most frequent air-dispersed alga. Over short distances from the origin reservoir pool (less than 1 km) the wind was an important agent for the dispersal of phytoplankton organisms including the bloom-forming toxic cyanobacterium *Microcystis aeruginosa*.

An important agent for air transport of algae, that only sporadically has been investigated, is lichen photobionts. In particular, lichen soredia, which contain coenobia of algae, have been found to disperse mainly by wind (55). Marshall in 1996 (56), during an aerobiological monitoring programme, that was carried out for over a year on Signy Island, South Orkney Islands, Antarctica, found that lichen soredia were the most abundant airborne propagules, even more abundant than ascospores. Tormo *et al.* in 2001 found a total of 213 soredia in samples from pollen traps in Badajoz City, Spain, containing algal cells assembled in various groups, each group with 6-14 cells (52).

Table 1 presents all airborne algal and cyanobacterial taxa reported by investigators of aeroalgal communities included in this review. A total of 353 morphological taxa (genera or species) have been recognized, belonging to 175 genera. The majority of airborne taxa belongs to the Cyanobacteria (37.4% of the total number), followed by Chlorophyta (35.4% of the total number). Other taxonomic groups include Bacillariophyta (15.3%), Streptophyta (4%), Dinophyta, Euglenophyta, Haptophyta and Cryptophyta (Table 1). Chlorophyta appear to dominate the aeroalgal community of temperate areas (34, 43), while Cyanobacteria dominate the tropical regions (43). Successful air-dispersal of algae is supported by their life-styles including dormant life-stages, such as cysts, which allow them to survive in atmospheric conditions (57, 58). Among the 353 airborne morphological taxa of water and soil – rock origin, some are closely linked to tree barks and belong to the group of lichenized algae (e.g *Klebsormidium dissectum, K. flaccidum*) (59).

Freshwater algae and cyanobacteria, comprising benthic and planktic taxa, are present in higher frequency and greater numbers in the atmosphere than marine taxa. Only 13 out of 175 reported genera in the reviewed literature are known from marine phytoplankton (Table 1). This can be attributed to two reasons. First, there is a bias towards investigations of airborne algae in mainland, countryside or urban areas. Rarely studies have focused on collecting algae only of marine origin. Preliminary observations on marine airborne phytoplankton were carried out for the first time by Stevenson and Collier in 1962 (60). They exposed glass plates washed with culture medium 200 m from the sea and collected marine taxa, mainly diatoms, with most frequent being *Chaetoceros* sp. and small unidentified flagellates. Maynard in 1968 also discovered marine algae in the atmosphere, sampling on a ship, as well as 40 miles away of the nearest coast. They retrieved dinoflagellate genera, such as *Oxytoxum, Gyrodinium, Gymnodinium, Ceratium* and *Peridinium,* the prasinophyte *Pyramimonas* and the haptophyte species *Emiliania* (*Coccolithus*) *huxleyi* (61). Lee and Eggleston in 1989 used marine culture media to grow viable algae from the atmosphere of a near coast area and obtained taxa previously reported from Stevenson and Collier in 1962 and Maynard in 1968, suggesting that they had marine origin, although no samplings from the nearby marine system were made (62). The second reason for the few airborne marine phytoplankton genera found could be that studies over or near the sea surface produced negative results (34, 43). A possible explanation could be that airborne marine taxa are difficult to retrieve with sampling methods based on the cultivability of viable airborne algae. Marine in contrast to freshwater and soil algae benefit from water flow in the global ocean for their dispersal at small, large and even coarse spatial scales (more than 1000 km), so their need for viability in atmosphere conditions might be less essential.

Starting from the long-lasting hypothesis of Baas Becking (63) "everything is everywhere, the environment selects", the interest for biodiversity and biogeography of microorganisms has risen over the last years (e.g. 64 – 68). Based on the publications presented here given their methodological limitations such as diverse methodologies and different target-microorganisms used, it appears that the "ubiquity" of airborne algae and cyanobacteria is supported only for particular taxa. Less than 20% of the total number of identified taxa was found to be common independently of the latitude, longitude and altitude of the samplings, as well as the atmospheric conditions governing the period of the samplings, while others are found only scarcely in different locations. The most frequently reported members of the aeroalgae belong to the genera *Chlorella*, *Chlamydomonas, Scenedesmus* and *Chlorococcum.* These are morphologically simple organisms, like "small green-balls", poorly distinguishable in the stages of sporangia and autospores transferred by the air. Therefore, microscopic analysis of air samples may lead to incorrect identification of such algae (69), especially if cryptic species exist. Several cases of inaccurate description of algae and cryptic species diversity are exposed with molecular methods (70). For example, hidden diversity among *Chlorella*-like algae has been revealed primarily by molecular phylogeny (71).

5. HEALTH EFFECTS OF AIRBORNE ALGAE AND CYANOBACTERIA

The human health risks resulting from bad air quality has brought to front the interest in a wide array of airborne microorganisms. However, airborne algae and cyanobacteria as causative agents of adverse health effects are the least studied microorganisms (15). Although in total, more than 15% of the worldwide identified airborne algae and cyanobacteria are known as allergy inducing or toxin producing taxa (Tables 1, 2), only approximately 50% of them have been used in order to be verified as the causative agent of health problems when aerosolized and inhaled. Little is known about the role of airborne algae and cyanobacteria in transportation of radionuclides, heavy metals, pesticides, herbicides and carcinogenic and mutagenic agents, as well as possible effects on the survival of bacteria that could impose health risks (15, 42, 43).

Very early, in 1866, Salisbury proposed that intermittent and remittent fever in rich malaria districts of Ohio and Mississippi valleys could be caused by the chlorophyte *Palmella* sp. (72). In 1948, Woodcock for the first time assumed that human respiratory irritation, cough and nasopharyngeal burning were connected with harmful phytoplankton blooms possibly causing mass mortality of marine organisms (73). Heise in 1949 was the first to associate airborne algae with hay fever, asthma and allergy (74). He also observed positive skin reactions in patients tested with cyanobacterial extracts (75).

McElhenney *et al.* in 1962 selected four strains of chlorophytes (*Neochloris* sp., *Chlorosarcinopsis* sp., *Bracteacoccus* sp. and *Klebsormidium* sp., found in previous aerobiological studies, and examined whether they are capable of inducing sensitivity by the respiratory route in children. They concluded that these airborne algae could cause asthma and rhinitis (76). In a similar experiment, Bernstein and Safferman, in 1966, confirmed that six

Table 1. Taxa list of airborne algae and cyanobacteria found in aerobiological studies.

Baccilariophyceae	Chlorosphaeropsis sp.	Chrysocapsa sp.	!Oscillatoria sp.[har]
Achnanthes sp.	Coccochloris stagnina	!Dinobryon cf. balticum	Pelogloea bacillifera
!Amphora ovalis	Coccomyxa dispar[har]	!Dinobryon sp.	Phormidium cf. ambiguum
Amphora sp.[har]	Coelastrum sp.		Phormidium angustissimum[har]
Campylodiscus clypeus	Coenococcus sp.	**Cryptophyta**	Phormidium bohneri
Chaetoceros sp.[mar]	Dictyochloris sp.	Rhodomonas lacustris	Phormidium dictyothallum
Cocconeis sp.[mar]	Dictyosphaeria sp.		Phormidium foveolarum
Coscinodiscus-like	!Didymocystis bicellularis	**Cyanobacteria**	Phormidium cf. inundatum
Cyclotella cf. ocellata	Dimorphococcus sp.	Anabaena anomala	Phormidium jenkelianum
!Cyclotella cf. striata	Diogenes bacillaris	Anabaena circinalis[har]	Phormidium luridum
Cyclotella sp.	Eudorina californica	Anabaena fertilissima[har]	Phormidium minnesotense
!Diatoma elongatum	Friedmannia sp.	Anabaena helicoidea	Phormidium orientale
!Diatoma cf. vulgare	Gleotila sp.	!Anabaena cf. inaequalis	Phormidium tenue
Eunotia amphioxys	Gloeococcus schroeteri	Anabaena oscillarioides	Phormidium uncinatum
Eunotia gibberula	Gloeocystis gigas	Anabaena sphaerica	!Phormidium spp.[har]
!Eunotia cf. pectinalis	Gloeocystis sp.	Anabaena spp.[har]	!Planktolyngbya circumcreta
Fragilaria capucina	!Haematococcus sp.	Anabaenopsis circularis[har]	Planktolyngbya limnetica
!Fragilaria cf. construens	Hormotilopsis sp.	Anacystis dimidiata	!Planktolyngbya sp.
!Fragilaria cf. pinnata	Microspora sp.	Anacystis marina	Plectonema carneum
Fragilaria sp.	Monoraphidium minutum	Anacystis montan	Plectonema gracillimum
Gomphonema cf. rotundatum	!Monoraphidium sp.	Anacystis thermalis	Plectonema notatum
Gomphonema sp.	Myrmecia sp.[har]	Anacystis sp.	Pleurocapsa minor
!Grammatophora sp.	Nannochloris bacillaris	Aphanocapsa delicatissima	Pseudanabaena cf. limnetica
!Hantzschia amphioxys	Nannochloris sp.	Aphanocapsa pulchra	Pseudanabaena papillaterminata
Hantzschia sp.	Neochloris pseudoalveolaris	Aphanocapsa spp.	!Pseudanabaena sp.
Himantidium arcus	Neochloris sp.[har]	Aphanothece castagnei	Schizothrix calcicola[har]
Himantidium papilio	Oedogonium sp.	Aphanothece naegelii	Schizothrix friesii
!Leptocylindrus sp.[mar]	Oocystis sp.[har]	Aphanothece saxicola	Schizothrix mexicana
!Licmophora sp.[mar]	Ourococcus sp.	Arthrospira sp.[har]	Schizothrix purpurascens
Melosira granulata	Palmella sp.[har]	Calothrix fusca	Schizothrix rivulis
Melosira sp.	Palmellococcus protothecoides	Calothrix marchica	Schizothrix rubella
Navicula cf. affinis	Palmellococcus sp.	Calothrix parietina	Schizothrix sp.
Navicula lineolata	Pandorina morum	Calothrix sp.	Scytonema bohneri[har]
Navicula minuscula	Pediastrum boryanum	Chlorogloea microcystoides	Scytonema hofmanni
Navicula semen	!Pediastrum duplex	Chroococcus limneticus	Scytonema rivulare
!Navicula spp.	!Pediastrum simplex	Chroococcus minutus	Scytonema spp.
Nitzschia frustulum	Pediastrum sp.	Chroococcus turgidus	Spirulina major
!Nitzschia longissima[mar]	Planctonema gracillimum	!Chroococcus sp.	Spirulina sp.
Nitzschia palea	Planctonema lauterbonii	Cylindrospermum spp.[har]	Stigonema sp.
!Nitzschia sp.	Planctonema radiosum	Entophysalis sp.	Symploca muscorum
Pinnularia borealis	Planctonema spp.	Fischerella ambigua	Synechocystis spp.[har]
Pinnularia gibba	Planktosphaeria sp.	Fremyella sp.	Synechococcus spp.[har, mar]
Pinnularia sp.	Pleodorina californica	!Geitlerinema sp.	Tolypothrix byssoidea[har]
!Pleurosigma normanii	Pleodorina sp.	Gloeocapsa crepidinum	Tolypothrix spp.
!Surirella ovalis	Pleurococcus vulgaris	Gloeocapsa decorticans	Trichodesmium hildebrandtii
!Surirella ovata	Pleurococcus sp.	Gloeocapsa magma	Westiellopsis prolifica[har]
Surirella cf. peruviana	Pleurastrum sp.	Gloeocapsa montana	Xenococcus kerneri
!Surirella cf. tenera	!Pseudochlorella sp.	Gloeocapsa spp.	Xenococcus sp.
!Surirella sp.	Prasiola crispa	Gloeothece rupestris	
!Synedra acus	Prasiola sp.	Gloeothece sp.	**Dictyochophyceae**
!Synedra cf. rumpens	Protococcus viridis	Gomphosphaeria sp.	!Dictyocha fibula[mar]
!Synedra cf. ulna	Protococcus sp.	Hapalosiphon spp.	
!Synedra cf. vaucheriae	Protosiphon sp.	!Homoeothrix sp.	**Dinophyta**
!Tabellaria cf. flocculosa	Pyramimonas sp.[mar]	Hydrocoleum heterotrichum	!Ceratium sp.
!Tabellaria sp.	!Radiococcus nimbatus	!Jaaginema sp.	Gymnodinium sp.[har, mar]
	Radiococcus sp.	Limnothrix redekei	Gyrodinium sp.[har, mar]
Chlorophyta	Radiosphaera sp.	!Limnothrix sp.	Oxytoxum sp.[mar]
Actinastrum sp.	Rhizoclonium sp.	Lyngbya borgertii	!Peridinium sp.
Ankistrodesmus convolutus	Scenedesmus acutus	Lyngbya cryptovaginata	!Prorocentrum sp.[har, mar]
Ankistrodesmus falcatus[har]	Scenedesmus bijuga	Lyngbya holsatica	
Ankistrodesmus sp.	Scenedesmus denticulatus	Lyngbya lagerheimii	**Euglenophyta**
Asterococcus superbus	Scenedesmus obliquus	Lyngbya major[har]	!Euglena spp.
Asterococcus-like	!Scenedesmus cf. quadricaudata	Lyngbya perelegans	Trachelomonas volvocina
Botryokoryne simplex	Scenedesmus spp.[har]	Lyngbya versicolor	
Bracteacoccus grandis	Selenastrum sp.	!Lyngbya spp.[har]	**Haptophyta**
Bracteacoccus sp.[har]	Sphaerocystis schroeteri	Mastigocladus laminosus	Emiliania (Coccolithus) huxleyi[mar]
Characium sp.	Sphaerocystis sp.	Merismopedia sp.	
Chlamydomonas agloeformis	Spongiochloris minor	Microchaete spp.	**Streptophyta**
Chlamydomonas nivalis	Spongiochloris sp.	Microcoleus chthonoplastes	!Closterium aciculare
Chlamydomonas polypyrenoideum	Spongiococcum sp.	Microcoleus vaginatus	Closterium sp.
!Chlamydomonas sp.[har]	Stichococcus bacillaris	Microcoleus sp.[har]	Coleochaete irregularis
Chlorella ellipsoidea	Stichococcus minor	Microcystis aeruginosa[har]	!Cosmarium sp.
Chlorella luteo-viridis	Stichococcus subtilis	Microcystis flos-aquae[har]	Cylindrocystis sp.
Chlorella minutissima	Stichococcus spp.[har]	Microcystis sp.[har]	Klebsormidium (Hormidium) dissectum

Chlorella pyrenoidosa[har]	*Tetracystis dissociata*	*Myxosarcina concinna*	*Klebsormidium* (*Hormidium*) *flaccidum*
Chlorella saccharophila	*Tetracystis excentrica*	*Myxosarcina spectabilis*	*Klebsormidium* (*Hormidium*) *subtile*
Chlorella vulgaris[har]	*Tetracystis* sp.[har]	*Myxosarcina* sp.[har]	*Klebsormidium* (*Hormidium*) spp.[har]
!Chlorella spp.[har]	*Tetraspora* sp.	*Nodularia harvenyana*	*Mesotaenium micrococcum*[har]
Chlorococcum diplobionticum	*Tetraëdron bifurcatum*	*Nostoc commune*[har]	*Mougeotia* sp.
Chlorococcum ellipsoideum	*!Tetraëdron minimum*	*Nostoc ellipsosporum*	*Roya* sp.
Chlorococcum humicola[har]	*Tetraëdron* sp.	*Nostoc linckia*[har]	*!Staurastrum* sp.
Chlorococcum hypnosporum	*Trebouxia cladoniae*	*Nostoc muscorum*[har]	*Zygnema* sp.
Chlorococcum infusionum	*Trebouxia* sp.[har]	*Nostoc palmelioides*	
Chlorococcum intermedium	*Trentepohlia* sp.	*Nostoc paludosum*[har]	**Xanthophyceae**
Chlorococcum polymorphum	*Treubaria*-like	*Nostoc punctiforme*	*Botrydiopsis* sp.
Chlorococcum scabellum	*Ulothrix tenerrima*	*Nostoc sphaericum*	*Heterococcus* sp.
Chlorococcum sp.[har]	*Ulothrix* sp.	*Nostoc spumigena*	*Heterothrix* sp.
Chlorogloea microcystoides	*Westella botryoides*	*Nostoc* spp.[har]	*Heteropedia* sp.
!Chloroplana terricola	*Westella* sp.	*Oscillatoria chlorina*	*Monallantus* sp.
Chlorosarcina sp.		*Oscillatoria lutea*	*Monocilia* sp.
Chlorosarcinopsis sp.[har]	**Chrysophyceae**	*Oscillatoria simplicissima*[har]	*Tribonema* sp.
Chlorosphaera antarctica		*Oscillatoria subbrevis*	*Vaucheria* sp.
Chlorosphaera sp.	*Chromulina* sp.		

!: taxa found with microscopic observation in the air of Thessaloniki City, [har]: taxa found to induce allergy or produce toxins, [mar]: taxa with marine origin.

different chlorophytes, including the common airborne *Chlorella, Chlorococcum* and *Scenedesmus* species, induce respiratory allergy and bronchial mucosal secretions to patients (77). Bernstein *et al.* in 1969 used chlorophyte strains, from which those of the species *Chlorella vulgaris, Chlorella pyrenoidosa* and *Ankistrodesmus falcatus* have been recovered from air samples in aerobiological studies, in order to reveal patterns of immunological cross – reactivity in experimental animals. Although no definite causative role of these algae to human respiratory allergy was established, the high potential for immunological reaction to animals provided with evidence that they contain allergenic properties (78).

Champion in 1971 reported six cases of allergic reactions to chlorophytes provoked by inhalation (79). Benaim-Pinto in 1972 suggested that airborne algae were the most probable etiological factor in respiratory allergy in Caracas, Venezuela (80). Mittal *et al.* in 1979 identified 10 airborne algae and cyanobacteria from the Delhi area, India, which could cause skin irritation and allergic reactions to patients suffering from naso-bronchial allergy (81). Bernstein and Safferman in 1973 demonstrated that the chlorophyte *Chlorella* sp., probably the most common airborne microorganism, could cause skin, nasal and bronchial reactions (82). Later, in 1995, Tiberg *et al.* proved that *Chlorella* sp. is an allergen, although relatively "weak" in comparison to other algae, and may be of clinical significance to a certain group of patients (83). Sharma and Rai studying the allergenic potency of two cyanobacterial species (*Nostoc muscorum* and *Phormidium fragile*) common in the air of Varanasi city in India revealed their allergenic nature (84).

Airborne algae are present indoors and are found in collections of house dust. Bernstein and Safferman in 1970 identified viable algae in aliquots of house dust and tested 84 patients on *Chlorococcum* and *Chlorella* extracts that were present in house dust samples. 58% of the patients showed positive responses to the algal allergens, suggesting that house dust is a likely source of human exposure to a variety of algae, which could induce clinical allergy problems (85). Holland *et al.* in 1973 also supported this idea when they identified 40 viable algal taxa (species or genera), including *Chlorella*-like organisms, from house dust samples and attempted to correlate them with allergenic reactions to humans (86).

Schlichting has calculated that a human inhales about 7 L of air per minute. Therefore, he estimated that at least 2880 algal and cyanobacterial cells are inhaled per day (43). Although these organisms usually constitute a minority of airborne bioaerosols, compared to fungi, pollen and bacteria (52), in certain cases the quantity of airborne algal particles can far exceed that of fungi spores and pollen grains (87). Brown *et al.* in 1964 found over 3000 algae m^{-3} in samples taken from a car moving through a dust cloud. In such cases, airborne algae should be considered of allergenic importance, since they could contribute considerably to the total abundance of airborne particles (35). The World Health Organization (WHO) has developed updated air quality guidelines, an international orientation on health threats of exposure to high levels of air pollution and a policy tool for lowering these consequences globally (88). Nevertheless, these guidelines were based on PM, ozone (O_3), nitrogen dioxide (NO_2) and sulfur dioxide (SO_2) concentrations, not including new data on impacts of bioaerosols on human health (WHO, 2006). Currently no standard levels of airborne algal and cyanobacterial particles have been determined (23).

Airborne algae and cyanobacteria occur in conjunction with other biotic and abiotic airborne particles. It is proposed that biological particles in combination with inorganic particulate matter, such as air pollutants, produce more severe effects than anticipated (13, 89). Immunological cross-reactivity of different airborne cyanobacteria (*Nostoc muscorum* and *Phormidium fragile*) (84) or among airborne algae and other allergens, especially molds (90) has been suggested that enhance human health adverse effects. Brown and Lester in 1965 studied the antigens from the chlorophytes *Tetracystis* and *Chlorococcum* and concluded that cross-reactivity between these taxa could be significant in human respiratory allergy (91). McGovern *et al.* in 1966 examined the allergenic potency of four algal strains *Klebsormidium* sp.,

Table 2. List of potentially harmful taxa, their frequency of occurrence in aerobiological studies, the sampling method used to collect them, the climatic region from where they were found and the health risks they impose.

Harmful airborne algae and cyanobacteria	Frequency of occurrence in aerobiological studies	Sampling method	Climatic regions from where airborne algae were recovered	Health risks
!*Chlorella* spp.	15	Air Bubbler, Membrane Filters, Exposed Culture Media, Rotorod Sampler	Temperate, Subtropical, Tropical	Allergy, Rhinitis, Hyper-sensitivity
!*Scenedesmus* spp.	13	Air Bubbler, Membrane Filters, Exposed Culture Media, Rotorod Sampler	Temperate, Subtropical, Tropical	Dermatitis, Allergy
Chlorococcum sp.	11	Air Bubbler, Membrane Filters, Exposed Culture Media, Rotorod Sampler	Temperate, Subtropical, Tropical	Allergy
Klebsormidium (*Hormidium*) spp.	10	Air Bubbler, Membrane Filters, Exposed Culture Media	Arctic, Temperate, Subtropical, Tropical	Allergy
!*Lyngbya* spp.	10	Air Bubbler, Membrane Filters, Exposed Culture Media, Rotorod Sampler	Arctic, Temperate, Subtropical, Tropical	Toxin producer, Allergy, Dermatitis, Swelling of mucous membrane of eyes and nose
!*Chlamydomonas* sp.	9	Air Bubbler, Membrane Filters, Exposed Culture Media, Experimental Containers	Arctic, Temperate, Tropical	Dermatitis, Rhinitis, Asthma
!*Oscillatoria* sp.	9	Air Bubbler, Membrane Filters, Exposed Culture Media, Rotorod Sampler	Arctic, Temperate, Subtropical, Tropical	Toxin producer, Hay fever
!*Phormidium* spp.	8	Air Bubbler, Membrane Filters, Exposed Culture Media, Rotorod Sampler, Experimental Containers	Arctic, Temperate, Subtropical, Tropical	Allergy
Nostoc spp.	6	Air Bubbler, Membrane Filters, Exposed Culture Media, Rotorod Sampler	Temperate, Subtropical, Tropical	Toxin producer, Allergy
Oocystis sp.	6	Air Bubbler, Membrane Filters, Exposed Culture Media	Temperate, Tropical	Allergy
Stichococcus spp.	6	Air Bubbler, Membrane Filters, Exposed Culture Media, Rotorod Sampler	Temperate, Subtropical, Tropical	Dermatitis, Rhinitis, Asthma
Anabaena spp.	5	Air Bubbler, Membrane Filters, Exposed Culture Media, Rotorod Sampler	Temperate, Subtropical, Tropical	Toxin producer, Allergy, Dermatitis, Rhinitis
Neochloris sp.	5	Air Bubbler, Membrane Filters, Exposed Culture Media	Temperate, Tropical	Allergy
Bracteacoccus sp.	4	Air Bubbler, Membrane Filters, Exposed Culture Media	Temperate, Tropical	Allergy
Palmella sp.	4	Air Bubbler, Membrane Filters, Exposed Culture Media	Temperate	Fever
!*Prorocentrum* sp.	4	Air Bubbler	Temperate	Toxin producer
Chlorella vulgaris	3	Air Bubbler, Experimental Containers	Temperate, Tropical	Allergy
Chlorosarcinopsis sp.	3	Air Bubbler, Membrane Filters, Exposed Culture Media	Temperate, Tropical	Allergy
Microcoleus sp.	3	Air Bubbler, Membrane Filters, Exposed Culture Media, Rotorod Sampler	Temperate, Subtropical, Tropical	Dermatitis
Microcystis sp.	3	Air Bubbler, Membrane Filters, Exposed Culture Media, Experimental Containers	Temperate, Subtropical	Toxin producer
Arthrospira sp.	2	Air Bubbler, Membrane Filters, Exposed Culture Media, Rotorod Sampler	Temperate, Subtropical	Toxin producer
Chlorella pyrenoidosa	2	Air Bubbler, Membrane Filters, Exposed Culture Media	Tropical, Subtropical	Allergy
Mesotaenium micrococcum	2	Air Bubbler	Subtropical	Dermatitis, Rhinitis, Asthma
Myxosarcina sp.	2	Air Bubbler, Membrane Filters, Exposed Culture Media	Subtropical	Allergy
Nostoc muscorum	2	Exposed Culture Media	Subtropical	Allergy
Synechococcus sp.	2	Air Bubbler, Membrane Filters, Exposed Culture Media	Temperate, Subtropical	Toxin producer
Synechocystis spp.	2	Exposed Culture Media, Rotorod Sampler	Temperate, Subtropical	Toxin producer
Tetracystis sp.	2	Air Bubbler, Membrane Filters, Exposed Culture Media	Tropical	Allergy
Anabaena circinalis	1	Air Bubbler, Membrane Filters, Exposed Culture Media	Tropical	Toxin producer
Anabaena fertilissima	1	Exposed Culture Media	Subtropical	Allergy
Anabaenopsis circularis	1	Exposed Culture Media	Subtropical	Allergy
Ankistrodesmus falcatus	1	Exposed Culture Media	Temperate	Allergy
Amphora sp.	1	Air Bubbler, Membrane Filters, Exposed Culture Media	Temperate	Toxin producer
Chlorococcum humicola	1	Exposed Culture Media	Subtropical	Allergy
Coccomyxa dispar	1	Air Bubbler, Membrane Filters, Exposed Culture Media	Tropical	Dermatitis, Rhinitis, Asthma

Cylindrospermum spp.	1	Exposed Culture Media, Rotorod Sampler	Subtropical	Toxin producer
Gymnodinium sp.	1	Wind nets	Tropical	Toxin producer
Gyrodinium sp.	1	Wind nets	Tropical	Toxin producer
Hapalosiphon spp.	1	Exposed Culture Media, Rotorod Sampler	Subtropical	Toxin producer
Lyngbya major	1	Exposed Culture Media	Subtropical	Allergy
Myrmecia sp.	1	Air Bubbler, Membrane Filters, Exposed Culture Media	Tropical	Dermatitis, Rhinitis, Asthma
Microcystis aeruginosa	1	Experimental Containers	Temperate	Toxin producer, Pneumonia
Microcystis flos-aquae	1	Air Bubbler, Membrane Filters, Exposed Culture Media	Tropical	Toxin producer
Nostoc commune	1	Exposed Culture Media	Subtropical	Allergy
Nostoc linckia	1	Exposed Culture Media	Subtropical	Toxin producer, Allergy
Nostoc paludosum	1	Exposed Culture Media	Subtropical	Toxin producer
Oscillatoria simplicissima	1	Exposed Culture Media	Subtropical	Allergy
Phormidium anguletissimum	1	Exposed Culture Media	Subtropical	Allergy
Scytonema bohneri	1	Exposed Culture Media	Subtropical	Allergy
Tolypothrix byssoidea	1	Air Bubbler, Membrane Filters, Exposed Culture Media	Tropical	Toxin producer
Trebouxia sp.	1	Air Bubbler, Membrane Filters, Exposed Culture Media	Tropical	Dermatitis, Rhinitis, Asthma
Westiellopsis prolifica	1	Exposed Culture Media	Subtropical	Allergy

!: taxa found in the air of Thessaloniki City.

Bracteacoccus sp. and two strains of *Tetracystis*) and the cross-reactivity between those strains to individuals of all ages. They suggested that allergenic reactivity is enhanced when the patients are exposed to increased concentrations of the algal extracts (87). However, there are only a limited number of studies analyzing the synergistic or antagonistic interactions of different allergenic airborne particles, warranting the study of these relationships an imperative need (23).

Table 2 presents the potentially harmful airborne photosynthetic microorganisms (algae and cyanobacteria) found in aerobiological studies that either have been experimentally shown to induce allergenic reactions or are known toxin producers. Overall, 52 taxa posing a threat to human health have been identified, out of which, 38 have been experimentally shown to induce health problems, while the other 14 are known to produce toxins in their natural environments. The most common method of sampling airborne algae and cyanobacteria is exposing plates with selected culture media, where the microorganisms are grown after passive transfer or active impact. With this technique it is possible to sample only the viable fraction of airborne algae and cyanobacteria, overlooking the non-viable ones that are transferred through air and still can cause allergic reactions. Moreover, most of the known microorganisms cannot be presently maintained in cultures (92), meaning that possibly a large number of these airborne microorganisms cannot be detected. The most frequently occurring airborne taxa mentioned above (Table 2) are the chlorophytes *Chlorella* sp., *Scenedesmus* sp., *Chlorococcum* sp., *Klebsormidium* sp. and the cyanobacterium *Lyngbya* sp. These taxa including harmful members have been reported in more than 40% of the areas where samplings were made. These taxa have been reported from virtually every climatic zone, suggesting a widespread dispersal and distribution, posing a threat to air quality and human health. Twenty six out of 27 taxa of airborne cyanobacteria, with members known to produce toxins have been found in tropical or subtropical areas. Only the cyanobacterium *Microcystis aeruginosa* has not been reported from a tropical area. *M. aeruginosa* has been found in a Mediterranean area transferred to the air during a water bloom event in a nearby reservoir (3). Given the positive effect of temperature increase on cyanobacterial growth, extended blooms and toxin production of certain taxa in their natural environments (93), research is needed to evaluate airborne harmful cyanobacteria and associated health risks in respect to global warming.

Additionally to their dominance in warm climates, cyanobacteria comprise also an important fraction of the airborne photosynthetic microorganisms in all climatic zones. Certain airborne taxa, such as species belonging to the genera *Microcystis, Anabaena, Lyngbya, Anabaenopsis, Hapalosiphon, Nodularia, Nostoc* and *Oscillatoria* are known toxin producers in their natural environments (freshwater, brackish, marine and terrestrial systems). *Microcystis, Anabaena, Oscillatoria*, and more rarely *Anabaenopsis, Hapalosiphon* and *Nostoc* species produce hepatotoxic microcystins. *Oscillatoria* and *Anabaena* produce also neurotoxic anatoxins, while *Lyngbya* produce skin irritating lyngbyatoxins and saxitoxins (94). Nodularins appear to be limited to *Nodularia* among the free-living cyanobacterial genera (95). *Nostoc* and other diverse cyanobacterial members produce the neurotoxic β-*N*-methylamino-L-alanine amino acid (BMAA) (96). Cyanobacterial toxins can cause gastroenteritis and related diseases, allergic and irritation reactions and liver diseases (97). Humans can be exposed to cyanobacterial toxins via skin contact, haemodialysis, ingestion and inhalation of the airborne cyanobacteria or aerosolized toxins (98).

Cheng *et al.* in 2007 showed with laboratory experiments that cyanotoxins in water could be transferred to air via a bubble-bursting process (99). It has been found that inhaled toxins have toxic effect on different target organs at lower doses in comparison with ingested toxins in experimental animals (100 – 102). Backer *et al.* in 2010 considered the recreational exposure to microcystins during algal blooms in two Californian Lakes. They measured the aerosolized microcystin concentration in personal air samplers and in nose swabs and found low concentrations of the toxin. This suggests that harmful cyanobacterial blooms can produce aerosolized toxins, which can cause adverse health problems if inhaled (103). Caller *et al.* in 2009 suggested that high incidences of amyotrophic lateral sclerosis (ALS) in New Hampshire could be related to frequent events of toxic cyanobacterial blooms from the nearby Lake Mascoma. Chronic exposure to cyanobacterial neurotoxins, such as BMAA, including through inhalation of aerosolized toxins, was associated with the unusually high numbers of occurring ALS in the population (104). BMAA also enhances the influence of other neurotoxins (105). Due to the worldwide distribution of diverse airborne cyanobacteria, which are known BMAA producers and the possible implications of BMAA neurotoxic properties in public health, more research towards this direction is needed (106).

Although the secondary metabolites mentioned above can induce serious health problems, endotoxins or lipopolysaccharides (LPS), characteristic components of the outer membrane of most cyanobacteria (107), should also be considered. Endotoxins are suspected to cause gastroenteritis, fever and allergy (108). According to Annadotter *et al.* (2005), endotoxins in aerosols were the most probable etiological agent behind outbreaks of an acute temporary, influenza-like syndrome described from four Scandinavian towns and Harare, Zimbabwe. The symptoms included fever, melancholy, muscle pain, tightness of the chest and respiratory-tract symptoms (109). However, recent research suggests that cyanobacterial LPS are not likely to cause allergenic reactions to healthy population and incidents attributed to cyanobacterial endotoxins should probably be ascribed to exotoxins instead (110).

Cyanobacteria species have also been found to facilitate the survival and growth of the bacterium *Legionella pneumophila*, causative agent of the Legionnaires disease, in aerosols (111). Turner *et al.* in 1990 associated a toxic bloom of the cyanobacterium *Microcystis aeruginosa*, with two cases of pneumonia in people who were in contact with the bloom, probable through the respiratory tract. Additionally, 16 soldiers, from whom 8 were in contact with the bloom, were admitted to medical centers with symptoms of cyanobacteria intoxication, such as sore throat, headache, abdominal pain, cough and gastrointestinal problems (112).

Algae that have been found to produce toxins that aerosolize are *Ostreopsis* sp. and *Karenia brevis*. Gallitelli *et al.*, in 2005, associated two algal blooms consisting of species of the dinoflagellate genus *Ostreopsis* with concurrent symptoms to people exposed to marine aerosol. In particular, the symptoms included rhinorrhea, cough, dyspnoea, wheezing and fever (113). However, the most studied phenomenon of airborne toxins that cause adverse health effects is the case of *Karenia brevis* Florida red tides, which are known to cause respiratory problems to human because of the aerosolization of the red tide brevetoxins (PbTx) (114 – 117). Among the symptoms, conjunctive irritation, copious catarrhal exudates, rhinorrhea, nonproductive cough and bronchoconstriction are reported. Few people also reported dizziness, tunnel vision and skin rashes. A comprehensive work has been published and an extensive list of references exists on the subject (for a review of the earlier literature see 118). Cheng *et al.* in 2005 estimated the particle size of the PbTx to a mass median diameter between 6 and 10 µm. This size signifies the high deposition efficiency of the PbTx in the upper and lower airways of the respiratory tract (119).

6. A CASE STUDY: AIRBORNE ALGAE AND CYANOBACTERIA AND RELATED HEALTH RISKS IN THESSALONIKI, GREECE

The city of Thessaloniki (40^{o} 37΄N and 22^{o} 57΄E) is located at the northern part of Thermaikos Gulf. It is a densely populated (16000 inhabitants km^{-2}), industrialized city (120) and one of the most polluted urban areas in Europe, with high ambient concentrations of airborne particles all year, higher than the recommended daily and annual limit value according to Council Directive 83/399/ECC (121). Furthermore, daily airborne pollen (122) and fungal spore (123) records were collected for fifteen years from 1987 to 2001, adding to the knowledge concerning the potentially hazardous or allergenic components of the atmosphere of Thessaloniki. In particular, Damialis *et al.* in 2007 predicted that pollen production for certain plant species in Thessaloniki will increase with ongoing climate change, implying a high risk for induced respiratory allergies (124). However, similar studies examining the occurrence and diversity of airborne algae in the area have only recently initiated (Genitsaris *et al.*, accepted).

The diversity and abundance of airborne algae and cyanobacteria in the city of Thessaloniki were studied from August 2007 to November 2008. The questions posed in this case study were: how many and which taxa were present in the air of Thessaloniki in respect to the reported airborne algae and cyanobacteria all around the world? Which of these taxa are related to allergies or other health problems in humans? Is the density of algal and cyanobacterial particles in the air sufficient to impose health risks?

The experimental site was set at the rooftop of the Biology School (*ca*. 50 m height), Aristotle University of Thessaloniki, located at the centre of the city, in an open area of the university campus with growing vegetation all

Table 3. Meteorological data in the city of Thessaloniki during the study (sunshine, rainfall, air temperature, relative humidity – RH and wind speed)

	Average Daily Air Temperature (°C)			Daily Raindrop (mm)			Average Daily Sunshine (min)			Average Daily RH (%)			Average Daily Wind Speed (m s^{-1})		
	min	max	mean	min	max	mean	min	max	mean	min	max	mean	min	max	mean
August 2007	17.75	29.87	26.5	0	23	1.63	3.6	729.4	621.7	37	79	56	1.08	2.15	1.55
Sep 07	16.59	27.6	20.9	0	8.8	1.4	0	674.1	493.6	35.2	83.7	58.6	0.98	5.58	2.29
Oct 07	10.38	22.61	16.54	0	18.3	2.08	0	606.6	294.9	36.9	88.1	69.1	0.9	4.75	1.86
Nov 07	7.1	17.1	10.76	0	10.7	1.47	0	494.5	194.9	33.2	90.5	68.8	0.95	5.54	2.21
Dec 07	0.84	10.76	6.3	0	4.8	0.5	0	443.5	185.3	43.9	91.1	70.6	0.84	5.92	2.02
Jan 08	-0.38	9.55	6.34	0	3.6	0.61	0	483	201	33.7	92.7	73	0.83	7.68	1.86
Feb 08	-0.68	11.54	7.72	0	17.8	0.67	0	555.7	296.8	24.5	86.1	62	0.95	4.21	2
Mar 08	8.47	14.85	12.58	0	8.6	0.44	0	659.1	423.1	36.8	85.4	62.1	1.16	3.59	1.95
April 08	9.46	18.67	14.76	0	34.7	3.17	0	674.4	371.8	43.8	90.6	70.2	1.15	3.56	1.69
May 208	12.56	25.33	19.47	0	13	0.88	0	755.5	604.3	33.8	84.1	60.2	1.24	3.83	1.94
June 08	19.13	29.58	24.41	0	7.7	0.57	47.2	787.5	633.4	43	78.1	58.1	1.28	3.07	1.99
July 08	23.25	28.75	26.56	0	6.8	0.39	523.4	795.7	714.8	35.1	60.6	49.1	1.48	5.36	2.4
Aug 08	23.94	30.8	27.58	0	0	0	296.9	745.5	662	31.8	66.9	49.9	1.32	4.22	2.1
Sept 08	24.14	27.62	25.14	0	27.7	2.36	48.21	650.9	556.2	50.1	72.5	63.5	1.34	2.54	1.72
Oct 08	-	-	-	0	8.7	0.66	-	-	-	-	-	-	-	-	-
Nov 08	-	-	-	0	7.3	0.5	-	-	-	-	-	-	-	-	-

Provided by Theodoros Mavrommatis. -: No data

around. This site is representative of the university area of the centre of the city, due to its height and proximity to the centre. Air was drawn into a conical flask containing 300 ml of sterile water at a rate of 16 L min^{-1} from August 2007 to November 2008 in a total of 93 samplings, with each sample being 3 m^3. Airborne particles were trapped and collected in the water. Fresh and Lugol-preserved sub-samples from the water were taken for microscopic analysis. Fresh sub-samples were examined within 3 h of collection. Fresh and Lugol preserved sub-samples were examined in sedimentation chambers using an epifluorescence-inverted microscope with phase contrast (Nikon SE 2000). Airborne algae were identified using taxonomic keys, to genus or species level (referred to as taxa in the rest of the text). Additional phylogenetic analysis of algae by sequencing the 18S rRNA gene in two sub-samples was performed, after the water was filtered through 0.2 μm pore size membrane filters (Whatman, USA) and stored immediately at -20°C until further analysis. The molecular techniques used are described in Genitsaris *et al.* in 2009 (125). Meteorological data for the periods of the study were provided (Table 3; Mavrommatis, Department of Geology, Aristotle University of Thessaloniki, personal communication).

During the 93 air-samplings throughout the year a total of 59 morphological airborne algal and cyanobacterial taxa were identified (Table 1). This number of taxa represents a large local pool in relation to the species pool of globally reported taxa in this review. This finding, given the methodological limitations for comparison, may indicate "ubiquitous" airborne microorganisms (65). However, 21 out of 59 taxa found in the air of centre of Thessaloniki in this first aerobiological attempt are reported for the first time and contributed 6% to the total number of known airborne taxa (Table 1), supporting the hypothesis of undiscovered aero-microbial diversity (61). Among the 21 new reported airborne taxa the marine diatoms *Leptocylindrus* sp., *Licmophora* sp., *Nitzschia longissima,* the dictyochophyte *Dictyocha fibula* and the dinoflagellate *Prorocentrum* sp. most probably had a local source, as they were simultaneously found in samplings from the nearby aquatic system of Thermaikos Gulf (Genitsaris *et al.*, accepted).

Diatoms were the predominant group with 25 taxa, followed by 15 taxa of chlorophytes and 12 taxa of cyanobacteria, accounting for 42.4%, 25.4% and 20.3% of the total number of airborne taxa, respectively. More than 50% of these taxa appeared to be viable under fluorescence microscopy (e.g. *Pediastrum* species), while a few number of airborne taxa established in experimental water containers in high numbers (e.g. *Scenedesmus* species) (Genitsaris *et al.*, accepted). Diatoms included not only viable cells but also empty frustules. Furthermore, phylogenetic analysis revealed 4 new species with more than 99% resemblance with the chlorophytes *Haematococcus lacustris*, *Podohedriella falcata, Scenedesmus vacuolatus* and *Scenedesmus obliquus*, which were added to the list of airborne algae of the air in the centre of Thessaloniki. These algae were most probably transported through air in the form of dormant life-stages (Genitsaris *et al.* accepted), making the microscopic identification impossible. The combination of microscopic and phylogenetic analysis facilitates the detection of the undiscovered diversity in environmental samples (125). Diatoms, chlorophytes and cyanobacteria are the usual suspects in the air of urban areas, as previously reported in related studies (36, 46, 49). Chlorophytes and cyanobacteria are the groups with the highest number of taxa, while diatoms represent only a small portion of the total number of airborne taxa (46, 49, 52). However, diatoms are frequently observed in the aeroalgal community of Antarctica (27). In this study, airborne marine diatoms, both planktic and benthic, contributed considerably to species richness indicating not only their local origin, but also the limited knowledge on airborne microbial diversity.

The number of total algal cells reached a maximum of 458 m^{-3} of air in the centre of Thessaloniki (Figure 1). Gregory *et al.* in 1955 reported mean values of 110 individuals m^{-3} of *Gloeocapsa* sp. in the air in different locations in England (32). Schlichting in 1969 (43) measured up to 260 algal cells m^{-3} in Texas area and considerably lower numbers in Michigan (up to 60 cells m^{-3}) and North Carolina (up to 15 cells m^{-3}). Rosas *et al.* in 1989 found a maximum number of 2220 Chlorophyta m^{-3} in the air of a city in Mexico (46), while Tormo *et al.* in 2001 found mean values of Chlorophyta of only 1.3 coenobia m^{-3} and 1.5 individuals m^{-3} for Bacillariophyceae, for 315 sampling days in Badajoz city in Spain (52). The largest values ever recorded for airborne algae and cyanobacteria were mentioned by Brown *et al.* in 1964,

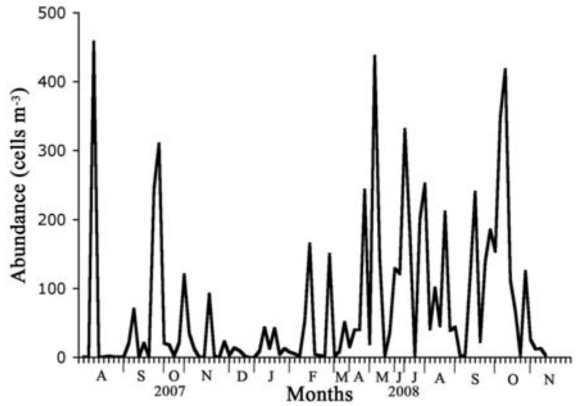

Figure 1. Abundance (cells m^{-3} of air) of airborne algae and cyanobacteria in the air samples from Thessaloniki City, during the study period (August 2007 – November 2008).

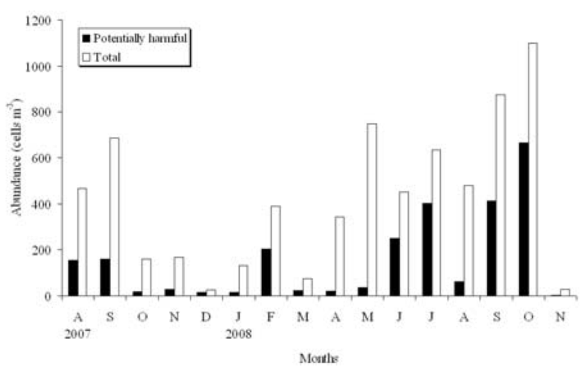

Figure 2. Monthly changes of potentially harmful (with black colour) and total airborne algae and cyanobacteria abundance (cells m^{-3}) in the air samples from Thessaloniki City during the study period (August 2007 – November 2008).

who calculated that the air in Texas might contain up to 3000 cells m^{-3} (34). If a person inhales about 0.5 m^3 of air per hour, then according to the results from Thessaloniki area it is possible that he could breath in, more than 5000 algal and cyanobacterial cells per day.

During the study, a total of seven genera, which members have been associated with human health problems were identified. *Chlamydomonas*, *Chlorella*, *Lyngbya*, *Prorocentrum, Phormidium, Oscillatoria* and *Scenedesmus* are all genera including species known to produce toxins and/or induce allergic reactions (Table 2). Their abundance ranged from zero to 451 cells m^{-3} of air during the sampling period. In Figure 2 the abundance of potentially harmful algae and cyanobacteria out of the total cells every month is presented. In all months, at least one taxon including potentially harmful members was present in the photosynthetic airborne microorganisms. In December 2007 and February, June, July and October 2008 more than 50% of the total airborne algal and cyanobacterial cells in the air of Thessaloniki comprised of members related to health problems. This means that during a day with the maximal measured abundance of airborne photosynthetic microorganisms a person could inhale at least 2500 potentially harmful microorganism cells per day. Although no limits on the abundance of airborne algae and cyanobacteria have been established (23), it is possible that such high numbers may impose prominent health risk, especially to the susceptive part of the population. It has been proposed (8) that certain groups of the population, with underlying conditions, such as asthmatics, patients with emphysema and bronchitis, patients with cardiovascular conditions and nutrient deficiencies, as well as the elderly, pregnant women and children are more sensitive to health problems created by airborne particles. Airborne algae and cyanobacteria have been proven to be associated with increased allergic reactions to patients with pre-existing allergenic conditions (81). In addition, certain algae and cyanobacteria may act synergistically to increase the patients' symptoms (84).

7. CONCLUSIONS AND PERSPECTIVES

Airborne algae and cyanobacteria represent a considerable part of atmospheric bioaerosols. However, they are the most under-studied microorganisms in aerobiological studies. The most common airborne photosynthetic microorganisms are Cyanobacteria, found more often in tropical regions and Chlorophyta, mostly in temperate areas. Less than 20% of airborne photosynthetic microorganisms were found to be common in relevant studies independently of location and meteorological conditions. Thirty-eight taxa have been linked with adverse health effects on humans, such as allergy, asthma, bronchitis, dermatitis, rhinitis, skin irritation and respiratory problems, caused either by inhalation of the algae individuals or by the toxins produced by some of them. Despite these health related issues, little scientific effort has been made to investigate airborne algae and cyanobacteria as causative pathogenic agents. The very few available estimations of the relative abundance of photosynthetic microorganisms in atmospheric particles, along with our study from a Mediterranean coastal urban area, show that airborne algae and cyanobacteria comprise a considerable amount of inhaled material. Therefore the need for considering their presence in health-oriented aerobiological studies in the future is imperative.

The paucity in the number of focused studies on airborne algae and cyanobacteria, and subsequently their potential health risks, is mostly due to the lack of consideration of the atmosphere as a habitat to these microorganisms and the scarcity of well-established, universally approved methodology. Inflows and outflows of algae and cyanobacteria from aquatic and terrestrial sources to the air affected directly and indirectly by climatic factors and anthropogenic activities are fundamental for a holistic study of airborne microorganism diversity and abundance dynamics. This ecological approach is associated with social and economical needs as a basis for applied research on air and water quality and their impacts.

In order to tackle the above-mentioned issues in future studies, the establishment of standard methods for aerobiological sampling is needed for observations from different places and times simultaneously with sampling in source habitats. For the identification of airborne algae, additionally to the microscopic analysis, appropriate new technologies, such as phylogenetic and metagenomic analysis should also be applied to uncover the full diversity of airborne photosynthetic microorganisms in air samples but also the possible existence of cryptic species. Identification in species level is needed because of species differences in physiological peculiarity, such as allergic and poisonous property, among species. Future perspectives could involve the description of biogeographical processes, patterns and factors governing the dispersal of potentially harmful airborne algae and cyanobacteria. Furthermore, global warming and pollution effects on their distribution are unexplored. Establishment of airborne microorganism monitoring system aiming to protect human health and the environment is needed. Furthermore, additional research both *in vitro* and *in situ*, on the health effects of toxic and allergenic algae and cyanobacteria when aerosolized is warranted. Finally, studies on the relationship between airborne algae and cyanobacteria abundance and respiratory-related outbreaks or other related incidents, especially in urban areas nearby to water bodies with harmful algal or cyanobacterial blooms, can serve as a complementary tool in human health protection.

8. ACKNOWLEDGEMENTS

The authors are grateful to Theodoros Mavrommatis for kindly providing meteorological data. We are also grateful to three reviewers for their constructive comments that improved the manuscript. The authors would like to thank Aikaterini Asvesti for comments on an early version of the manuscript.

All co-authors contributed to the writing. S. G. performed literature search, provided original data and analyzed the data. M. M-G. led the writing, literature search and analysis. Original data of this paper are part of S.G. PhD thesis.

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Abbreviations: PM: particulate matter; PbTx: brevetoxin; LPS: lipopolysaccharides; ALS: amyotrophic lateral sclerosis; BMAA: β-*N*-methylamino-L-alanine amino acid; WHO: World Health Organization.

Key Words: Airborne Algae And Cyanobacteria, Allergy, Bioaerosols, Dispersal, Inhalation, Phytoplankton, Toxin, Review

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