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Functional relations of airborne fungi to meteorological and pollution factors in a Mediterranean urban environment

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ABSTRACT

The link between airborne fungal spores and environmental parameters was investigated for 4 years in the city of Athens, Greece. Spearman rank order correlation analysis (r_s), stepwise multiple linear regression analysis and Canonical Correspondence Analysis evidenced significant interrelationships between the fungal spore concentration and meteorological factors, and less significance with the air pollutants. Air temperature, solar radiation and wind speed influenced positively ($p < 0.01$) the total fungal count as well as the genera *Cladosporium*, *Aspergillus* and *Alternaria*, and negatively the genus *Penicillium*. Relative humidity and atmospheric pressure had negative effects to the prevalent genera except *Penicillium*. Pollution factors were weakly associated positively with *Penicillium* and negatively with the other fungal constituents. Regression analysis indicated that air temperature exerted a consistently strong influence and was the single best predictor of the fungal aerosol concentration in the atmosphere.

1. Introduction

Airborne fungi constitute a significant part of global bioaerosols and belong to the coarse fraction of air particulate matter (Sesartic and Dallafior, 2011). Fungal material is emitted from the earth's surface to the air both via active discharge and passively through the action of wind. Fungal aerosol interacts in various ways with the biosphere, with an impact on human health, agro economy, food production, cultural heritage, etc. Also, it has a potential role in regulating atmospheric chemistry and modulating climate by acting as ice and cloud condensation nuclei (Ariya et al., 2009; Fröhlich-Nowoisky et al., 2009; Sesartic et al., 2013).

The profile of fungal particulate matter present in the atmosphere of a geographic area is determined by the continuous interaction of biotic and abiotic factors. The relation between climatic conditions and fungal aerosol is complex. Meteorological factors influence the growth of fungi as well as spore production, release and dispersal while fungal aerosol influences atmospheric processes. Therefore, it is crucial to elucidate the interaction between fungi and meteorological parameters.

Air pollution interacts with the atmospheric processes and affects the climatic conditions. It may also influence the diversity, concentration and distribution of airspora. However, the impact of air pollutants on airborne fungi has been evaluated only in a few studies (Lin and Li, 2000; Ho et al., 2005; Adhikari et al., 2006; Bauer et al., 2008; Sousa et al., 2008; Grinn-Gofroń et al., 2011).

Greece has a typical Mediterranean climate with mild, wet winters and hot, dry summers, along with intensive solar radiation throughout the year. A great variety of climate subtypes are encountered in Greece due to its geomorphological diversity with unique topographical and geological characteristics. The city of Athens is located in the center of the Attica basin and is characterized by urban land cover and use. Several factors define the microclimate in the city of Athens that may influence pollution dynamics, as well as the diversity and concentration of airborne fungal particulate matter.

Athens has serious air pollution problems mainly due the closed topography of the Attica basin and the densely built city centre. The air pollutants present seasonal variation with episodes of certain pollutants exceeding threshold values. The concentration of CO, NO, SO_2 and smoke increase during winter months due to central heating and heavy traffic, whereas ozone concentration increases during summer months (Paschalidou and Kassomenos, 2006). The tropospheric ozone is a photochemical reaction product and the increased solar radiation during summer has a key role in its

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concentration. The high concentration of NO_2 is attributed to increased NO_2 emission from cars in winter and to the photochemical conversion of NO to NO_2 in summer.

Various studies with diverse methodological procedures have been conducted worldwide to investigate the composition and concentration fluctuations of fungal spores in ambient air and to elucidate the association between fungal aerosol and environmental factors. Most studies are based on results obtained by non-culture based methods and focus on certain allergenic genera that produce spores easily recognizable. So, there is limited information for the genera *Aspergillus* and *Penicillium,* which constitute a significant part of fungal aerosol and have implications in human affairs, since their spores are usually indistinguishable in slide recording methods. Concerning the Mediterranean region, the interactions of airborne fungi with meteorological factors or air pollutants are underexplored. In a study of airborne fungi in Italy by a culture-based method, the meteorological factors and air pollutants have been evaluated (Filipello Marchisio and Airaudi, 2001). Also, the distribution patterns of fungal aerosol sampled by a non-viable method in Thessaloniki, Greece has been evaluated in relation to meteorological parameters (Gioulekas et al., 2004; Damialis and Gioulekas, 2006).

The objective of the present study was to investigate the influence of meteorological variables and air pollutants on the concentration fluctuations of the total fungal load and predominant genera, during a multi-annual study on fungal aerosol sampled by two complementary methods, in Athens city center.

2. Materials and methods

2.1. Study area

Athens is located in central Greece and it is the largest city. Athens is densely populated and dominates the Attica basin which is bisected by a number of hills and surrounded by Aegaleo, Parnitha, Penteli and Hymettus mountains, with an opening to the Saronic Gulf at the southwest. The average altitude of the city is 136.1 m. The climate is temperate Mediterranean. Athens is characterized by a long-term mean annual temperature of 18.5 °C, but the years 1999, 2000 and 2001 were the warmest since 1897 with annual mean temperature 19.5 °C, 19.2 °C, and 19.4 °C respectively (Founda et al., 2004). The annual mean relative humidity is 62% and the annual mean rainfall is 414.1 mm. The coldest month is January with average temperature 6.7 °C and the hottest is July with average temperature 31 °C. Precipitation takes place mainly in autumn and winter months.

2.2. Aeromycological data

Air sampling was performed in the center of Athens city, for 4 years. The sampling site was on the rooftop of the Ministry of the Environment building at a height of 30 m from ground level. It is located in a densely populated area with tall buildings and narrow streets, near Areos Park. Two portable air samplers (Burkard Manufacturing Company, Ltd), one for agar plates and one for glass slides were used. Twelve Petri dishes with Malt Agar (MA) 2% and Potato Dextrose Agar (PDA) were exposed for 1 min each, three times a week and then incubated at 25 °C in the dark for 7–21 d. Also glass slides covered with glycerin jelly as an adhesive were exposed for 30 min, mounted with three drops of the glycerin jelly and examined with a Zeiss KF-2 light microscope at 400X magnification.

The colony counts were corrected by the multiple infection transformation factors (Gregory, 1948) and converted to colony forming units per cubic meter (CFU/m^3) of air sampled, whereas the spore counts were converted to spores per cubic meter ($spores/m^3$) of air sampled. Details of the sampling protocols have been previously reported (Pyrri and Kapsanaki-Gotsi, 2012, 2015).

2.3. Environmental data

Meteorological data were provided by the National Observatory of Athens which is located on a hill about 3 km away from the sampling site. The variables used were air temperature (T), relative humidity (RH), solar radiation (SR), atmospheric pressure (AP), wind speed (WS), wind direction (WD) and total precipitation (P). Temperature was given in degrees Celsius (°C), relative humidity as a percentage (%), atmospheric pressure in mmHg, solar radiation in MJ m^{-2}, wind speed in m sec^{-1} and precipitation in mm.

Air pollution parameters were provided by the PERPA station of the Greek Ministry of Environment and Energy located at the building where the air sampling took place. The parameters measured were carbon monoxide (CO), nitric oxide (NO), nitrogen dioxide (NO_2), sulphur dioxide (SO_2), ozone (O_3), and smoke and concentrations are given in $\mu g\ m^{-3}$. Greece monitored black smoke as a surrogate for particulate matter (PM) concentrations during the time of this study.

For the statistical analysis, the hourly mean values of the environmental parameters measured concurrently with fungal sampling were used, except the total precipitation (P) for which the total amount of rain of the last 5 d before the sampling was used.

2.4. Statistical analysis

The concentrations of the total fungi and the predominant genera as well as the meteorological and the pollution parameters were computed in Excel, in SPSS (v.22) and in C (v4.5) for statistical processing.

The linear association between fungal spore concentration and individual meteorological and pollution factors was explored with the implementation of the Spearman rank order correlation coefficient (r_s). The concentration of fungal spores was not normally distributed as tested by the Kolmogorov-Smirnov (data not shown).

Canonical Correspondence Analysis (CCA) was performed to determine which environmental factors explain the fluctuations and variance of the outdoor fungal spores. A multivariate direct gradient analysis was performed so that fungal spore occurrences could be directly associated with environmental variables. The unimodal distribution model was selected. The constrained ordination technique with weighted averaging was applied. The statistical significance of the first and all canonical axes was determined with the Monte Carlo permutation test available in C. Automated forward selection was used to determine the importance of each environmental variable in explaining the total variance observed in the aerobiological data. A CCA was conducted for the total count and the major constituents including seven meteorological variables: mean air temperature (T), relative humidity (RH), solar radiation (SR), atmospheric pressure (AP), wind speed (WS) and wind direction (WD). A separate CCA was performed for fungal spores and five pollution parameters: carbon monoxide (CO), nitric oxide (NO), nitrogen dioxide (NO_2), sulphur dioxide (SO_2), ozone (O_3), and smoke (SM). Fungal spore concentrations were not analyzed in relation to all twelve environmental variables since an association was found among meteorological and pollution factors.

Stepwise multiple linear regression analysis was implemented to forecast the change of the fungi (dependent variable) in relation to meteorological parameters (independent variables) and to identify the best combination of predictors. The aerobiological data used for the analysis of total fungi, *Cladosporium*, *Alternaria*,