World Academy of Science, Engineering and Technology
International Journal of Environmental and Ecological Engineering
Vol:2, No:8, 2008

Use of Multiple Linear Regressions to Evaluate the Influence of O_3 and PM_{10} on Biological Pollutants

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***Abstract*—**Exposure to ambient air pollution has been linked to a number of health outcomes, starting from modest transient changes in the respiratory tract and impaired pulmonary function, continuing to restrict activity/reduce performance and to the increase emergency rooms visits, hospital admissions or mortality. The increase of allergenic symptoms has been associated with air contaminants such as ozone, particulate matter, fungal spores and pollen.

Considering the potential relevance of crossed effects of non-biological pollutants and airborne pollens and fungal spores on allergy worsening, the aim of this work was to evaluate the influence of non-biological pollutants (O_3 and PM_{10}) and meteorological parameters on the concentrations of pollen and fungal spores using multiple linear regressions.

The data considered in this study were collected in Oporto which is the second largest Portuguese city, located in the North. Daily mean of O_3, PM_{10}, pollen and fungal spore concentrations, temperature, relative humidity, precipitation, wind velocity, pollen and fungal spore concentrations, for 2003, 2004 and 2005 were considered. Results showed that the 90th percentile of the adjusted coefficient of determination, P_{90} (R^2aj), of the multiple regressions varied from 0.613 to 0.916 for pollen and from 0.275 to 0.512 for fungal spores. O_3 and PM_{10} showed to have some influence on the biological pollutants. Among the meteorological parameters analysed, temperature was the one that most influenced the pollen and fungal spores airborne concentrations. Relative humidity also showed to have some influence on the fungal spore dispersion.

Nevertheless, the models for each pollen and fungal spore were different depending on the analysed period, which means that the correlations identified as statistically significant can not be, even so, consistent enough.

***Keywords*—**Air pollutants, meteorological parameters, biological pollutants, multiple linear correlations.

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I. Introduction

EXPOSURES to ambient air pollution has been linked to a number of health outcomes, starting from modest transient changes in the respiratory tract and impaired pulmonary function, continuing to restrict activity/reduce performance and to the increase of emergency room visits, hospital admissions or mortality [1]. Asthma is one of the respiratory diseases that has been directly associated with air pollution featuring an accumulation of inflammatory cells and mucus in the airways, with bronchoconstriction and a generalised airflow limitation [2]. The increase of asthma symptoms has been associated with air contaminants such as ozone, sulphur dioxide, nitrogen dioxide, particulate matter, pollen and fungal spores [3], [4].

It is currently accepted that tropospheric ozone is one of the most important atmospheric pollutant for the twenty-first century; the increase of its concentrations has been a problem of international impact [5]. Standard values that should not be exceeded were fixed by the European Union, according to the Directive 2002/3/EC: i) information threshold (IT) - 180 $\mu g/m^3$ (1-hour average); ii) alert threshold (AT) - 240 $\mu g/m^3$ (1-hour average); and iii) target value for the protection of human health (TVPHH) - 120 $\mu g/m^3$ (8-hour running average).

Particulate air pollution is a mixture of solid, liquid, or solid and liquid particles suspended in the air. The size of suspended particles varies, from a few nm to tens of μm. In practical terms, a distinction is made between PM_{10} ("thoracic particles" that are particulates with aerodynamic diameters smaller than or equal to 10 μm and can penetrate into the lower respiratory system) and $PM_{2.5}$ ("respirable particles" that are particulates with aerodynamic diameters smaller than or equal to 2.5 μm and can penetrate into the deeper part of the lung) [6]. PM_{10} include traffic and combustion derived carbon ultrafine particles, secondary particles (nitrates and sulphates), and wind-blown dust of geological origin, potentially containing endotoxin and some biological particles [7]. Standard values for the protection of human health, that should not be exceeded, were fixed by the European Union according to the Directive 1999/30/EC: i) 24-hour limit value (24-h LV) – 50 $\mu g/m^3$, not to be exceeded more than 35 times a year; and ii) annual limit value (annual LV) - 40 $\mu g/m^3$.

Pollen is the male gametophyte of seed plants and is produced as part of the sexual reproduction cycle. Fungal spores, uni- or multicellular, are reproductive or distributional structures produced during the life cycle of fungi. About 100,000 species of fungal spores are known today, being about 80 related with allergic pathologies, whereas allergenic proteins have been identified in 23 fungal genera [8]. Pollen grains and fungal spores are inert particles being seasonal air pollutants. Their presence and dispersion in the atmosphere depends on a wide range of factors including meteorological (temperature, rain, humidity, wind, etc), biological (physiological state of plants, plant distribution, pollinators, etc) and topographical issues.

In the last decades, epidemiological studies have showed that those particles may be responsible for various pathologies in the respiratory tract [9] and an increasing number of aerobiological studies has been conducted around the world [10]-[14].

Also the relation between meteorological parameters and the biological particles has been worldwide documented [12], [15]-[21]. However, studies concerning the relations between non-biological pollutants and biological particles are still few. The relationships between inhalable airborne pollen and fungal spores with some chemical air pollutants were examined by [22] using basic linear correlations and linear regression models for different pairs of variables. [23] analyzed correlations between fungal spores (not considering pollens), non-biological pollutants and meteorological factors using correlation coefficients and multiple linear regressions; they concluded that the correlations were complex, showing that further studies are needed on these topics. Furthermore, as far as the authors know, the influence of multiple parameters on pollen was not yet studied.

Considering the potential relevance of crossed effects of non-biological pollutants and pollens and fungal spores on allergy worsening [24], the aim of this work was to evaluate the influence of ozone, PM_{10} and meteorological parameters on the concentrations of pollen and fungal spores using multiple linear regressions.

II. Methods

A. Data

The data considered in this study were collected in Oporto which is the second largest Portuguese city, located in the North. With about 263 thousand inhabitants and a population density of 5787 inhabitants per square kilometre, Oporto is limited on the west by the Atlantic Ocean and on the south by the Douro River. The annual average temperature is around 15ºC and the difference between warmer and colder monthly averages is less than 10ºC. Annual air humidity is between 75% and 80%, and the total annual mean precipitation varies between 1000 mm and 1200 mm, with about 40% in the winter season, and with more than one hundred days per year with precipitation equal to or higher than 1.0 mm. Prevailing winds are from the W and NW in summer and from the E and SE in winter [25], [26].

Samplers were located at one urban site with traffic influences. O_3 and PM_{10} samplers were located in the area covered by pollen and fungal spores sampling. Ornamental and nonornamental trees, shrubs and herbaceous species can be found in the surroundings of the sampling site (some of this species are considered to be allergenic pollen producers and can act as an inoculation source for fungal).

The study here reported considered the daily mean of ozone (O_3) and PM_{10} concentrations, temperature (T), relative humidity (RH), precipitation (PP), wind velocity (WV), pollen and fungal spore concentrations, for 2003, 2004 and 2005.

The non-biological data were recorded by the Air Quality Monitoring Network of Oporto-MA, managed by the Regional Commission of Coordination and Development of Northern Portugal (*Comissão de Coordenação e Desenvolvimento Regional do Norte*), under the responsibility of the Ministry of Environment.

O_3 measurements were performed through UV-absorption photometry using the equipment 41MUV Photometric Ozone Analyser from Environment S.A., according to ISO 13964, *Portaria n*◦ 623/96 and EU Directive 2002/3/CE. PM_{10} concentrations were obtained through the beta radiation attenuation method, considered equivalent to the one advised by EU Directive 1999/30/CE and by *Decreto-Lei n*◦ 111/2002, using the equipment MPSI 100 I et E from Environment S.A. All the equipment was submitted to a rigid maintenance program being periodically calibrated.

The meteorological parameters were continuously measured by the Geophysical Institute of Oporto University (*Instituto Geofísico da Universidade do Porto*) at *Serra do Pilar* on the left edge of the Douro River at an approximate altitude of 90 m. The analysis of the wind patterns in different places of the city, allowed concluding that the wind pattern at *Serra do Pilar* can be considered representative of the wind patterns at the site here considered.

Airborne pollen and fungal spores were continuously monitored, using a seven day Hirst type volumetric spore trap manufactured by Burkard Manufacturing Company Limited UK. This sampler has a 2x14 mm intake orifice through which the sampled air is impacted onto a drum, rotating once every 7 days. A vane tail keeps the intake orifice facing the wind and a vacuum pump allows a suction of ten litres of air per minute that is equivalent to the human inhalation. Airborne particles were trapped on a Melinex tape coated with silicone oil, which was cut in seven pieces of 48 mm (each one corresponds to one day) and mounted on the slides with a mounting media of glycerol jelly. Pollen grains and fungal spores were identified and counted under an optical microscope (400X) using four and two traverses lines, respectively, evenly spread over the glass slide.

The pollinic types considered in this study were: *Acer* spp., *Alnus* spp., Asteraceae, Betulaceae, Caryophylaceae, *Castanea* spp., Chenopodiaceae/Amaranthaceae, *Corylus* spp., Cupressaceae, Ericaceae, *Fraxinus* spp., Myrtaceae, *Olea europaea*, Pinaceae, *Plantago* spp., *Platanus* spp., Poaceae,

Quercus spp., *Rumex* spp., *Salix* spp., *Ulmus* spp. and Urticaceae. Due to the difficulty on distinguishing some pollen of plant species belonging to the same family, because of its similar morphology, it is common to aggregate them by the family names.

The fungal spore types considered were: *Alternaria spp.*, Aspergillaceae, *Botrytis* spp., *Cladosporium* spp., *Coprinus* spp., *Corynespora* spp., *Didymella* spp., *Drechslera* spp., *Epicoccum* spp., Rusts, *Fusarium* spp., *Ganoderma* spp., *Leptospheria* spp., *Oidium* spp., *Periconia* spp., *Pithomyces* spp., *Pleospora* spp., *Polythrincium* spp., *Rhizopus stolonifer*, *Torula* spp. and *Ustilago* spp.. The sampling method used in this study does not allow the distinction between *Aspergillus* spp. and *Penicillium* spp. spores due to their reduced size (*Aspergillus* spp.: 2-10 µm; *Penicillium* spp.: 3-5 µm), being both spore types included in the family Aspergillaceae.

B. Statistical Model

Multiple linear regression (MLR) was used to model pollen and fungal spores, considering non-biological pollutant concentrations (O_3 and PM_{10}) and meteorological parameters (T, PP, RH and WV) as predictors.

MLR is a commonly used method in environmental sciences. The measured variable y is given by:

$$y = P_0 + \sum_{i=1}^{k} P_i x_i + \varepsilon \quad (1)$$

where x_i are the predictor variables, P_i the regression coefficients and ε the error associated to the regression (assumed to have expectation of zero). The predicted variable given by the regression model $\hat{y}$ can be written as:

$$\hat{y} = P_0 + \sum_{i=1}^{k} P_i x_i \quad (2)$$

The regression parameters P_i are calculated by minimizing the sum of square errors, through:

$$P_i = \arg\min \sum_{i=1}^{k} (y_i - \hat{y}_i)^2 \quad (3)$$

The statistical significance of the regressions obtained was also analysed calculating the critical correlation coefficient, R_{crit}:

$$R_{crit} = \pm \frac{t_{crit}}{\sqrt{DF + t_{crit}^2}} \quad (4)$$

with a significance level of 0.05 (two-tailed test), being DF=n-k degrees of freedom, k the number of independent variables and n the number of data.

The correlation is statistically valid if the absolute value of R_{crit} is lower than the absolute value of the correlation coefficient which is given by:

$$R = \pm \sqrt{\frac{\sum_{i=1}^{n}\left(Y_i - \overline{Y}_i\right)^2 - \sum_{i=1}^{n}\left(Y_i - \hat{Y}_i\right)^2}{\sum_{i=1}^{n}\left(Y_i - \overline{Y}_i\right)^2}} \quad (5)$$

The behaviour of MLR was evaluated calculating the adjusted correlation of determination (R^2_{aj}), and the root mean squared error (RMSE) according to the following equations:

$$R^2_{aj} = R^2 - \frac{k-1}{n-k} \times \left(1 - R^2\right) \quad (6)$$

$$RMSE = \sqrt{\frac{1}{n}\sum_{i=1}^{n}\left(Y_i - \hat{Y}_i\right)^2} \quad (7)$$

where R^2 is the coefficient of determination.

$R^2_{aj,}$ is lower than R^2, adjusting it for the number of explanatory terms in a model. Unlike R^2, the R^2_{aj} increases only if the new term improves more the model than would be expected by chance. As this test does not give the model accuracy, RMSE was also reported (7).

This statistical parameter measures residual errors, which provides a global idea of the difference between the observed and modelled values [27], [28].

III. Results

The multiple linear regressions were performed using as input variables the minimum, average and maximum values of O_3 and PM_{10} concentrations, T, RH, PP and WV. For each period, regressions were performed for all types of pollen and fungal spores. Tables I and II show the statistically valid multiple linear regressions, for pollen and fungal spores, respectively, with statistically valid parameters. These parameters were calculated using *t*-test (significance level 0.05); the selection of the parameters for the best regression was performed using genetic algorithms [29], [30].

The 90th percentile of R^2_{aj} (P_{90} *(R^2aj)*) of multiple linear regressions were 0.609 and 0.274, respectively, for pollen and fungal spores. As examples, the superior part of the tables shows the regressions achieved with P_{90} *(R^2aj)*; the lower part of the tables shows the multiple linear regressions for some pollen and fungal spores (2003-2005).

Results showed that P_{90} *(R^2aj)* for pollen multiple linear regressions varied from 0.613 to 0.916. The statistically valid multiple linear regressions with R^2aj superior to P_{90} *(R^2aj)* were obtained for: i) *Acer* spp. (2003 and 2005); ii) *Alnus* spp. (2003); iii) Caryophylaceae (2005); iv) *Castanea* spp. (2003); v) Pinaceae (2005); and vi) *Ulmus* spp. (2004). The pollen multiple regression models for 2003-2005 presented R^2_{aj} between 0.039 and 0.544

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TABLE I
MULTIPLE LINEAR REGRESSIONS ACHIEVED FOR EACH TYPE OF POLLEN

Period	Regression [a]	n [b]	R^2_{aj} [c]	RMSE [d]
2003	$Aceraceae = 0.369 + 0.221 \times O_3 - 0.191 \times O_{3Min} - 0.288 \times RH_{Max}$	26	0.721	0.231
2005	$Aceraceae = 0.644 - 0.216 \times O_{3Max} + 0.198 \times PM_{10Max} + 0.907 \times T - 0.594 \times T_{Max} - 0.173 \times PP_{Max} + 0.214 \times WV_{Min}$	20	0.916	0.133
2003	$Alnus\ spp. = 0.201 - 0.079 \times O_3 + 0.142 \times T_{Max} - 0.062 \times PP_{Min}$	36	0.680	0.124
2005	$Caryophylaceae = 0.028 - 0.061 \times O_{3Min} + 0.120 \times O_3 - 0.128 \times O_{3Max} - 0.066 \times PM_{10Min} + +0.081 \times PM_{10Max} - 0.063 \times HR_{Min} + 0.086 \times HR_{Max} - 0.026 \times PP_{Min} - 0.057 \times WV_{Min} + 0.067 \times WV_{Max}$	33	0.631	0.042
2003	$Castanea\ spp. = 0.328 - 0.172 \times O_{3Max} + 0.510 \times T + 0.168 \times HR + 0.225 \times WV_{Max}$	38	0.634	0.236
2005	$Pinaceae = 0.601 - 0.154 \times PM_{10Min} + 0.393 \times T_{Min}$	52	0.613	0.314
2004	$Ulmus\ spp. = 0.653 + 0.447 \times O_3 + 0.461 \times PM_{10} + 0.220 \times T_{Min} - 0.280 \times PP_{Max}$	26	0.629	0.329
2003-2005	$Aceraceae = 0.568 + 0.189 \times O_3 - 0.139 \times PM_{10} + 0.118 \times PM_{10Max} + 0.356 \times T - 0.132 \times PP_{Max} + 0.141 \times WV_{Max}$	75	0.544	0.354
	$Alnus\ spp. = 0.441 - 0.093 \times PM_{10Max} - 0.153 \times T_{Min} + 0.180 \times T_{Max} - 0.075 \times PP_{Min} - 0.072 \times WV_{Min}$	83	0.378	0.267
	$Betulaceae = 0.225 + 0.199 \times AvgO_3 - 0.094 \times MinPM_{10} + 0.192 \times AvgPM_{10} - 0.086 \times MaxPM_{10} - 0.103 \times MinWV$	130	0.254	0.319
	$Caryophylaceae = 0.107 - 0.047 \times O_{3Min} + 0.105 \times O_3 - 0.056 \times O_{3Max} - 0.035 \times PM_{10Min} + 0.076 \times PM_{10Max} - -0.101 \times T_{Min} + 0.095 \times T$	119	0.385	0.139
	$Castanea\ spp. = 0.264 + 0.251 \times T + 0.119 \times HR + 0.114 \times WV_{Max}$	116	0.235	0.344
	$Olea\ europaea = 0.348 + 0.221 \times O_3 + 0.131 \times PM_{10} - 0.135 \times WV$	68	0.269	0.320
	$Platanaceae = 0.463 + 0.305 \times O_{3Max} + 0.144 \times PM_{10Min} + 0.153 \times PP_{Min}$	79	0.223	0.582
	$Pinaceae = 0.642 + 0.195 \times O_3 + 0.106 \times PM_{10Max} + 0.188 \times T_{Min} - 0.196 \times RH + 0.177 \times RH_{Max}$	142	0.365	0.388
	$Ulmus\ spp. = 0.398 + 0.155 \times PM_{10Min} - 0.099 \times T_{Min} - 0.196 \times HR_{Max}$	91	0.265	0.410
	$Urticaceae = 0.809 + 0.102 \times O_3 + 0.086 \times O_{3Max} + 0.138 \times PM_{10} - 0.068 \times PM_{10Max} + 0.191 \times T_{Max} + +0.111 \times HR_{Min} - 0.048 \times WV_{Max}$	277	0.385	0.313

[a] Logarithm of pollen concentrations were used to develop the regression models; [b] n: data number; [c] R^2_{aj}: adjusted determination coefficient; [d] RMSE: root mean squared error

Table II shows that the P_{90} (R^2aj) for fungal spores multiple linear regressions varied from 0.275 to 0.512. The statistically valid multiple linear regressions with R^2aj superior to P_{90} (R^2aj) were obtained for: i) *Alternaria* spp. (2003, 2005 and 2003-2005); ii) *Cladosporium* spp. (2003); iii) *Epicoccum* spp. (2003); iv) *Ganoderma* spp. (2003, 2005 and 2003-2005); and v) Total fungi (2003).

The fungal spore multiple regression models for 2003-2005 presented R^2_{aj} between 0.009 and 0.175.

IV. DISCUSSION

For pollen, the multiple linear regressions with statistically valid parameters varied with the year and the type of pollen.

However, it was possible to perceive that O_3 influenced these types of pollen, with exception of Pinaceae. The influence of PM_{10} was only observed for *Acer* spp. (2005), Caryophylaceae and Pinaceae. The influence of T was present in almost all the regression models with exception of *Acer* spp. (2003) and Caryophylaceae. RH influenced *Acer* spp. (2003), Caryophylaceae and *Castanea* spp.; PP was present on the regressions of *Acer* spp. (2005), Caryophylaceae and *Ulmus* spp.; and WV influenced *Acer* spp. (2005), Caryophylaceae and *Castanea* spp.. For 2003-2005 period, the parameters that most influenced the airborne pollen were PM_{10} and O_3, influencing, respectively, 17 and 12 of the 22 pollinic types studied. T was also a parameter with great influence, being present in 17 regression models. The types of pollen without T influence were: Betulaceae, Myrtaceae, *Platanus* spp., *Salix* spp., and *Olea europaea*. At a general level, RH, PP and WV presented almost no influence on the pollen.

Pollens in PM_{10} were not considerable, since the sizes of pollen are larger than 10μm. Therefore, the use of PM_{10} as independent variable does not seem to be very biologically meaningful, indicating perhaps a coincidence in these particles pattern of occurrence rather than a cause-effect relationship.

The values of the R^2aj obtained for fungal spores were quite inferior to those of pollen. As well as for pollen the regressions obtained for fungal spores varied with the year and the type of fungal spore. These values of the R^2aj obtained during the 2003-2005 period for the fungal spores with sporadic occurrence corroborate the hypothesis, that the occurrence of these spores can be associated to other factors such as the vegetative development of plants and the existence of vegetable material in decomposing. Nevertheless, *Alternaria* spp. and *Ganoderma* spp. presented P_{90} (R^2aj) correlations for three periods: 2003, 2005 and 2003-2005. The other fungal spores that showed good R^2_{aj} were *Cladosporium* spp. (2003), *Epicoccum* spp. (2003) and the total fungal spores (2003). In general, O_3 showed significant negative influence on the concentrations of fungal spores. The influence of PM_{10} was not very significant.

TABLE II
MULTIPLE LINEAR REGRESSIONS ACHIEVED FOR EACH TYPE OF FUNGAL SPORE

Period	Regression [a]	n [b]	R^2_{aj} [c]	RMSE [d]
2003	*Alternaria* spp. $= 0.389 - 0.050 \times O_3 - 0.043 \times PM_{10Min} - 0.076 \times PM_{10Max} + 0.147 \times T_{Min} + 0.118 \times T_{Max} - 0.093 \times RH + +0.091 \times RH_{Max} - 0.036 \times PP_{Min} - 0.041 \times PP_{Max}$	351	0.480	0.292
2005	*Alternaria* spp. $= 0.528 - 0.056 \times O_3 + 0.154 \times T_{Min} + 0.131 \times T_{Max} - 0.062 \times RH$	364	0.325	0.383
2003-2005	*Alternaria* spp. $= 0.408 - 0.034 \times O_3 - 0.084 \times PM_{10Min} - 0.082 \times PM_{10Max} + 0.217 \times T - 0.071 \times RH + +0.038 \times RH_{Max} - 0.041 \times PP$	1068	0.309	0.354
2003	*Cladosporium* spp. $= 1.820 + 0.123 \times O_{3Min} - 0.210 \times O_3 - 0.152 \times PM_{10} + 0.519 \times T - 0.118 \times RH - -0.070 \times WV_{Min} - 0.093 \times WV_{Max}$	351	0.500	0.497
2003	*Epicoccum* spp. $= 0.233 - 0.112 \times O_3 + 0.078 \times O_{3Max} - 0.039 \times PM_{10Max} + 0.149 \times T_{Min} - 0.042 \times PP_{Min} - 0.033 \times PP_{Max}$	351	0.274	0.267
2003	*Ganoderma* spp. $= 0.915 - 0.162 \times O_3 + 0.156 \times PM_{10Min} - 0.343 \times PM_{10} + 0.497 \times T - 0.108 \times WV_{Min} - 0.146 \times WV_{Max}$	351	0.512	0.517
2005	*Ganoderma* spp. $= 0.838 + 0.378 \times T_{Min} + 0.171 \times RH - 0.116 \times WV$	364	0.376	0.548
2003-2005	*Ganoderma* spp. $= 0.944 + 0.051 \times O_{3Max} + 0.382 \times T + 0.140 \times RH - 0.047 \times PP_{Max} - 0.085 \times WV$	1068	0.275	0.625
2003	Total fungi $= 2.410 + 0.064 \times O_{3Min} - 0.179 \times O_3 - 0.099 \times PM_{10Max} + 0.262 \times T - 0.048 \times PP$	351	0.277	0.392
2003-2005	Aspergillaceae $= 0.387 - 0.042 \times O_3 + 0.059 \times WV_{Max}$	1068	0.009	0.570
	Botrytis spp. $= 0.445 + 0.068 \times O_{3Min} - 0.106 \times O_3 + 0.047 \times T - 0.038 \times RH$	1068	0.024	0.416
	Cladosporium spp. $= 1.908 + 0.092 \times O_{3Min} + 0.086 \times PM_{10Min} - 0.142 \times PM_{10} + 0.139 \times T_{Min} + 0.142 \times T_{Max} - -0.095 \times RH - 0.048 \times PP$	1068	0.142	0.655
	Coprinus spp. $= 0.521 + 0.043 \times O_{3Min} - 0.052 \times O_3 - 0.039 \times PM_{10} + 0.033 \times RH - 0.039 \times PP$	1068	0.030	0.424
	Epicoccum spp. $= 0.230 - 0.028 \times O_3 - 0.024 \times PM_{10Max} + 0.122 \times T + 0.055 \times RH_{Min} - 0.099 \times RH + 0.046 \times RH_{Max}$	1068	0.175	0.270
	Rusts $= 0.453 - 0.047 \times O_{3Min} + 0.100 \times O_3 + 0.031 \times PM_{10Min} - 0.042 \times PM_{10} - 0.037 \times T - 0.098 \times RH + 0.047 PP_{Max}$	1068	0.065	0.391
	Fusarium spp. $= 0.086 + 0.073 \times T_{Min} - 0.078 \times T + 0.030 \times RH + 0.036 \times PP + 0.021 \times WV_{Max}$	1068	0.060	0.279
	Ustilago spp. $= 0.131 + 0.034 \times PM_{10} - 0.086 \times T_{Min} + 0.064 \times T_{Max} - 0.044 \times PP - 0.044 \times PP_{Max} + 0.037 \times WV$	1068	0.053	0.307
	Total fungi $= 2.381 + 0.084 \times O_{3Min} - 0.200 \times O_3 + 0.070 \times O_{3Max} - 0.077 \times PM_{10Max} + 0.115 \times T$	1068	0.055	0.567

[a] Logarithm of pollen concentrations were used to develop the regression models; [b] n: data number; [c] R^2_{aj}: adjusted determination coefficient; [d] RMSE: root mean squared error.

T and RH were the parameters that showed to have more influence. The spores without T influence were Aspergillaceae, *Coprinus* spp. and *Oidium* spp.; and those not influenced by RH were Aspergillaceae, *Corynespora* spp., *Drechslera* spp, *Oidium* spp., *Ustillago* spp and the total fungal spores. In general, the other meteorological parameters did not show a great influence.

Similar results were achieved by [23]: multiple linear regressions of total fungal spores, with contributions of temperature, PM_{10} and O_3 on the fungal spores. For Aspergillaceae, *Cladosporium* spp. and *Ganoderma* spp., the results were different; even so, the influence of temperature was frequent on both studies.

Nevertheless, the models for each pollen and fungal spore were different depending on the year, i.e., different influences were found for each year and for 2003-2005, which makes the models somehow inconsistent.

Accordingly, it should be remarked that the results previously published supported in only one period, may not be safe enough, which means that the comparison of the results here presented with those obtained by other authors should be made carefully (the differences can also be due to regional specificities, such as climate, vegetation, species diversity and fungal growth substrates).

However, in spite of the inconsistent trends between air pollutants and pollen and fungal spores, further research should be made concerning their crossed interaction with health. In fact, pollen and fungal spore allergens may reach peripheral airways, leading to airway reactivity and symptoms exacerbation, due to its transference to other small particles such as PM [31], especially in regions such as Oporto, where its concentrations exceeded the standard levels (Directive 1999/30/EC). Additionally, the concentrations of other pollutants, such as O_3, can produce an inflammatory effect on the airways causing increased permeability and easier penetration of pollen and fungal spore allergens [2], [33].

V. CONCLUSION

In general, statistically significant correlations were observed randomly. The parameters that influenced the multiple linear regressions, performed for each pollen and fungal spore, depended on the analysed period, which means that the correlations identified as statistically significant can be inconsistent. Thus, it seems that the concentrations of ozone and PM_{10} do not influence most of the airborne pollen and fungal spore concentrations. According to the different conclusions reached, that depended on the periods analysed, it should be remarked that the results previously published supported in only one period, may not be safe enough.

Therefore, the comparison of the results here presented with those obtained by other authors should be made carefully, because the differences can also be due to regional

specificities, such as climate, vegetation, species diversity and fungal growth substrates.

Nevertheless, in spite of the inconsistent trends between air pollutants and pollen and fungal spores, further research should be made concerning their crossed interaction with health.

ACKNOWLEDGMENTS

Authors are grateful to *Comissão de Coordenação da Direcção Regional-Norte* and to *Instituto Geofísico da Universidade do Porto* for kindly providing the air quality and meteorological data. The authors also thank to *Fundação Calouste Gulbenkian* (project 77161). S. I. V. Sousa also thanks the FCT for the fellowship SFRD/BD/38843/2007.

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