

# Fungal Spores of *Cladosporium* in the Air of Tetouan Meteorological Parameters and Forecast Models



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## Abstract

*Cladosporium* is fungal taxa whose spores are included in aerobiological studies of outdoor environments. This spore type is present in the atmosphere of Tetouan (Morocco) throughout almost the entire year, although they reach their highest concentrations during spring to autumn. Fungal spore's concentrations were monitored during 2009-12 using Burkard volumetric spore traps. Stepwise multiple regressions were constructed taking into account meteorological and biological parameters with data from 2009 to 2011 and tested on data from 2012. Differences between observed and expected values were examined using Wilcoxon test.

*Cladosporium* showed maximum daily values in March, August, September or October depending on year. The forecast models explained 64.5 to 66.9% of the variation of daily spore concentrations when previous day or week concentration ( $Clado_{t-1}$ ) and mean concentration of the same day or week in other years ( $C_{mean}$ ), frequency of the wind first quadrant, rainfall and maximum relative humidity were introduced in the regression equations. The predictive models based on weekly values achieved 65.4 to 67.3% of variance. The Wilcoxon test demonstrated that there were no significant differences when model 4 was selected for main spore season and pre-peak and model 2 for weekly concentration, validated therefore satisfactory for predicting *Cladosporium* spore in the atmosphere of Tetouan. This study demonstrates that the input of meteorological and biological ( $C_{mean}$  and  $Clado_{t-1}$ ) parameters increased the goodness of models fitting.

**Keywords:** Fungal Spores; *Cladosporium*; Aerobiology; Meteorological Parameters; Modeling; Tetouan; Morocco

## Introduction

Conidia of *Cladosporium* species represent the most common fungal component isolated from air [1,2]. Their small conidia, usually formed in branched chains are well adapted to be spread easily in large numbers over long distances [3]. A number of studies have confirmed that *Cladosporium* spores are an important aeroallergen and exposure to high concentrations affects human health by increasing the incidence of bronchial ailments [4,5] and they can cause several respiratory diseases in man such as asthma and rhinitis when inhaled [6,7]. They act saprophytically on dead organic material or as plant pathogens, infecting all stage of plant growth, causing plant diseases, prompting considerable economic losses worldwide [8], or they occur as hyperparasites on other fungi [9]. In view to establish preventive measures against such allergies and the fungal infection of crops, different groups of aerobiologists have studied the relationship between the daily atmospheric concentration of fungal spores and meteorological parameters

making use of correlations, linear and multiple regressions analysis. Most common aerobiological models use almost uniformly meteorological data as the main controlling variables [10]. Environmental factors such as temperature, humidity, wind, nutrients availability, circadian periodicity and seasons play an important role on fungal spore's production, dispersion and/or transport in the air for short and long distances. When spores deposited on solid or wet surface and if appropriate conditions are met, they germinate [11,12]. Multi-factorial effect of climatic conditions and delayed effect are poorly understood.

Aerobiologists are concerned to develop forecast models providing prediction of atmospheric particles concentrations. Forecast of airborne fungal spores are few and with limited accuracy [13-17]. These predictive models for both short-and long-term variation in spore concentrations provide accurate information in order to take preventive measures against exposure to these particles and to improve diagnostics therapy.

In this study, an attention was paid to the spore concentration mean value for that day or week as do Recio et al. [18]. According to Rodriguez-Rajo et al. [19], daily mean pollen concentration substantially improve the prediction accuracy. As demonstrated in the previous paper for *Alternaria* [10], lagged values from the series of daily or weekly spore concentrations fit quite well into the regression models and should be taken into account. The aim of this study was to examine the annual and seasonal dynamics of atmospheric *Cladosporium* spores in Tetouan (Morocco) during 2009-2012 and the relationship between spore levels and some meteorological factors. As no predictive models has been developed up to date for *Cladosporium* in Morocco, this work aims also to construct forecasting models enabling accurate predictions of the daily and weekly spore concentrations of *Cladosporium*.

### Material and Methods

The province of Tetouan is located in the eastern sector of the Tingitan Peninsula in the northwest of Morocco (Figure 1), covering an area of 2,574 km<sup>2</sup>. In bioclimatic terms, it belongs to the thermo mediterranean climate. It is characterized by two seasons, the first humid and fresh from October to April and the second drier and hot from May to September. For the years 2009-2012, the average mean temperature was 19.2°C and mean annual rainfall was 765.5mm. The mean annual maximum temperature varied between 22.3 and 23.1°C, whereas in the case of the minimum temperature, the averages ranged from 14.3 to 17.6°C. The coldest months are January, February or December, respectively. The warmest month corresponds to July or August. The city, far 10 km from the Mediterranean Sea, is located between two mountain ranges; therefore, the prevailing winds are those blowing from the NE (known locally as “Chergui”) and SW (locally known as “Gharbi”) [20].

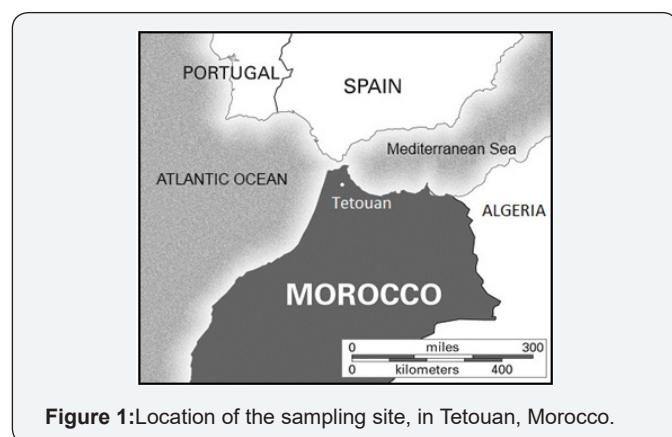


Figure 1: Location of the sampling site, in Tetouan, Morocco.

Fungal spores sampling was performed during 2009-2012 using Burkard 7-day volumetric spore traps located on the flat roof of the Department of Biology of the Faculty of Sciences of Tetouan 15 m above ground level. The sampler was calibrated to aspirate an air flow of 10 l/min. To collect daily airborne spores, a trapping surface of Melinex film coated with 2% silicon

solution was mounted on the drum rotating inside the sampler at a speed of 2 mm/h. The drum was changed weekly and the exposed tape was cut into seven segments for daily spore counts. The spores were counted in the microscope slide using two longitudinal transects according to the Spanish Aerobiological Network (REA) [21]. Daily mean concentrations were expressed as the number of spores per cubic metre of air. The main spore season (MSS) was determined by the 90% method of Nilsson and Person [22] taking the beginning and end of the MSS as 5 and 95% of the annual accumulated sums.

The correlation coefficient between mean daily spore concentrations and meteorological parameter values was calculated using Spearman's correlation test for statistical significance  $p < 0.01$  and  $p < 0.05$ . Values were computed by the program Statistica (Software program). The meteorological parameters used were maximum temperature ( $T_{max}$ ), mean temperature ( $T_{mean}$ ), minimum temperature ( $T_{min}$ ), maximum relative humidity ( $R_{Hmax}$ ), mean relative humidity ( $R_{Hmean}$ ), minimum relative humidity ( $R_{Hmin}$ ), precipitation, mean and maximum wind speed (WSmean and WSmax), frequencies of four directions of wind (NE, SE, NW, SW) and calm. For each wind direction, total hours per day were expressed as a percentage.

Meteorological data were obtained from the Physics Department of the Faculty of Science, which has an automatic meteorological station located 500 m from the sampling site. A multiple stepwise regression by backward elimination was made to evaluate the parameters forming part of the mathematical model that enables us to predict *Cladosporium* spore concentrations using data from 2012 as external values to validate the forecast model. This analysis was applied between dependent variable (daily *Cladosporium* spore concentrations of the MSS, weekly means of the year and averaged daily values of the second pre-peak period) and 15 independent variables. The pre-peak was defined as the period when the daily spore concentration curve starts to increase accumulating 1000 sp/m<sup>3</sup> until a maximal value is reached. The 15 independent variables were firstly the lagged values from the series of spore concentrations, previous day or week concentration ( $Clad_{t-1}$ ) and mean concentration for the same day or week of other years 2009-2011 ( $C_{mean}$ ) and secondly the same meteorological parameters selected above for the correlation analysis at the exception of  $T_{mean}$ . The Wilcoxon test was made to select the reasonable predictor variables by comparing positive or negative rankings of differences between observed and predicted series. Spearman's correlation test was also performed to estimate the degree of association between these two series.

### Results

#### Spores Data

Spores of *Cladosporium* were found in the air of Tetouan throughout the whole study period with very important

concentrations. The highest number of spores was recorded in 2012 with 421,997 spores, while the lowest value occurred in 2010 with 296,707 spores (Table 1). The monthly spore levels showed the highest spore concentrations during spring to autumn. Thus, the maximal load of *Cladosporium* is found in September for 2009, August for 2010 and 2011 or October for 2012 (Table 1).

**Table 1:** Monthly total of *Cladosporium* spores in the air of Tetouan, 2009-2012.

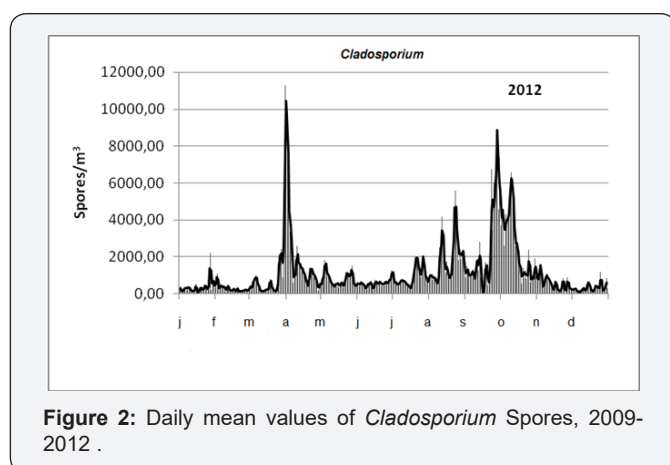
	2009	2010	2011	2012
January	3,865.32	6,724.08	10,682.28	10,987.92
February	2,403.76	4,412.92	5,572.80	8,271.72
March	5,934.60	2,286.36	9,401.40	32,544.72
April	10,016.96	18,735.84	25,008.48	59,161.32
May	15,127.56	16,771.32	28,704.24	23,843.48
June	25,040.88	38,044.08	27,227.88	16,881.48
July	24,746.04	27,113.40	19,406.52	28,124.64
August	38,505.24	60,745.64	69,523.92	57,992.76
September	78,297.84	34,320.24	46,718.64	75,778.20
October	71,175.24	53,976.24	31,204.44	81,895.26
November	27,799.20	18,246.60	23,669.28	16,920.36
December	17,136.36	15,330.60	10,943.64	9,594.68
Total	320,049	296,707	308,064	421,997
Mean ± SD	26,671±24,925.63	24,726±18,823.96	25,672±17,995.48	35,166±26,532.08

During the four analyzed years, the MSS lasted between 244 and 295 days. Start dates of the spore season varied from February to April. End dates were in the first fortnight of December in 2009 and 2010, and in 20<sup>th</sup> November and 06<sup>th</sup> November in 2011 and 2012, respectively (Table 2). The maximum daily spore concentration reached 6,242 sp/m<sup>3</sup> on 19<sup>th</sup> August in 2011, 7,712 sp/m<sup>3</sup> on 16<sup>th</sup> September in 2009, 7,753 sp/m<sup>3</sup> on 30<sup>th</sup> October in 2010 and 11,276 sp/m<sup>3</sup> on 31<sup>th</sup> March in 2012 (Table 2), (Figure 2).

**Table 2:** Data of the main spore season (MSS) of *Cladosporium*, 2009-2012.

	MSS-start	Main Spore Season (MSS)	MSS-end	Duration	Total recorded Day	Spore Peak	Day
	Value	20 November	269 277,102	19 August	6,242		
	04 March	06 November	248 380,541	31 March	11,276		
2009	17 April	16 December	244	299,065	16 September	7,712	
2010	04 February	11 December	295	282,091	30 October	7,753	
2011	25 February	20 November	269	277,102	19 August	6,242	
2012	04 March	06 November	248	380,541	31 March	11,276	

MSS-start: starting date of MSS, MSS-end: end date of MSS.



**Figure 2:** Daily mean values of *Cladosporium* Spores, 2009-2012 .

### Correlation with meteorological parameters

During the seasons concerned, the correlation between *Cladosporium* spore concentration and Temperature showed a positive correlation with a significance level of  $p < 0.01$  (Table 3). Only in one year (2011), it was observed that the mean relative humidity correlated negatively with a significant coefficient. The association between wind speed and *Cladosporium* spore concentration was often positive and significant. A negative and significant correlation was found between first quadrant wind direction (NE) and spore concentrations of *Cladosporium* in 2012, whereas the positive influence of fourth quadrant wind direction (NW) was noted in 2011, 2012 and all the period in 2009-2012.

**Table 3:** Spearman's correlation coefficients between *Cladosporium* spore concentrations and meteorological factors.

	2009	2010	2011	2012	2009-2012
Minimum Temperature	0,42	0,29	0.48**	0,23**	0,34**
Mean Temperature	0,39	0,37	0.44**	0,27**	0,32**
Maximum Temperature	0,42	0,42	0.48**	0,23**	0,39**
Minimum Relative Humidity	-0,04	-0,48	-0.10	0,03	-0,06
Mean relative Humidity	0,00	0,04	-0.13	0,02	0,07
Maximum Relative Humidity	0,03	0,01	-0.03	0,04	0,02
Maximum Wind Speed	0,16	0,12*	0.16**	0,07	0,13
Mean wind Speed	0,15	0,18*	0.29**	0,12	0,16**
Rainfall	-0,06	-0,01	0.04	0,08	0,07
% NE	0,02	0,00	-0.06	-0.14	0,02
% SE	-0,1	0,03	-0.05	0,02	0.13**
% SW	-0,02	-0,06	0.03	0,06	0.16**
%NW	-0,01	0.1	0.16**	0,30**	0,07
Calm	0,03	0,09	0.29**	0,01	0,06

### Stepwise multiple regression

In the stepwise backward elimination regression, which not included  $T_{\text{mean}}$  (model 1), the percentage variance explained up to 52.1% for the MSS, 51.1% for the pre-peak and 65.4% for the weekly values (Table 4). When more meteorological parameters were eliminated from the equation, the resulting  $R^2$  satisfactory

predicts future concentration with an adjusted regression coefficient ranging from 66.9 to 67.3% when averaged daily data for the pre-peak and averaged weekly data were used (Table 4). As regards the Wilcoxon test, it was used to compare the results from the expected values and observed data for 2012 of MSS, pre-peak and weekly values of the year.

**Table 4:** Multiple regression models proposed for predicting daily *Cladosporium* spore concentration for Spearman's correlation (r) Wilcoxon test the MSS, the pre-peak and the weekly concentration.

Models	Equations	R2
<b>Main spore season</b>		
1 <sup>a</sup>	Clado = 0.720 Cladot-1+298.758	0.521
2 <sup>a</sup>	Clado = 0.490 Cladot_1 + 0.595	0.638
3 <sup>a</sup>	Clado = 0.480 Cladot_1 + 0.606 Cmoy - 2.033 Fr1Q - 9.278	0.642
4 <sup>a</sup>	Clado = 0.480 Cladot_1 + 0.599 Cmoy - 3.211 Fr1Q+ 7.315 RHmax - 548.419	0.645
<b>Pre-peak</b>		
1 <sup>b</sup>	Clado = 1.140 Cmoy - 86.290	0.511
2 <sup>b</sup>	Clado = 0.771 Cmoy + 0.521 Cladot_1 - 238.599	0.656
3 <sup>b</sup>	Clado = 0.794 Cmoy + 0.503 Cladot_1 + 19.995 Rain - 312.784	0.664
4 <sup>b</sup>	Clado = 0.776 Cmoy + 0,491 Cladot_1 + 17,043 Rain + 19.998 Fr1Q - 302.860	0.669
<b>Weekly concentrations</b>		
1 <sup>c</sup>	Clado = 0.999 Cmoy + 13.587	0.654
2 <sup>c</sup>	Clado = 0.846 Cmoy + 0.191 Cladot_7 - 212.708	0.673

R2: regression coefficient; Cladot-1: Cladosporium spore concentration recorded one day before; Cmean: mean concentration for the same day; Fr 1Q: frequency of the wind 1st quadrant; RHmax: maximum relative humidity; Rain: Rainfall; Cladot-7: Cladosporium

lum spore concentration recorded seven days before.

a) Parameters eliminated for Main Spore Season

1: Tmean; 2: Tmean and Tmin; 3: Tmean, Tmin and WSmax; 4: Tmean, Tmin, WSmax and WSmean

b) Parameters eliminated for pre-peak:

1: Tmean; 2: Tmean and Tmin; 3: Tmean, Tmin and WSmax; 4: Tmean, Tmin, WSmax and WSmean

c) Parameters eliminated for weekly concentrations

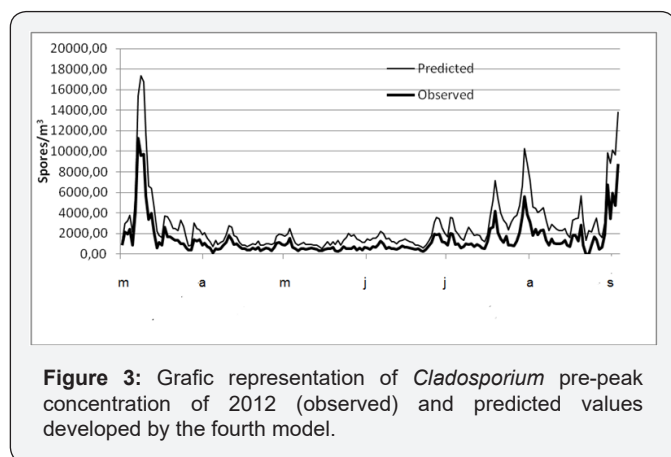
1: Tmean and Tmin; 2: Tmean, Tmin, WSmax and WSmean

The test indicates that there were no significant differences between series compared when model 4 was selected for MSS and pre-peak and model 2 for weekly values (Table 5). The meteorological parameters that the stepwise regression analysis selected were Fr1Q and R<sub>Hmax</sub> in the case of MSS, and Rain and Fr1Q for the pre-peak period (Table 4). In the stepwise backward elimination regression modeling approach performed with weekly values, the variables C<sub>mean</sub> and Clado<sub>t-1</sub> were

maintained in the second model and R<sup>2</sup> reached 67.3% (Table 4). The Spearman's correlation test indicated a very high degree of association (r = 0.782) between the observed and expected series showing model 4 (Clado = 0.776 Cmean + 0,491 Clado<sub>t-1</sub> + 17,043 Rain + 19.998 Fr1Q - 302.860) as the most efficient to predict the overall trend of the pre-peak Cladosporium spores in the air (Table 5). Figure 3 shows the predicted spore season for 2012. Compared to the observation, the pattern fits quite well.

**Table 5:** Spearman's correlation coefficients and comparison of averages by the Wilcoxon on test between the main spore season, the pre-peak period and Cladosporium spore weekly values of the year 2012 (observed) and data from the linear regression equations (predicted).

	Spearman's correlation			Wilcoxon test	
		Rank+	Rank -	z	p
<b>Main Spore Season</b>					
Observed 2012 vs predicted Mod.1 <sup>a</sup>	0.809***	146	102	-1.119	0.263 n.s.
Observed 2012 vs predicted Mod.2 <sup>a</sup>	0.814***	121	127	-1.081	0.280 n.s.
Observed 2012 vs predicted Mod.3 <sup>a</sup>	0.815***	117	131	-1.104	0.269 n.s.
Observed 2012 vs predicted Mod.4 <sup>a</sup>	0.807***	118	130	-0.961	<b>0.336 n.s.</b>
<b>Pre-peak</b>					
Observed 2012 vs predicted Mod.1 <sup>b</sup>	0.534***	97	90	-0.507	0.612 n.s.
Observed 2012 vs predicted Mod.2 <sup>b</sup>	0.740***	100	87	-0.694	0.694 n.s.
Observed 2012 vs predicted Mod.3 <sup>b</sup>	0.728***	84	103	-1.152	0.249 n.s.
Observed 2012 vs predicted Mod.4 <sup>b</sup>	0.748***	88	99	-0.277	<b>0.782 n.s.</b>
<b>Weekly concentrations</b>					
Observed 2012 vs predicted Mod.1 <sup>c</sup>	0.868***	25	26	-1.219	0.223 n.s.
Observed 2012 vs predicted Mod.2 <sup>c</sup>	0.891***	26	25	-0.759	<b>0.448 n.s.</b>
n.s. not significant					



**Discussion**

Cladosporium spores are the most prevalent spores to occur in the air of Tetouan accounting around 64% of the annual total [23]. Atmospheric Cladosporium spore concentrations registered maximum emissions in spring, summer and early autumn. The monthly total spore spectrum had generally two relevant peaks, the first in March-April and the second in August-October. In the south of the Iberian Peninsula, a similar pattern was observed [24]. The spore season shows that the annual spore index

varies between 296,707 and 421,997. In Malaga (south Spain), annual spore totals showed a high differences between the years studied, being lower in 1996 than 1997 (16,501 and 241,288, respectively) [24]. Based on data from 12 sampling stations in the Iberian Peninsula, mean annual total spores ranged from 54,459 in Cartagena to 933,485 in Sevilla [25]. In other sampling sites in Portugal and Spain, this annual load varies from 70,000 spores/year [26] to 285,000 [27]. These differences could be explained by geographical characteristics, climatological conditions of each locality, plant substrates colonized, fungal sources and farmer activities.

Spearman's correlations performed between daily spore concentrations and meteorological parameters indicated that air temperature is the most potential factor. Similar results were found by other authors in other countries [27-30]. Our results showed significant correlations between Cladosporium spore and wind speed. In the same way, wind speed was clearly associated with spore dispersal in Finland [31], in Turkey [32] and in Portugal [26]. Daily spores variations are related to variations in temperatures, relative humidity, rainfall and wind. In Tetouan, gradual increase of temperature starting from spring and the resulting dry conditions favour liberation of Cladosporium conidia into the atmosphere. Rainfall and relative humidity have a negative effect on the release, dispersion and

suspension of the spores in the atmosphere. Dry conidiospores of *Cladosporium* grow in an exposed position on conidiophores and are released passively when disturbed by wind.

The forecast model constructed using the mean spore concentration for the same day in other years and spore concentration on the previous day or week, as well as frequency of the first quadrant, maximum relative humidity and rainfall seemed to be very effective as they explained 64.5 to 67.3 % accuracy. When the observed and expected numerical series were correlated, higher coefficient of correlations were obtained and selected independent variables were considered as potential model variables. When model 4 was applied for MSS and pre-peak and model 2 for weekly concentration, no significant differences were revealed between actual and predicted values in 2012. Stepwise multiple regression model 4 used to predict daily variation of spore concentrations reached a high percentage of forecast (66.9%), validated with data from 2012 (Table 5).

The presence of spores in the outside air is related to climatic factors, making it possible to design predictive models for spore concentrations in centers with an extensive database. Different authors have indicated mean temperature as the most influential meteorological factor on this fungal type, meaning that it is the most suitable parameter for designing predictive models of atmospheric concentrations of *Cladosporium* [33] at the opposite of our results. In the same way Angulo et al [14] obtained the best results when they used the mean temperature accumulated over one week. In Tulsa (USA), researchers also indicated temperature as being the most consistent meteorological variable for the prediction of high spore concentrations in the atmosphere [16,34]. Ballero et al. [35] founded the mean temperature, maximum temperature and relative humidity as the appropriate predictive variables. Damialis & Gioulekas [36] obtained the efficient models when they used solar radiation in the daily prediction models. According to Burge [37], *Cladosporium* seems to have a relationship with temperature and dew point. In the models proposed by Aira et al. [38], rainfall and mean temperature, in the case of *Cladosporium cladosporioides*, proved to have the best predictive goodness. However, the percentage of variance explained increases considerably when the concentration of spores from the previous day is introduced into the model. It may be considered that this variable reflects the conditions on that the concentration of spores in the air depends, both for their formation, release and transport.

In the present study, the mean concentrations of spore of the same day or week in other years and the previous spore concentrations recorded 1 day or week before and meteorological parameters mainly frequency of the wind first quadrant, rainfall and maximum relative humidity best fitted the models and are highly satisfactory. These meteorological parameters are determinants from a mycological and physiological point of view on the growth and spore production and release of *Cladosporium*.

These results are specific to biogeography characteristics and climatic conditions of Tetouan.

## Conclusion

The highest number of *Cladosporium* spores was recorded in spring to autumn. *Cladosporium* spores are dispersed passively by the wind and found in greatest abundance when temperature rises and wind speed increases. The daily fluctuation in spore concentrations is positively associated to inland winds from NW.

From the results of predictive models using spore and meteorological variables, it was concluded that daily or weekly spore concentrations can be predicted using the mean concentrations of spore of the same day or week in other years and the previous spore concentrations recorded 1 day or week before and meteorological parameters mainly frequency of the wind first quadrant, rainfall and maximum relative humidity.

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