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# Outdoor Allergenic Fungal Spores: Comparison Between an Urban and a Rural Area in Northern Portugal

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## Abstract

*Background:* The frequency and concentration of many airborne fungal spores associated with respiratory allergy symptoms are influenced by geographical and climatic characteristics.
*Objective:* The aim of this work was to monitor the distribution of 11 potentially allergenic fungal spore types in 2 regions with different urbanization levels in Northern Portugal: Porto (urban area) and Amares (rural area).
*Methods:* Airborne fungal spore levels were monitored from 2005 to 2007 using Hirst-type spore traps. The Spearman correlation test was used to analyze the influence of meteorological factors (temperature, relative humidity, and rainfall) on spore concentration. Meteorological data from both areas were compared using the *t* test, and spore concentrations were compared using the sign test.
*Results:* In both areas, *Cladosporium, Agaricus, Aspergillus/Penincillium, Alternaria, Coprinus*, and rusts were the most abundant fungal types observed. Most of the analyzed spore types presented maximum values during the summer months, with the exception of *Polythrincium*, *Stemphylium*, and *Torula*, which reached a peak earlier in the year, whereas *Aspergillus/Penicillium* and *Botrytis* showed a wider distribution. Temperature had a positive effect on most spore concentrations, and relative humidity and rainfall negatively influenced concentrations of *Alternaria, Cladosporium, Epicoccum*, and *Torula*.
*Conclusions:* The concentration of all selected spore types was higher in the rural than in the urban area, with higher values registered during summer and autumn and lower values found during winter and spring. Meteorological parameters, such as air temperature, humidity and rainfall, influence airborne concentrations of major allergenic fungal spores.

Key words: Aerobiology. Allergens. Fungal spores. Rural area. Urban area.

## Resumen

*Antecedentes:* Las características geográficas y climáticas influyen en la frecuencia y la concentración de un gran número de esporas fúngicas en el aire asociadas a síntomas de alergia respiratoria.
*Objetivo:* El objetivo de este trabajo fue controlar la distribución de 11 tipos de esporas fúngicas potencialmente alergénicas en dos regiones del norte de Portugal con diferentes niveles de población: Porto (zona urbana) y Amares (zona rural).
*Métodos:* Se midieron los niveles de esporas fúngicas en el aire desde 2005 hasta 2007 mediante captadores de esporas tipo Hirst. Se utilizó la prueba de correlación de Spearman para analizar la influencia de los factores meteorológicos (temperatura, humedad relativa y precipitaciones) en la concentración de esporas. Se compararon los datos meteorológicos de ambas zonas mediante la prueba *t*, y las concentraciones de esporas, mediante la prueba de los signos.
*Resultados:* Los tipos de hongos más abundantes observados en ambas zonas fueron *Cladosporium, Agaricus, Aspergillus/Penincillium, Alternaria, Coprinus* y royas. La mayoría de los tipos de esporas analizados presentaron valores máximos durante los meses de verano, a excepción de *Polythrincium, Stemphylium* y *Torula*, que alcanzaron sus valores máximos a principios de año, mientras que *Aspergillus/Penicillium* y *Botrytis* mostraron una distribución más amplia. La temperatura tuvo un efecto positivo en la mayoría de concentraciones de esporas, y la humedad relativa y las precipitaciones influyeron de forma negativa en las concentraciones de *Alternaria, Cladosporium, Epicoccum* y *Torula*.
*Conclusiones:* Las concentraciones de todos los tipos de esporas seleccionados fueron mayores en la zona rural que en la zona urbana; los valores más elevados se registraron durante el verano y el otoño y los valores más bajos durante el invierno y la primavera. Los parámetros meteorológicos, como la temperatura del aire, la humedad y las precipitaciones, influyen en las principales concentraciones en el aire de las principales esporas fúngicas alérgenicas.

Palabras clave: Aerobiología. Alérgenos. Esporas fúngicas. Zona rural. Zona urbana.

## Introduction

Atmospheric air contains a wide variety of components that can affect human health. These components include inorganic gaseous and particulate pollutants and biological particles. Among biological particles, fungal spores are associated with allergic sensitization and respiratory symptoms.

The 11 allergenic spores chosen for our analysis were *Alternaria, Aspergillus/Penicillium, Botrytis, Cladosporium, Drechslera*-type, *Epicoccum, Ganoderma, Pithomyces, Polythrincium, Stemphylium*, and *Torula*.

Exposure to fungal spores, vegetative cells, and metabolites is associated with a number of allergic diseases in humans, and the main manifestations are rhinitis, allergic bronchopulmonary mycoses, and hypersensitivity pneumonitis [1]. Prevalence is highly dependent on the fungal species and population studied. Prevalence of sensitization to *Alternaria* among patients with allergic diseases in the United States and Greece ranges from 13.5% to 31.9% [2,3]. In the case of *Aspergillus*, prevalence ranges from 5% to 17.4% [2,3], whereas sensitization to *Penicillium* has been shown to be 7.2% in a group of American patients [2]. Cross-reactivity has been observed between *Alternaria* and *Aspergillus* [4]. A study performed on asthmatic patients in the Netherlands showed that 7% of patients were sensitized to fungal spores [1]. In European and American populations, the prevalence of sensitization ranges from 0.7% to 24.1% [2,3]. Reactivity to *Drechslera*-type spores (including *Bipolaris, Drechslera, Exserohilum*, and *Helminthosporium*), as demonstrated by skin testing, is significant (around 26%) in Finnish asthmatic children [5]. Allergy to *Epicoccum* is becoming more widespread—it affects between 5% and 15.4% of the population in Europe [2,6]—and cross-reacts with other fungi such as *Alternaria, Cladosporium*, and *Penicillium* [7]. In a group of Indian patients who underwent skin prick tests with crude extracts of *Ganoderma*, 11% had positive results, indicating the allergenic potential of this species. *Pithomyces* spores can potentially produce mycotoxins such as cyclodepsipeptides, sporidesmolides, and sporidesmin. To our knowledge, no data are available on the prevalence of sensitization to *Pithomyces, Polythrincium, Stemphylium*, and *Torula*, even though these fungi are considered allergenic.

The environmental differences between urban and rural areas reveal extremes of exposure and provide us with a unique insight into the potential causes of increased prevalence of allergy [8]. In urban areas, high levels of vehicle emissions have been associated with increased prevalence of respiratory allergy [9]. Although the role of air pollution in allergic disease is unclear, there is increasing evidence that higher levels of traffic-related particulate matter and the ability of this allergen to affect the allergenicity of biological particles may contribute to the increasing prevalence and severity of allergic disease in urban areas [10]. These pollutants lead to the release of proteins and allergens by the pollen grain and induce morphological changes on its surface [11]. Whether such interactions can also occur with fungal spores remains unknown. In rural areas, on the other hand, exposure has been shown to protect against sensitization to common aeroallergens, asthma, and allergic diseases [12].

The aim of this study was to compare the genera of allergenic fungal spores present in the air of an urban area and a rural area in northern Portugal, and to study their association with meteorological factors.

## Material and Methods

In the rural area, the sampler (Lanzoni, Bologna, Italy) was located on a farm at Amares (Braga District) (41°38'N, 8°23'W; height, 5 m), surrounded by greenhouses, woodland, and vineyards. The district has an area of 2673 km² and a population of around 830 000 inhabitants. In Porto (urban area), the sampler (Burkard Manufacturing Co., Rickmansworth, UK) was located on the roof of Faculdade de Ciências (41º11' N, 8º39' W; height, 20 m), surrounded by trees, shrubs, and herbaceous species. The Porto district, in the Douro Litoral Region, has an area of 2395 km$^2$ and a population of nearly 1 900 000 inhabitants. The west of this city borders the Atlantic Ocean and the south borders the River Douro (Figure 1).

Daily spore concentrations were sampled from January 2005 to December 2007 using 2 Hirst-type volumetric spore traps (Burkard Manufacturing Co., Lanzoni) with a flow rate of 10 L/min. Spores were trapped onto a Melinex adhesive tape (Burkard Manufacturing Co.) that was cut into segments daily. The slides on which the adhesive segments were placed were mounted in fuchsin-glycerine jelly and covered with glass. The daily mean concentration of the number of fungal spores was determined using an optical microscope at a magnification of ×400 along 2 full lengthwise traverses. Spore counts were converted to spores/m$^3$/d. Fungal spores were identified by morphological characteristics and comparison with a reference work [13].

To allow comparisons between the 2 sampling sites, data were standardized by converting annual values into *z* scores. The standard score is

$$z = \frac{x - X}{S}$$

where $x$ is a raw value to be standardized, $S$ is the standard deviation of the sample, and $X$ is the mean of the sample.

The meteorological data and daily spore concentrations for both locations were compared. The Kolmogorov-Smirnov test was used to check normality. The *t* test was used when data were normally distributed, and the nonparametric sign test was applied when data were nonnormally distributed. In both cases, the significance level was adjusted to the number of comparisons performed.

In order to verify the degree of association between daily atmospheric spore concentrations and daily meteorological values (temperature, relative humidity, and rainfall), the Spearman rank correlation test was used with significance set at 95% and 99%.

## Results

We analyzed the meteorological conditions of Amares and Porto, and observed statistically significant differences in the relative humidity values of both locations during 2006 and 2007 (Figure 2 and Table 1).

We identified 42 spore types, and the most abundant were *Cladosporium, Agaricus, Aspergillus/Penincillium, Alternaria*,

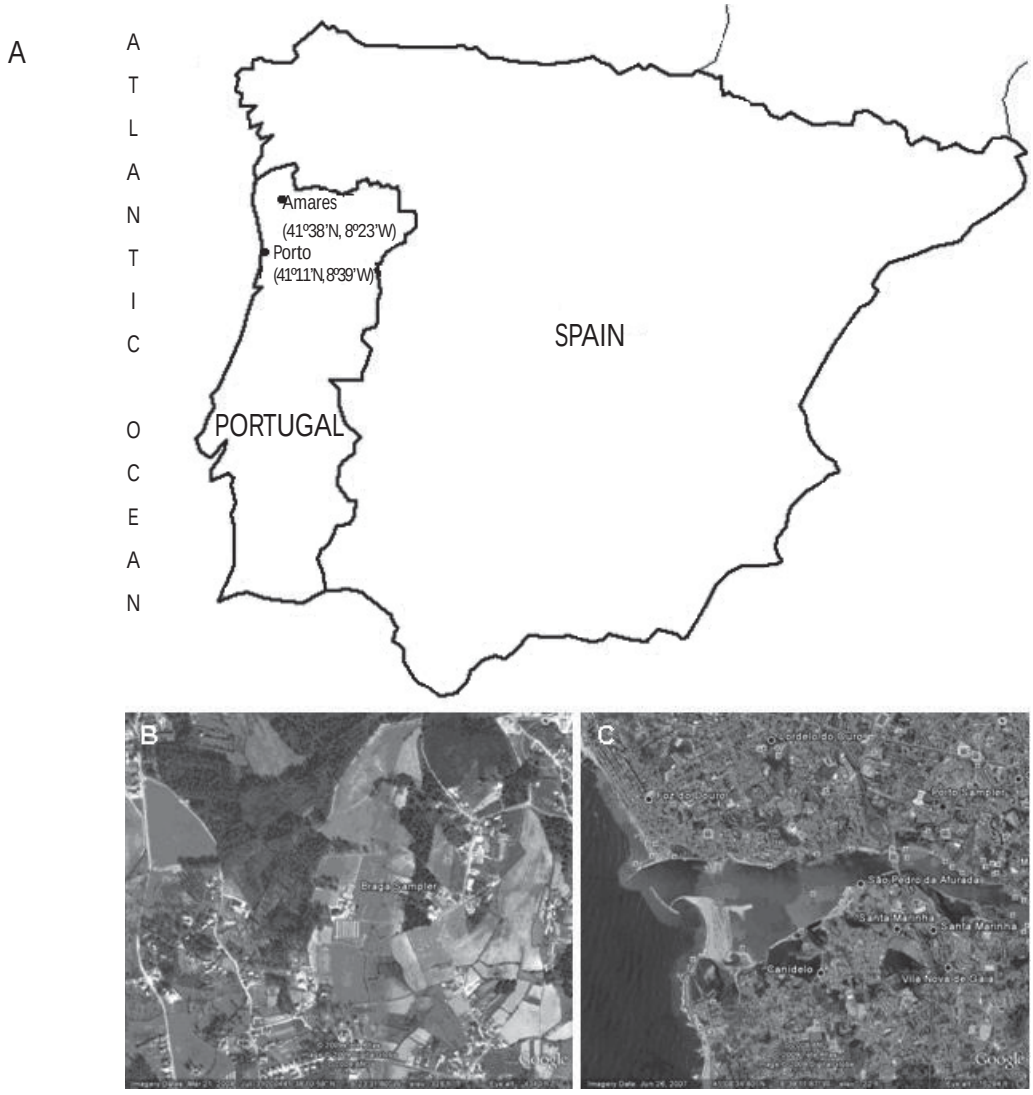



Figure 1.Sampler location in the 2 study areas. A, Amares (rural). B, Porto (urban). C, Aerial photographs were obtained using Google Earth.

Table 1. Kolmogorov-Smirnov and *t* Test Results for Daily Spore Concentrations According to Meteorological Conditions[a]

| | | Kolmogorov-Smirnov Test | | | | | *t* Test |
|---|---|---|---|---|---|---|---|
| Meterological Factor | Year | K-S Value (Amares) | *P* | K-S Value (Porto) | Significance | T | *P*[a] |
| Mean Temperature | 2005 | 0.451 | .987 | 0.431 | 0.992 | 0.285 | .389 |
| | 2006 | 0.416 | .995 | 0.392 | 0.998 | 0.246 | .404 |
| | 2007 | 0.423 | .994 | 0.559 | 0.914 | 0.040 | .484 |
| Mean relative humidity | 2005 | 0.746 | .634 | 0.672 | 0.758 | 0.169 | .055 |
| | 2006 | 0.400 | .997 | 0.626 | 0.828 | 0.214 | .022 |
| | 2007 | 0.449 | .988 | 0.456 | 0.986 | 0.364 | .001 |
| Rainfall | 2005 | 0.630 | .822 | 0.700 | 0.711 | 0.998 | .165 |
| | 2006 | 0.827 | .501 | 0.615 | 0.844 | 0.267 | .058 |
| | 2007 | 0.930 | .389 | 0.722 | 0.675 | 0.637 | .266 |

Abbreviation: K-S, Kolmogorov-Smirnov.
[a] The significance level was adjusted to the 9 comparisons performed (.0056).

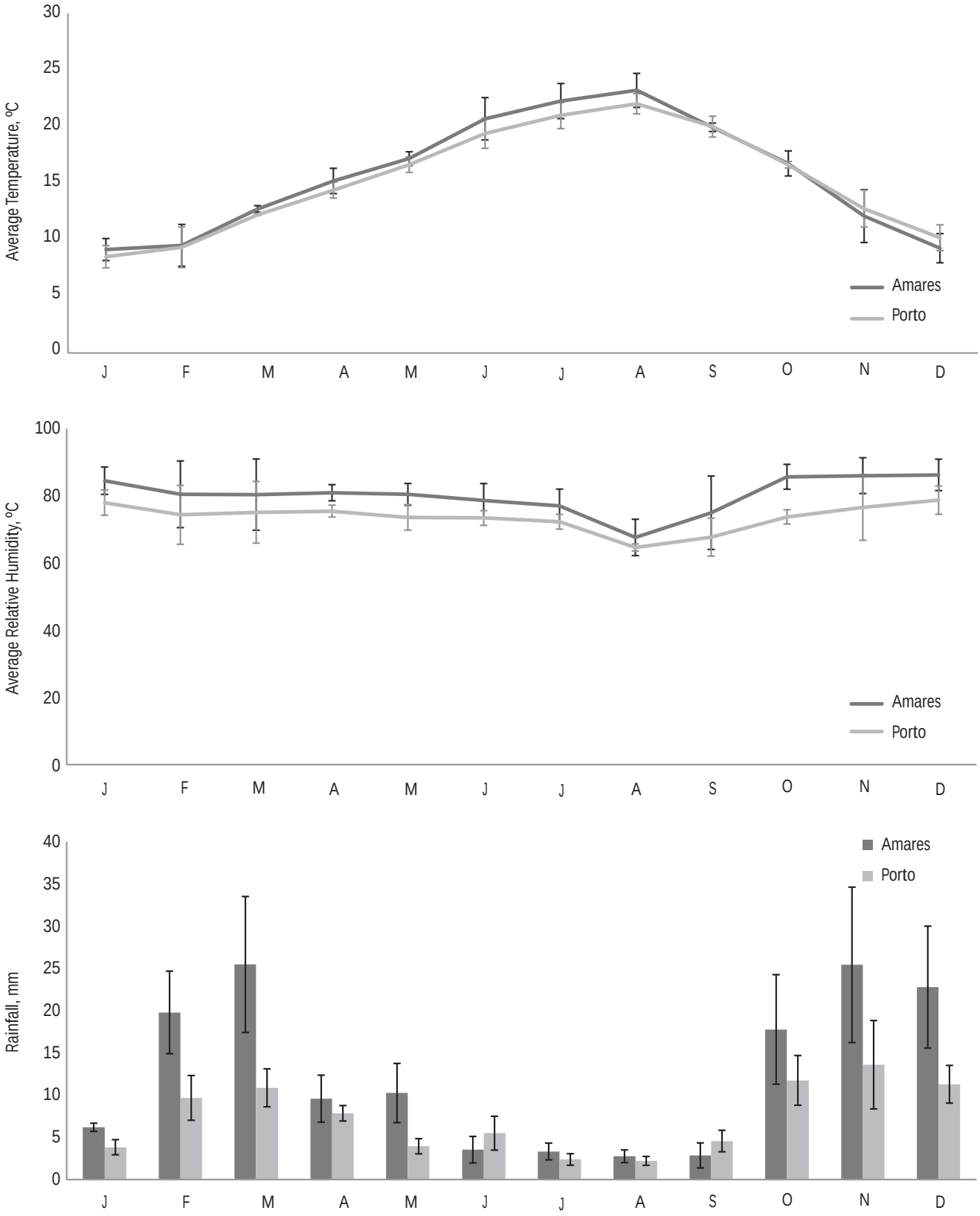
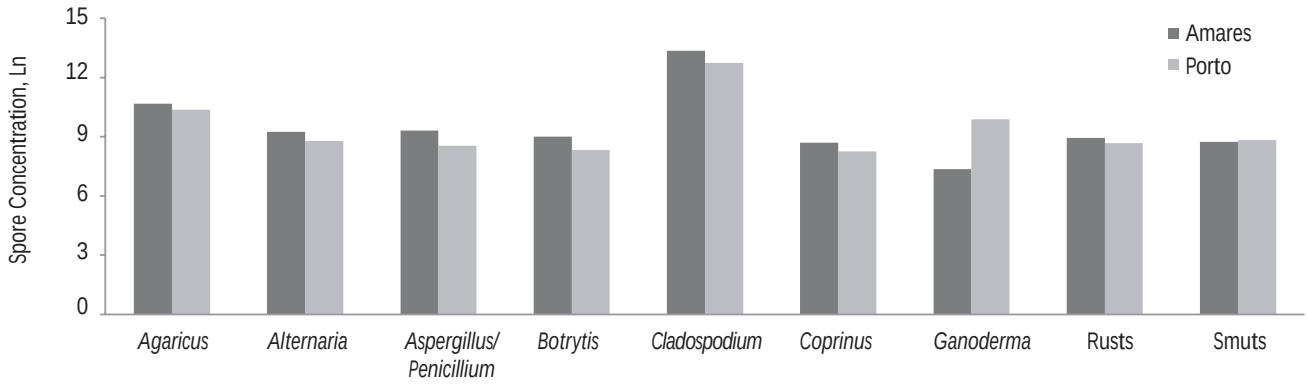



Figure 2.Mean (SD) monthly meteorological conditions (temperature, relative humidity, and total rainfall level) registered in Amares and Porto from 2005 to 2007.




Ln indicates natural logarithm.

Figure 3. Spore concentrations by species and location.

*Coprinus*, and rusts. Values above 1% for *Botrytis* were recorded in Amares, whereas smuts and *Ganoderma* were recorded in Porto (Table 2).

The lowest monthly values were detected in January, February, and December, and the highest values were recorded from August to October. *Cladosporium, Epicoccum, Ganoderma,* and *Pithomyces* presented their maximum monthly spore counts during this period; maximum values for *Alternaria* were registered from August to September. *Polythrincium, Stemphylium,* and *Torula* peak values were reached earlier in the year, whereas *Aspergillus/Penicillium* and *Botrytis* showed a wider temporal distribution (Figure 3 and Table 2).

During the study period, *Alternaria, Aspergillus/ Penicillium, Cladosporium,* and *Torula* presented higher *z* scores in Amares than in Porto. The remaining fungal spores presented different behaviors: *Botrytis, Drechslera*-type, *Epicoccum, Polythrincium,* and *Stemphylium* revealed higher values in the rural area in 2005 and 2007, and higher values in the urban area in 2006; *Ganoderma* presented higher values in the rural area, except in 2007, when higher values were recorded in Porto (Table 2).

We observed statistically significant differences between rural and urban values for *Alternaria, Aspergillus/Penicillium* (2005 and 2006), *Botrytis, Cladosporium, Epicoccum, Ganoderma, Polythrincium* (2006 and 2007), and *Torula* (Table 3).

*Alternaria, Cladosporium,* and *Epicoccum* presented a significant positive correlation with air temperature and a negative correlation with relative humidity and rainfall, whereas *Torula* exhibited negative correlations with relative humidity and rainfall only. We observed positive correlations between *Drechslera*-type, *Ganoderma, Pithomyces, Polythrincium,* and *Stemphylium* concentrations and temperature values. Moreover, *Botrytis* presented a different correlation for both location and year: in the urban area, positive correlations with temperature (2007), relative humidity, and rainfall (2005); in the rural area, negative (2005) and positive correlations (2006 and 2007) with temperature. Finally, *Aspergillus/Penicillium* spores did not present significant correlation coefficients with any of the meteorological factors analyzed (Table 4).

## Discussion

Airborne fungal spore levels were monitored from 2005 to 2007 in 2 different regions (urban/rural) in northern Portugal. The most frequent spore was *Cladosporium*, followed by *Aspergillus/Penincillium* and *Alternaria*. Moreover, *Botrytis, Drechslera*-type, *Epicoccum, Pithomyces, Polythrincium, Stemphylium,* and *Torula* constituted a diminutive portion of the total airborne fungal spore spectrum (around 2%). *Ganoderma* presented a different behavior depending on the area: in Porto it accounted for more than 4% of the fungal spectrum, whereas in Amares it only accounted for 0.2%. We observed 2 distinct groups: *a)* spores with an occurrence higher than 50% (*Alternaria, Botrytis, Cladosporium Epicoccum*, and *Ganoderma*); and *b)* spores with an occurrence below 50% and sporadic behavior (*Aspergillus/Penicillium, Drechslera*-type, *Pithomyces, Polythrincium,* and *Stemphylium*). *Torula* varied with the area: in Amares it was included in the first group, whereas in Porto it was in the second group. A study performed in Poland also observed 2 distinct groups: a high-frequency group composed of *Alternaria, Cladosporium, Botrytis, Epicoccum, Ganoderma,* and *Drechslera*-type; and a low-frequency group composed of *Pithomyces, Polythrincium, Stemphylium,* and *Torula* [14]. The main difference between the 2 studies is the inclusion of *Drechslera*-type in the second group.

When compared with previous studies from other regions of the Iberian Peninsula [15], the spore counts we recorded in Porto were low and the values recorded in Amares were more associated with inland regions. This could be explained by the geographical characteristics of Porto (close to the River Douro and the Atlantic Ocean), which negatively affect concentrations of these airborne biological particles. The decrease in spore concentrations due to the presence of rivers and oceans has been described elsewhere [16].

Atmospheric fungal spore concentrations varied according to the season, with higher values during summer and autumn and lower values during winter and spring. In these regions, rainfall is mainly concentrated during the cool months of January,

Table 2. Descriptive Statistics of the Selected Spore Types Present in the Atmosphere of Amares and Porto (2005 to 2007)[a]

| | | Total | Abundance, % | Maximum | Date | Mean (SD) | *z* Score | Days of Occurrence | Occurrence, % |
|---|---|---|---|---|---|---|---|---|---|
| **Amares** | | | | | | | | | |
| *Alternaria* | 2005 | 3742.82 | 1.15 | 80.35 | 30 Jul | 10.25(13.29) | 280.82 | 315 | 86 |
| | 2006 | 3175.39 | 0.94 | 104.16 | 22 Jun | 8.70 (14.09) | 224.72 | 274 | 75 |
| | 2007 | 3542.43 | 1.01 | 109.12 | 04 Oct | 9.98 (13.39) | 263.91 | 279 | 76 |
| *Aspergillus/ Alternaria* | 2005 | 4980.83 | 1.53 | 302.56 | 22 Nov | 13.65 (29.13) | 170.52 | 187 | 51 |
| | 2006 | 3704.13 | 1.10 | 242.05 | 02 Oct | 10.15 (22.08) | 167.28 | 156 | 43 |
| | 2007 | 2419.49 | 0.69 | 98.21 | 17 Apr | 6.82 (13.58) | 177.72 | 126 | 35 |
| *Botrytis* | 2005 | 3293.44 | 1.01 | 124.99 | 23 Jan | 9.02 (12.35) | 265.89 | 333 | 91 |
| | 2006 | 1783.62 | 0.53 | 44.64 | 26 Mar | 4.89 (6.89) | 257.99 | 300 | 82 |
| | 2007 | 3123.81 | 0.89 | 97.22 | 05 Dec | 8.80 (12.34) | 252.53 | 311 | 85 |
| *Cladosporium* | 2005 | 216 988.10 | 66.72 | 4682.24 | 04 Jun | 594.49 (679.01) | 318.69 | 365 | 100 |
| | 2006 | 188 296.48 | 55.89 | 5884.54 | 29 Sep | 515.88 (832.94) | 225.44 | 365 | 100 |
| | 2007 | 229 226.40 | 65.17 | 7566.98 | 04 Oct | 645.71 (973.99) | 234.68 | 355 | 97 |
| *Drechslera*-type | 2005 | 76.38 | 0.02 | 5.95 | 22 Mar | 0.21 (0.57) | 134.44 | 60 | 16 |
| | 2006 | 16.86 | 0.01 | 1.98 | 01 Apr | 0.05 (0.26) | 65.74 | 13 | 4 |
| | 2007 | 57.54 | 0.02 | 1.98 | 08 Jun | 0.16 (0.34) | 133.42 | 49 | 13 |
| *Epicoccum* | 2005 | 1310.43 | 0.40 | 27.78 | 18 Oct | 3.59 (4.68) | 279.47 | 276 | 76 |
| | 2006 | 706.30 | 0.21 | 33.73 | 03 Oct | 1.94 (3.77) | 186.84 | 189 | 52 |
| | 2007 | 1180.48 | 0.34 | 63.49 | 05 Oct | 3.33 (5.97) | 197.20 | 234 | 64 |
| *Ganoderma* | 2005 | 632.90 | 0.19 | 20.83 | 30 Aug | 1.73 (2.69) | 234.71 | 198 | 54 |
| | 2006 | 552.54 | 0.16 | 15.87 | 27 Aug | 1.50 (2.35) | 234.01 | 181 | 50 |
| | 2007 | 370.02 | 0.11 | 10.91 | 07 Sep | 1.04 (1.70) | 217.31 | 159 | 44 |
| *Pithomyces* | 2005 | 101.18 | 0.03 | 4.96 | 13 Sep | 0.28 (0.64) | 156.64 | 77 | 21 |
| | 2006 | 85.31 | 0.03 | 15.87 | 29 Sep | 0.23 (1.07) | 79.49 | 41 | 11 |
| | 2007 | 88.29 | 0.03 | 10.91 | 19 Aug | 0.25 (0.65) | 136.03 | 58 | 16 |
| *Polythricium* | 2005 | 68.45 | 0.02 | 4.96 | 08 Apr | 0.19 (0.63) | 108.67 | 42 | 12 |
| | 2006 | 242.05 | 0.07 | 15.87 | 24 Apr | 0.66 (2.59) | 93.28 | 61 | 17 |
| | 2007 | 143.84 | 0.04 | 13.89 | 30 Apr | 0.41 (1.09) | 131.84 | 81 | 22 |
| *Stemphylium* | 2005 | 149.79 | 0.05 | 4.96 | 27 Jun | 0.41 (1.09) | 186.02 | 99 | 27 |
| | 2006 | 152.77 | 0.05 | 8.93 | 15 Jun | 0.42 (1.07) | 142.46 | 83 | 23 |
| | 2007 | 228.16 | 0.06 | 11.90 | 03 Oct | 0.64 (1.27) | 178.72 | 117 | 32 |
| *Torula* | 2005 | 903.71 | 0.28 | 27.78 | 16 Feb | 2.48 (3.37) | 267.54 | 241 | 66 |
| | 2006 | 515.40 | 0.15 | 12.90 | 15 Oct | 1.41 (1.989) | 259.36 | 194 | 53 |
| | 2007 | 614.05 | 0.17 | 14.88 | 08 Jul | 1.73 (2.66) | 230.45 | 213 | 58 |

Continued

| | | Total | Abundance, % | Maximum | Date | Mean (SD) | *z* Score | Days of Occurrence | Occurrence, % |
|---|---|---|---|---|---|---|---|---|---|
| **Porto** | | | | | | | | | |
| *Alternaria* | 2005 | 1975.07 | 1.09 | 47.62 | 18 Aug | 5.43 (8.76) | 224.96 | 263 | 72 |
| | 2006 | 1881.82 | 0.96 | 81.34 | 29 Aug | 5.16 (9.259 | 202.90 | 255 | 70 |
| | 2007 | 2624.83 | 1.37 | 85.31 | 06 Aug | 7.72 (12.52) | 209.11 | 249 | 68 |
| *Aspergillus/ Penicillium* | 2005 | 2415.52 | 1.34 | 151.78 | 19 Aug | 6.64 (17.10) | 140.84 | 103 | 28 |
| | 2006 | 1697.31 | 0.87 | 122.02 | 09 Aug | 4.65 (13.42) | 126.15 | 92 | 25 |
| | 2007 | 1054.50 | 0.55 | 87.3 | 04 Jun | 3.10 (8.54) | 123.14 | 83 | 23 |
| *Botrytis* | 2005 | 1130.88 | 0.63 | 52.58 | 20 Aug | 3.11 (4.98) | 226.42 | 261 | 72 |
| | 2006 | 114.78 | 0.58 | 23.81 | 30 Mar | 3.13 (3.73) | 305.78 | 274 | 75 |
| | 2007 | 1802.46 | 0.94 | 83.33 | 17 Jun | 5.30 (9.57) | 187.75 | 252 | 69 |
| *Cladosporium* | 2005 | 111 535.52 | 61.80 | 2972.02 | 21 Aug | 306.42 (458.26) | 242.72 | 363 | 99 |
| | 2006 | 99 715.84 | 50.91 | 3958.08 | 21 Aug | 273.19 (493.57) | 201.47 | 364 | 100 |
| | 2007 | 127 054.37 | 66.21 | 8297.09 | 18 Jun | 373.69 (725.82) | 174.53 | 339 | 93 |
| *Drechslera*-type | 2005 | 54.56 | 0.03 | 3.97 | 30 Jul | 0.15 (0.45) | 120.92 | 45 | 12 |
| | 2006 | 32.74 | 0.02 | 3.97 | 28 Jun | 0.09 (0.37) | 88.78 | 27 | 7 |
| | 2007 | 70.43 | 0.04 | 3.97 | 20 Sep | 0.21 (0.59) | 119.90 | 47 | 13 |
| *Epicoccum* | 2005 | 545.60 | 0.30 | 14.88 | 30 Jul | 1.50 (2.34) | 232.11 | 201 | 55 |
| | 2006 | 296.61 | 0.15 | 11.90 | 27 Aug | 0.81 (1.43) | 206.62 | 153 | 42 |
| | 2007 | 622.98 | 0.32 | 51.58 | 07 Oct | 1.83 (4.22) | 147.18 | 183 | 50 |
| *Ganoderma* | 2005 | 7807.04 | 4.33 | 190.46 | 09 Sep | 21.45 (35.35) | 220.21 | 275 | 75 |
| | 2006 | 6629.54 | 3.38 | 179.55 | 11 Sep | 18.16 (28.42) | 232.65 | 288 | 79 |
| | 2007 | 4934.21 | 2.57 | 121.02 | 14 Sep | 14.51 (19.61) | 250.85 | 293 | 80 |
| *Pithomyces* | 2005 | 86.30 | 0.05 | 9.92 | 08 Aug | 0.24 (0.85) | 100.94 | 50 | 14 |
| | 2006 | 47.62 | 0.02 | 2.98 | 21 Jun | 0.13 (0.43) | 111.01 | 37 | 10 |
| | 2007 | 104.16 | 0.05 | 5.95 | 02 Oct | 0.31 (0.74) | 141.15 | 68 | 19 |
| *Polythricium* | 2005 | 29.76 | 0.02 | 2.98 | 10 May | 0.08 (0.32) | 93.05 | 26 | 7 |
| | 2006 | 43.65 | 0.02 | 2.98 | 19 Apr | 0.12 (0.42) | 104.08 | 34 | 9 |
| | 2007 | 53,.57 | 0.03 | 2.98 | 11 Aug | 0.16 (0.47) | 114.15 | 41 | 11 |
| *Stemphylium* | 2005 | 178.56 | 0.10 | 7.94 | 04 Jul | 0.49 (1.01) | 176.14 | 104 | 28 |
| | 2006 | 191.46 | 0.10 | 10.91 | 17 Jun | 0.52 (1.21) | 157.30 | 98 | 27 |
| | 2007 | 257.92 | 0.13 | 16.86 | 04 Jun | 0.76 (1.68) | 152.85 | 105 | 29 |
| *Torula* | 2005 | 398.78 | 0.22 | 14.88 | 13 Feb | 1.10 (1.83) | 217.43 | 170 | 47 |
| | 2006 | 331.22 | 0.17 | 11.90 | 29 Aug | 0.91 (1.55) | 213.21 | 158 | 43 |
| | 2007 | 393.82 | 0.21 | 15.87 | 12 Mar | 1.16 (1.91) | 205.20 | 165 | 45 |

[a] Annual values were converted into *z* scores to allow comparisons between the different locations.

Table 3. Kolmogorov-Smirnov and Sign Test Results for Daily Spore Concentrations

| Fungal spore | Year | Kolmogorov-Smirnov Test | | | | | Sign Test |
|---|---|---|---|---|---|---|---|
| | | K-S Value (Amares) | Significance | K-S Value (Porto) | Significance | Value | *P*[a] |
| *Alternaria* | 2005 | 4.207 | 0.000 | 5.108 | 0.000 | –7.440 | .000 |
| | 2006 | 5.130 | 0.000 | 5.515 | 0.000 | –5.400 | .000 |
| | 2007 | 4.296 | 0.000 | 4.954 | 0.000 | –3.834 | .000 |
| *Aspergillus/ Penicillium* | 2005 | 6.108 | 0.000 | 7.021 | 0.000 | –5.551 | .000 |
| | 2006 | 6.169 | 0.000 | 7.327 | 0.000 | –4.610 | .000 |
| | 2007 | 6.354 | 0.000 | 7.333 | 0.000 | –2.923 | .003 |
| *Botrytis* | 2005 | 4.443 | 0.000 | 5.084 | 0.000 | –11.247 | .000 |
| | 2006 | 4.571 | 0.000 | 3.860 | 0.000 | –4.206 | .000 |
| | 2007 | 4.481 | 0.000 | 5.345 | 0.000 | –5.805 | .000 |
| *Cladosporium* | 2005 | 3.746 | 0.000 | 4.847 | 0.000 | –11.826 | .000 |
| | 2006 | 5.185 | 0.000 | 5.540 | 0.000 | –9.618 | .000 |
| | 2007 | 4.786 | 0.000 | 5.593 | 0.000 | –6.881 | .000 |
| *Drechslera*-type | 2005 | 9.163 | 0.000 | 9.669 | 0.000 | –1.735 | .083 |
| | 2006 | 10.241 | 0.000 | 9.979 | 0.000 | –2.028 | .043 |
| | 2007 | 9.587 | 0.000 | 9.218 | 0.000 | 0.000 | 1.000 |
| *Epicoccum* | 2005 | 4.229 | 0.000 | 5.463 | 0.000 | –8.146 | .000 |
| | 2006 | 5.807 | 0.000 | 5.646 | 0.000 | –4.784 | .000 |
| | 2007 | 5.441 | 0.000 | 6.124 | 0.000 | –4.652 | .000 |
| *Ganoderma* | 2005 | 4,985 | 0.000 | 5.190 | 0.000 | –12.772 | .000 |
| | 2006 | 4.972 | 0.000 | 4.993 | 0.000 | –14.443 | .000 |
| | 2007 | 5.320 | 0.000 | 4.235 | 0.000 | –15.701 | .000 |
| *Pithomyces* | 2005 | 8.701 | 0.000 | 9.007 | 0.000 | –2.127 | .033 |
| | 2006 | 9.057 | 0.000 | 9.904 | 0.000 | –0.512 | .609 |
| | 2007 | 9.106 | 0.000 | 8.506 | 0.000 | –1.258 | .208 |
| *Polythricium* | 2005 | 9.595 | 0.000 | 10.106 | 0.000 | –2.016 | .044 |
| | 2006 | 8.291 | 0.000 | 9.921 | 0.000 | –4.875 | .000 |
| | 2007 | 7.856 | 0.000 | 9.426 | 0.000 | –4.577 | .000 |
| *Stemphylium* | 2005 | 8.100 | 0.000 | 7.639 | 0.000 | –0.710 | .478 |
| | 2006 | 8.114 | 0.000 | 7.615 | 0.000 | –1.443 | .149 |
| | 2007 | 6.848 | 0.000 | 6.731 | 0.000 | –0.583 | .560 |
| *Torula* | 2005 | 4.418 | 0.000 | 5.241 | 0.000 | –6.829 | .000 |
| | 2006 | 4.551 | 0.000 | 5.501 | 0.000 | –3.857 | .000 |
| | 2007 | 4.854 | 0.000 | 5.027 | 0.000 | –3.758 | .000 |

Abbreviation: K-S, Kolmogorov-Smirnov.
[a] The significance level was adjusted to the 33 comparisons performed (.0015).

February, and December, except for *Torula*, which reached peak concentrations during these months. Since excessive rain tends to wash the spores out of the atmosphere [17], the low concentration found during those months could be explained by the occurrence of higher rainfall levels.

The maximum monthly average of *Aspergillus/Penicillium*, *Cladosporium*, and *Epicoccum* was similar. Nevertheless, the maximum monthly average of *Ganoderma*, *Polythrincium*, *Stemphylium*, and *Torula* occurred first in Amares and 1 month later in Porto. Only *Alternaria* and *Pithomyces* presented the maximum monthly average first in the urban region and later in the rural region. *Botrytis* and *Drechslera*-type presented high concentrations throughout the year.

*Alternaria* spores, which were present in the atmosphere of Amares and Porto on most days, had a similar distribution pattern: the highest concentrations were found during the summer (from the end of June to early October in Amares; August in Porto). This pattern has also been observed in Poland [18] and the United Kingdom [16]. *Alternaria* values in Porto and Amares were lower than those found in southern Spain [15], but similar to those found in northern Spain [19], a region that is geographically similar to the 2 study areas presented in this work. In Porto, the prevalence

Table 4. Association Between Daily Fungai Spore Concentrations and Meteorological Factors[a]

| | Before | Tmean | Tmax | Tmin | RHmean | RHmax | RHmin | R |
|---|---|---|---|---|---|---|---|---|
| **Amares** | | | | | | | | |
| | 2005 | 0.755 | 0.761 | 0.673 | –0.282 | –0.162 | –0.274 | –0.302 |
| *Alternaria* | 2006 | 0.705 | 0.709 | 0.628 | –0.256 | 0.007 | –0.276 | –0.340 |
| | 2007 | 0.553 | 0.612 | 0.360 | –0.364 | –0.074 | –0.338 | –0.327 |
| *Aspergillus/* | 2005 | –0.059 | –0.024 | –0.085 | –0.034 | –0.067 | –0.050 | –0.029 |
| *Penicillium* | 2006 | –0.029 | –0.010 | –0.066 | –0.052 | 0.033 | –0.032 | –0.095 |
| | 2007 | 0.003 | –0.014 | –0.009 | –0.079 | –0.068 | –0.047 | –0.067 |
| | 2005 | –0.037 | –0.080 | 0.006 | 0.133 | 0.048 | 0.145 | 0.176 |
| *Botrytis* | 2006 | 0.035 | –0.028 | 0.093 | 0.313 | 0.243 | 0.311 | 0.280 |
| | 2007 | 0.320 | 0.287 | 0.272 | –0.071 | 0.009 | –0.050 | –0.044 |
| | 2005 | 0.608 | 0.585 | 0.587 | –0.109 | –0.064 | –0.124 | –0.101 |
| *Cladosporium* | 2006 | 0.633 | 0.619 | 0.602 | –0.170 | 0.058 | –0.191 | –0.210 |
| | 2007 | 0.333 | 0.378 | 0.179 | –0.164 | 0.061 | –0.164 | –0.199 |
| | 2005 | 0.148 | 0.140 | 0.123 | –0.048 | –0.072 | –0.034 | –0.044 |
| *Drechslera* | 2006 | 0.147 | 0.144 | 0.127 | –0.042 | 0.013 | –0.030 | –0.044 |
| | 2007 | 0.233 | 0.268 | 0.145 | –0.176 | –0.032 | –0.186 | –0.161 |
| | 2005 | 0.512 | 0.516 | 0.462 | –0.100 | –0.042 | –0.113 | –0.151 |
| *Epicoccum* | 2006 | 0.476 | 0.475 | 0.427 | –0.098 | 0.062 | –0.121 | –0.173 |
| | 2007 | 0.222 | 0.316 | 0.065 | –0.172 | 0.002 | –0.196 | –0.233 |
| | 2005 | 0.646 | 0.659 | 0.587 | –0.058 | 0.079 | –0.103 | –0.130 |
| *Ganoderma* | 2006 | 0.555 | 0.541 | 0.496 | –0.117 | 0.109 | –0.138 | –0.227 |
| | 2007 | 0.355 | 0.423 | 0.222 | –0.142 | 0.067 | –0.157 | –0.209 |
| | 2005 | 0.200 | 0.235 | 0.164 | –0.129 | –0.154 | –0.134 | –0.149 |
| *Pithomyces* | 2006 | 0.159 | 0.168 | 0.116 | –0.064 | 0.000 | –0.054 | –0.068 |
| | 2007 | 0.225 | 0.276 | 0.114 | –0.196 | –0.041 | –0.178 | –0.140 |
| | 2005 | 0.030 | 0.014 | –0.018 | –0.033 | –0.034 | –0.112 | –0.007 |
| *Polythricium* | 2006 | 0.101 | 0.166 | 0.000 | –0.144 | –0.036 | –0.151 | –0.173 |
| | 2007 | 0.156 | 0.160 | 0.135 | –0.197 | –0.113 | –0.173 | –0.101 |
| | 2005 | 0.391 | 0.364 | 0.381 | –0.071 | 0.013 | –0.053 | –0.100 |
| *Stemphylium* | 2006 | 0.325 | 0.320 | 0.283 | –0.071 | 0.039 | –0.088 | –0.100 |
| | 2007 | 0.490 | 0.441 | 0.452 | –0.171 | –0.085 | –0.127 | –0.091 |
| | 2005 | 0.049 | 0.122 | –0.068 | –0.335 | –0.194 | –0.332 | –0.271 |
| *Torula* | 2006 | –0.015 | 0.066 | –0.100 | –0.241 | –0.121 | –0.257 | –0.273 |
| | 2007 | 0.097 | 0.153 | 0.017 | –0.238 | –0.111 | –0.217 | –0.178 |
| **Porto** | | | | | | | | |
| | 2005 | 0.549 | 0.558 | 0.507 | –0.181 | –0.110 | –0.152 | –0.253 |
| *Alternaria* | 2006 | 0.693 | 0.701 | 0.638 | –0.338 | –0.152 | –0.344 | –0.285 |
| | 2007 | 0.650 | 0.703 | 0.558 | –0.375 | –0.284 | –0.308 | –0.356 |
| *Aspergillus/* | 2005 | 0.043 | 0.059 | 0.030 | –0.079 | –0.088 | –0.055 | –0.024 |
| *Penicillium* | 2006 | 0.033 | 0.029 | 0.037 | –0.038 | –0.051 | –0.029 | –0.023 |
| | 2007 | –0.068 | –0.053 | –0.102 | –0.037 | 0.051 | –0.020 | –0.049 |
| | 2005 | –0.112 | –0.113 | –0.105 | 0.016 | 0.013 | 0.021 | 0.044 |
| *Botrytis* | 2006 | 0.174 | 0.174 | 0.181 | 0.017 | 0.100 | 0.006 | 0.002 |
| | 2007 | 0.510 | 0.493 | 0.467 | –0.188 | –0.166 | –0.101 | –0.094 |
| | 2005 | 0.357 | 0.345 | 0.363 | –0.078 | –0.086 | –0.050 | –0.110 |
| *Cladosporium* | 2006 | 0.624 | 0.605 | 0.604 | –0.283 | –0.119 | –0.247 | –0.271 |
| | 2007 | 0.526 | 0.595 | 0.436 | –0.349 | –0.252 | –0.303 | –0.319 |

Continued

| | Before | Tmean | Tmax | Tmin | RHmean | RHmax | RHmin | R |
|---|---|---|---|---|---|---|---|---|
| | 2005 | 0.192 | 0.193 | 0.183 | 0.010 | –0.018 | 0.031 | –0.003 |
| *Drechslera* | 2006 | 0.132 | 0.123 | 0.125 | –0.041 | –0.027 | –0.023 | –0.062 |
| | 2007 | 0.269 | 0.307 | 0.235 | –0.093 | –0.022 | –0.080 | –0.178 |
| | 2005 | 0.330 | 0.336 | 0.309 | –0.081 | –0.080 | –0.036 | –0.162 |
| *Epicoccum* | 2006 | 0.373 | 0.384 | 0.344 | –0.176 | –0.057 | –0.161 | –0.199 |
| | 2007 | 0.410 | –0.456 | 0.347 | –0.215 | –0.191 | –0.191 | –0.304 |
| | 2005 | 0.478 | 0.426 | 0.507 | 0.288 | 0.234 | 0.268 | 0.082 |
| *Ganoderma* | 2006 | 0.592 | 0.586 | 0.573 | –0.084 | 0.000 | –0.062 | –0.096 |
| | 2007 | 0.584 | 0.612 | 0.505 | –0.098 | 0.046 | –0.100 | –0.205 |
| | 2005 | 0.240 | 0.246 | 0.239 | 0.013 | 0.029 | –0.019 | –0.023 |
| *Pithomyces* | 2006 | 0.167 | 0.175 | 0.164 | –0.057 | –0.038 | –0.052 | –0.010 |
| | 2007 | 0.330 | 0.348 | 0.285 | –0.227 | –0.244 | –0.176 | –0.211 |
| *Polythricium* | 2005 | 0.161 | 0.166 | 0.149 | 0.020 | 0.029 | 0.020 | –0.098 |
| | 2006 | 0.003 | 0.000 | 0.000 | –0.028 | 0.018 | 0.020 | –0.093 |
| | 2007 | 0.192 | 0.176 | 0.176 | –0.125 | –0.092 | –0.079 | –0.083 |
| | 2005 | 0.386 | 0.369 | 0.380 | –0.015 | 0.017 | 0.019 | –0.121 |
| *Stemphylium* | 2006 | 0.460 | 0.459 | 0.453 | –0.157 | –0.035 | –0.158 | –0.171 |
| | 2007 | 0.385 | 0.358 | 0.344 | –0.059 | –0.042 | 0.025 | –0.106 |
| | 2005 | –0.088 | –0.033 | –0.115 | –0.211 | –0.143 | –0.222 | –0.175 |
| *Torula* | 2006 | 0.063 | 0.090 | 0.035 | –0.173 | –0.115 | –0.126 | –0.151 |
| | 2007 | 0.232 | 0.269 | 0.170 | –0.254 | –0.181 | –0.217 | –0.333 |

Abbreviations: T, temperature; RH, relative humidity.
[a] Spearman correlation coefficients with a significance level of 95% are presented in bold, numbers in normal text correspond to a significance level of 99%, and nonsignificant correlations are presented in gray.

of sensitization to *Alternaria* is 2.5% [20], although data on fungal allergen sensitization in Amares are lacking. Moreover, a recent Iberian study reports a prevalence of 12% (demonstrated by skin prick testing) to *Alternaria* in the general population. In patients with allergic rhinitis, prevalence reached 39%, and the maximum values for sensitization to *Alternaria* were reached in patients with bronchial asthma (56%) [21].

The distribution of *Aspergillus/Penincillium* spores was similar in both areas, with the maximum concentrations during spring and autumn (April-May and October-November in Amares; April, June, August, and October-November in Porto), although when different sampling techniques were used, the highest concentrations of *Aspergillus* spores were found in October or during winter [22]. A previous work on *Penicillium* spore concentrations in a tropical region reported the spore concentration as constant between the dry and rainy seasons, while no differences were observed according to the degree of urbanization [23]. In Porto, the prevalence of sensitization to *Aspergillus* is 0.8% and to *Penicillium* it is 0.7% [20].

*Botrytis* spores presented a similar distribution in both locations, with the lowest values reached in February. In the rural area, the highest value was reached in July; in the urban area, it was reached in October. The same annual distribution has been described elsewhere [24], while other works report an earlier sporulation season [25].

*Cladosporium* constitutes the main source of inhaled fungal allergens. Unlike *Alternaria*, which is predominant in warm and humid climates, *Cladosporium* can be found in cooler climates [26], such as northern Portugal. These spores were present throughout the year and were the dominant fungal type, as observed by other authors in other locations [24]. The airborne concentration of *Cladosporium* spores was higher than that previously recorded for Porto [27], although the same seasonal pattern was observed. Once more, *Cladosporium* values in Porto and Amares were lower than those found in southern Spain [15], but similar to those found in Ourense and León [28]. In Amares, the value considered responsible for the induction of allergic symptoms (4000 spores/$m^3$) was surpassed. The prevalence of sensitization to this fungal spore in Porto is 1.2% [20].

*Drechslera*-type spores presented similar concentrations throughout the year, with the maximum values found in March, July, and September in Amares and August in Porto. In Poland the concentration of this spore type started to rise in May, and the maximum was registered from June to August. Concentrations remained high until October [14,24].

*Epicoccum* spores were found almost all year round, with the maximum values recorded between July (Porto) and October (Amares). In other locations, such as Poland, spore densities of *Epicoccum* peaked toward summer and autumn [24].

A study carried out over several years detected *Ganoderma*

spores from June through October, with peak concentrations occurring from August to October [24]. In the present study, these spores were present from July to October, and the maximum values were found in August (Amares) and September (Porto).

*Pithomyces* spores presented a sporadic distribution throughout the year, as previously reported [29]. The peak dates were different: in the urban area, the maximum value was registered in early August; in the rural area, it did not appear until mid-September. Other authors have reported higher concentrations of *Pithomyces* during summer and autumn [24].

In the atmosphere of Rzeszów, *Polythrincium* spores were present from April to November [14], although in Cracow these spores were registered from June to October, with the peak value in August [24]. In Amares and Porto, *Polythrincium* presented a different distribution, with high concentrations from March to May, and the peak dates recorded in April (Amares) and May (Porto).

In Amares and Porto, high values of *Stemphylium* spores were recorded from May to October, with maximum values recorded in June (Amares) and July (Porto). In a previous study performed in Poland this period was wider, ranging from March until November [14].

Analysis using *z* scores revealed that *Alternaria, Aspergillus/Penicillium, Cladosporium, Epicoccum,* and *Pithomyces* spores were 23% more abundant in the rural area. Urban environments have been widely studied, whereas rural areas receive far less attention in the literature. However, some studies do compare spore concentrations between the 2 environments and, despite the use of different sampling techniques, spore concentrations have been reported to be higher in rural areas than in urban areas [25]. *Aspergillus* and *Penicillium* spores are exceptional in that more isolates have been found in urban areas rather than in rural areas [30].

The differences in spore concentrations between urban and rural areas may be related to ecological factors such as the presence of woodland, crops, weeds, or even livestock, which can act as an intermediary host to moulds, thus leading to increased spore concentrations. In fact, a recent study demonstrated the presence of several fungal species among mycotic flora from the beak cavity and cloaca of geese [31]. Moreover, straw used for animal bedding may provide a substrate for fungi. In fact, the disturbance of bedding material during cage washing is an important source of airborne biological particles [32], and farming operations create mechanical disturbances that facilitate release of fungal spores [33].

For most of the allergenic fungal spore types analyzed, airborne concentrations were lower in urban areas than in rural areas. Nevertheless, the heavy pollution recorded in urban areas—particulate matter, nitrogen dioxide, sulfur dioxide, even ozone [34]—cannot only modify protein production and release of other biological particles, but can also induce higher permeability of the upper airways, thus affecting the sensitization of allergic patients. Future studies should address the sensitization rate in these areas and the relationship between atmospheric fungal spores and chemical pollutants.

*Aspergillus/Penicillium* spores did not present a significant correlation with any of the meteorological factors analyzed. This result was consistent with that of a previous study [35]. This lack of association can be explained by the sporadic behavior of these spores [36]. All the selected spore types—except *Botrytis* (Amares), *Polythrincium* (Amares), and *Torula*—presented positive correlations with temperature. Negative correlations were obtained between *Alternaria, Cladosporium, Epicoccum,* and *Torula* levels and relative humidity and rainfall. The remaining fungal spores presented a different behavior in the 2 locations during the study period. This fact may be explained by the interannual differences registered on spore distribution. In fact *Alternaria, Cladosporium,* and *Epicoccum* are considered dry-air fungal spores, found in higher concentrations during warm, dry weather conditions with high wind speeds [17].

Some airborne fungal spores are known for their allergenic potential. In the present work, *Alternaria, Aspergillus/Penicillium,* and *Cladosporium* were the most frequent allergenic genera, whereas *Botrytis, Epicoccum, Drechslera*-type, *Ganoderma, Polythrincium, Pithomyces, Stemphylium,* and *Torula* were less frequent.

Atmospheric fungal spore concentrations varied with the season, and were higher during summer and autumn and lower during winter and spring. However, high concentrations of *Torula* were found in February-March and of *Aspergillus/Penicillium* in November.

*Alternaria, Aspergillus/Penicillium, Cladosporium,* and *Torula* spore concentrations were higher in Amares than in Porto. The remaining fungal spores presented different behaviors over the 3 years. This is to be expected, since most of these spores, besides being important allergens, are well-known saprobes and phytopathogens.

Meteorological parameters affected airborne spore concentration, with the exception of *Aspergillus/Penicillium*. In general terms, temperature had a positive effect on the selected spores, while humidity and rainfall had the opposite effect.

This work emphasizes the need for atmospheric fungal spore monitoring combined with meteorological data, since climatic changes can influence the seasonal distribution of spore types and thus interfere with the occurrence of allergic symptoms.

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