Relationship between air pollution, meteorological factors and grass pollen counts, with seasonal allergic rhinitis in Madrid (1996 and 2009)

Brief running title: “Seasonal rhinitis and environment in Madrid”

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Abstract

Objective: The aim of this study is to assess the relationships of meteorological and pollutant variables on the symptoms of patients with seasonal allergic rhinitis due to sensitization to grass pollen during two different time periods in Madrid.

Methods: During the period March 23 to December 31 in 1996 and 2009, were carried out a daily count of grass pollen grains (Burkard spore trap) and rhinitis symptoms score in two groups of patients (n = 25 in 1996 and n = 23 in 2009) with a history of seasonal allergic rhinitis. Descriptive statistics of the same variables in 1996 and 2009 study periods, non-parametric paired samples Wilcoxon test and categorical principal component analysis (CatPCA, SPSS24 package) model, were performed to describe how the variables are related.

Results: The symptom score mean value in 1996 is low and in 2009 moderate. The 1996 and 2009 CatPCA analysis explains around 66.4 % and 70.5 % of the variance, respectively. The strongest relationship in 1996 was between symptoms and grass pollen counts (R = 0.55), and temperature and O₃ (R = 0.63). In 2009, the relationship between temperature and pollution variables is even higher than in the 1996 period: O₃ (R = 0.53) and with PM10 (R = 0.34), in both cases with a positive sign.

Conclusions: The effect of the temperature and pollution (mainly O₃, even at lower atmospheric concentrations than the established guidelines about its effects on health), could contributed to the observed higher seasonal allergic rhinitis symptom score in 2009.

Key words: Grass pollen grains, pollution, temperature, ozone, categorical principal component analysis.
Resumen

Objetivo: El objetivo de este estudio fue evaluar las relaciones de las variables meteorológicas y contaminantes en los síntomas de los pacientes con rinitis alérgica estacional con sensibilización al polen de gramíneas durante dos períodos diferentes en Madrid.

Métodos: Durante el período del 23 de marzo al 31 de diciembre de 1996 y 2009, se realizó un recuento diario de granos de polen de gramíneas (Burkard spore trap) y puntuación de síntomas de rinitis en dos grupos de pacientes (n = 25 en 1996 y n = 23 en 2009) con historia de rinitis alérgica estacional. Para describir cómo se relacionan las variables, se realizaron estadísticas descriptivas de las mismas variables en los períodos de estudio de 1996 y 2009, pruebas parciales no paramétricas, un análisis de componentes principales con la prueba de Wilcoxon y un análisis de componentes principales (CatPCA, SPSS24).

Resultados: El valor medio de la puntuación de síntomas en 1996 fue bajo y en 2009 moderado. El análisis CatPCA de 1996 y 2009 explica aproximadamente el 66,4% y el 70,5% de la varianza, respectivamente. La relación más fuerte en 1996 fue entre los síntomas y los recuentos de polen de gramíneas (R = 0.55), la temperatura y el O₃ (R = 0.63). En 2009, la relación entre la temperatura y las variables de contaminación fue incluso mayor que en el período de 1996: O₃ (R = 0.53) y con PM10 (R = 0.34), en ambos casos con un signo positivo.

Conclusiones: El efecto de la temperatura y la contaminación (principalmente O₃, incluso a concentraciones atmosféricas más bajas que las pautas establecidas sobre sus efectos en la salud), podría contribuir a la mayor puntuación de síntomas de rinitis alérgica estacional observada en 2009.

Palabras clave: Polen de gramíneas, contaminación, temperatura, ozono, análisis por componentes principales
Background

In Madrid, up to 44 types of pollen coexist, although there are basically four that most frequently produce seasonal allergic rhinitis: the Cupressaceae (January and February); Platanaceae (March and April), Poaceae and Oleaceae pollen (May and June) [1].

In Madrid, grass pollen is the most allergenic pollen and up to 88% of patients are polysensitized (allergic to more than one type of pollen) [2]. Thus, most allergy sufferers in the center of Spain are sensitized to 4 or 5 different types of pollen, which implies a longer duration of symptoms throughout the year and greater difficulty when applying pharmacological or prophylactic treatment to these patients. In the Alergológica (2015) study, the prevalence of rhinitis from all causes increased from 55.5 % to 62.0 % (p< 0.001), and grass pollen grains were the principal allergen implicated, 34.8 % to 73.7 % (in Madrid: 61.9 % to 87.1 %), from 2005 to 2015 respectively [3]. The maximum age range of incidence of seasonal allergic rhinitis is 25 - 34 years, and this confirms that it affects young adults, but the overall range is very wide, affecting patients of practically any age [3]

Climate change is increasing the length of the pollen season and its intensity, with significant impact on the millions of patients who already suffer from allergies [4]. Increasing temperatures lead to earlier and longer pollen and allergy seasons, due to more frost-free days and earlier and longer flowering seasons [5, 6]. Higher temperatures also increase ozone production, which sensitizes the respiratory tract to allergens [7]. Higher carbon dioxide levels cause greater plant growth, resulting in increased pollen production and increased pollen potency [8]. More fall-winter precipitation further contributes to increased pollen production [9].
The aim of this study is to assess the relationships of meteorological and pollutant variables on the symptoms of patients with seasonal allergic rhinitis due to sensitization to grass pollen during two different time periods in Madrid.

**Material and Methods**

During the period March 23 to December 31, 1996 and the period March 23 to December 31, 2009, the following studies were carried out:

- Daily grass pollen count using Burkard collector (Clinica Subiza, Madrid), as previously described [1]. The main pollen season was considered from the first date with 10 grains/m³ on three consecutive days, until the date of the last three consecutive records at the same level.
- Daily measurement of meteorological data (temperature, rain, humidity, wind speed) using data from Barajas-Madrid station (National Meteorological Agency).
- Daily measurement of pollution (O₃, CO, SO₂, NO₂, PM10) from the Escuelas Aguirre station (Madrid City Council).
- Daily count of rhinitis symptoms in two groups of patients (n = 25 in 1996 and n = 23 in 2009, completing daily symptom cards at home) selected on the basis of a history of seasonal allergic rhinitis in the last two years. Each patient was evaluated with a medical assessment that included a case history; clinical examination and skin prick tests. The mean age of patients was 30.95 (age range 16-47), all of them (100%) sensitized to grass pollen (with wheal of 4+ [(AR > AH): AR= resulting area (mm²): allergen area – saline area); AH (histamine area – saline area)] and also sensitized to Oleaceae, Platanaceae and Cupressaceae pollen grains (43.5 %, 30.4 % and 56.5 %, respectively with smaller wheal: 2+ - 3+). Two of the patients (2009) were sensitized to D.
pteronyssinus and four of them (2009) to cat dander, with no clinical relevance. Nasal symptoms (sneezing, itching, congestion, and rhinorrhea) were assessed in these patients with clinical sensitization to grass pollens in the last two years. An electronic card was used on a daily basis with the following scale: 0 - absence of symptoms; 1- mild symptoms; 2- moderate symptoms; and ≥3 - severe symptoms.

- Descriptive statistics of the same variables in 1996 and 2009 study periods, and non-parametric paired samples Wilcoxon test (SPSS24 package) used as variables which do not fit the normal distribution, to enable any significant differences to be seen at the two observation points for each one of the studied variables.

- The categorical principal component analysis (CatPCA, SPSS24 package) model [10], was used to describe how the variables that are framed within the allergy concept (grass pollen grains and symptoms) are related. A first analysis assessed all the variables, and the final analysis showed the most important or representative relationships. This test allowed us to reduce relationships of the variables to 2D and represent them graphically in both the periods studied. The variance explained by this methodology is a result that expresses the ability of the analysis to summarize or represent the relationships between the variables included.

Results:

Total yearly grass pollen counts in 1996 and 2009 were 6588 grains/m³ and 2556 grains/m³, respectively, (seasonal maximum counts day: June 1 with 552 grains/m³ and May 11 with 121 grains/m³, respectively). The main pollen season lasted from May 13 to July 9 and May 2 to July 11, respectively.
Table 1 includes descriptive statistics of all variables for both years, highlighting significant differences (p-value=0.000), non-parametric paired Wilcoxon test, especially for symptoms (higher score in 2009 studied period), except for temperature. This study is limited by the non-availability of the trend in the studied variables over the years, but we have seen that the temperature in Madrid has tended to increase over the last 38 years, with temperature increases of 1.4 °C May-July from 1979 to 2016. (i.e.: 0.36°C per decade) [11].

Fig. 1 shows plots of the main variables studied during both time periods; the samples show that when there is an increase in grass pollen grain counts, there is also an increase in the symptoms score (mean value for 1996, low score; mean value for 2009, moderate score).

The 1996 CatPCA analysis explains around 66.4 % of the variance and in 2009, 70.5 % of the variance. In both cases, these percentages are acceptable, i.e, enough to explain the relationships between the variables.

As is shown in Fig. 2, 1996 CatPCA analysis, symptoms are related mainly to grass pollen (R = 0.55), and to a lesser extent to temperature (R = 0.38), and O₃ (R = 0.28), all positive-sign relationships. With PM10 the correlation coefficient is much lower (R = 0.18). The temperature is related to pollen counts, symptoms, PM10 and O₃, especially with O₃ (R = 0.63). Due to the position and proximity of the lines on the graph, the variables showing a closer relationship are symptoms and grass pollen counts (R = 0.55), and temperature and O₃ (R = 0.63). In both cases, the relationships are positive, meaning that high values in one parameter correspond to high values in the other, i.e. higher pollen levels, higher symptoms, and higher temperatures, higher O₃ levels. The temperature is also clearly related to symptoms and with PM10.
(R = 0.34; R = 0.33), is also positive, the reason why high temperature levels are related to high levels of symptoms and PM10. With grass pollen counts, but still with a positive sign, the temperature relationship is much lower (R = 0.28).

Another point to note in the analysis is that the pollution variables O₃ and PM10, show a weak relationship, so they occupy distanced positions, almost perpendicular on the graph, and in addition this relationship has a negative sign (high levels of PM10 reduce O₃ levels).

On the other hand, the 2009 CatPCA analysis (Fig 3), explains 70.5 % of the variance. The relationship of symptoms to pollen increases (R = 0.81) and remains positive, and to a lesser extent to O₃ (R = 0.35), with both coefficients higher than in 1996 period studied.

The difference between the analyses of the 2009 and 1996 periods studied is in the relationship of the allergy variables to the rest of the variables (as can be clearly seen in the positions occupied by the variables on the graph). In contrast to the 1996 period, in the 2009 period these variables (symptoms and grass pollen counts) are mainly related to O₃ (R = 0.35 R = 0.26). Therefore, this is the closest variable in the 2009 graph, related to the PM10 and with almost no relationship with temperature (which is distant and almost perpendicular on the graph). The relationship between temperature and pollution variables is even higher than in the 1996 period: with O₃ (R = 0.53) and with PM10 (R = 0.34), in both cases with a positive sign. The pollution variables (O₃ and PM10) are still only slightly related (R = 0.06), although here the relationship is positive.

Discussion:
In this study, we found a higher symptom score in the 2009 period, despite lower pollen counts, than in the 1996 period, but this was a year with lower humidity, similar temperature and higher O\textsubscript{3} levels (but lower than threshold) which may have affected the longer duration and intensity of the grass pollen season that year. The particular atmospheric conditions in spring in Madrid, with great instability lifting the pollen deposited on the ground, may also have contributed.

It has been shown in S. Spain that the grass pollen season has increased as a result of rising temperatures [12], and also in the case of Oleaceae pollens [13]. The increase in temperature in this geographical area seems to have a positive effect on the intensity of flowering with longer pollination periods, in many cases more intense. In this study, the main pollen season in 2009 was 13 days longer than in 1996. The grass pollen forecast in Madrid is always announced by March. The rainfall from October 1995 - March 1996 in Madrid was twice that of the previous period (October 1994-March 1995), which led to an increase in the number of grains in 1996, four times higher than in 1995. On the other hand, the Spanish Society of Allergy and Clinical Immunology (SEAIC) estimated that the accumulated concentrations of grass pollen counts in 2009 exceeded 5100 grains per cubic meter of air, compared to 4000 recorded in 2008. Nevertheless, lower grass pollen counts were collected that year, probably due to the cleaner rain effect observed in May that coincided with the grass pollen peak period (Fig. 1), but in a more polluted atmosphere and with more allergenic activity, as the higher seasonal allergic rhinitis score showed.

We suggest that this fact could be attributed to the coadjuvant effect of the temperature and pollution (mainly O\textsubscript{3}) observed in this study on the greater potency of the allergens and also to the direct relationship between the greater level of atopy in the 2009 group compared to the 1996 group. It has been shown that ozone increases allergenicity (induces larger wheals and flares in skin prick tests) [14] and that the exposure of
Phleum pratense pollen to increasing ozone concentrations from 100 ppb up to 5 ppm results in a significant increase in the naturally released pollen cytoplasmic granules, which are also known to contain allergens [15].

Pollution has already been pointed out as one of the possible causes of oxidative stress and this stress as the origin of the higher prevalence of seasonal allergic rhinitis, intensifying activity as a defense from environmental pollution, thus strengthening allergenicity. [16]. Some pollutants such as SO$_2$, NO$_2$ and O$_3$ can alter the allergenic proteins of pollen [16, 17].

O$_3$ is a pollutant that usually reaches higher values in areas far from the emission sources, that is, in semi-urban and rural areas. The atmospheric conditions present in heat waves favor the formation of tropospheric ozone from precursors [18]. During the summer, exceeding the threshold temperature in Madrid (36.5 °C) coincides with the days in which the threshold for warning the population is exceeded for high concentrations of O$_3$, set at 180 µg/m$^3$ [18]. In 2009, no exceeding of this information threshold or the alert threshold is recorded, but the annual average values experienced an upward trend [19]. In Madrid in 2017, the number of exceedances of the O$_3$ target value for health protection (>25 times), was recorded in the 2011-2017 report [20]. As it is a secondary pollutant, the O$_3$ measures adopted refer to measures that reduce the emission of precursors, mainly NOx and volatile organic compounds [18]. In a recent report from Ecologistas en Acción (2018), in winter two of the stations measured high O$_3$ values similar to those recorded in spring in the same places (San Agustín de Guadalix and Puerto de la Morcuera): 70 and 80 µg/m$^3$ [21].

With regard to photochemical pollutants or the synergistic effect of ultraviolet radiation on pollutants, the effects of UV-B rays and O$_3$ have been studied, finding that the pollen tube was reduced considerably, more than by the stress itself, despite low levels of O$_3$ [22].
Differences have been found in the allergenicity of pollens of groups of trees of the same species and grass pollen, which although of relatively close taxonomy, are found in areas with different pollution levels (city / countryside) or temperatures (valley / mountain) [23]. In Spain, Armentia et al, (2002), found that the grass pollen of rural areas in the province of Valladolid is less damaged than the same species in urban areas, where grass pollen is subjected to a polluted environment, repeated mowing that lets very few spikes flourish, and pesticides applied by the local authorities [23]. Feo-Brito et al, (2007), reported that the air pollution levels in Puertollano (more polluted) were associated with an increased risk of asthma symptoms in pollen-allergic asthmatic patients compared with a similar group from Ciudad Real (less polluted) [24]. The largest contribution was by O₃, especially in Puertollano [24]. Mur Gimeno et al, (2007,2010), show that pollen-allergic asthmatics in Puertollano present poorer clinical progress and become decompensated earlier than those from Ciudad Real, and that this could be due to air pollution [25, 26]. This body of evidence supports the fact that urbanization and high levels of vehicle emissions induce more symptoms of bronchial obstruction (in particular bronchial asthma), in people living in urban areas compared to those living in rural areas.

In the eastern United States and Europe, the most severe regional ozone episodes occur when a slow-moving, high pressure system develops in summer. This is when the days are longest, when solar radiation is most direct (the solar zenith angle is lower) and air temperatures are high. As the slow-moving air in the shallow boundary layer passes over major metropolitan areas, precursor concentrations rise, and as the air slowly flows around the high-pressure system, photochemical production of O₃ occurs at peak rates [27]. We suggest that high temperatures in Madrid, with inclusive low levels of O₃ concentrations (but higher 13 years later), could be a factor that has contributed to the increase of symptoms even at lower grass pollen counts in 2009 compared with 1996.
Most of the times, O$_3$ levels are higher in rural areas than in cities. O$_3$ is degraded by nitrogen monoxide (NO) also involved in its formation. This degradation occurs more often in cities than in rural areas, because there is more NOx in cities. For this reason, O$_3$ concentrations are higher in rural areas than in cities [27].

According to the review of evidence on health aspects of air pollution – REVIHAAP Project (Technical Report) (2013) [28], the European threshold of 180 µg/m$^3$ for informing the population may thus not be viewed as an effective threshold value under which absolutely no one will suffer any effect at all. However, the WHO postulated that the effects of concentrations lower than 200 µg/m$^3$ will be limited in severity and will only prevail in less than 5% of the total population [29]. Warning the whole population at lower concentration levels is thus not advised. As such, it concerns a sliding scale and although somewhat artificially, it is possible to talk about a mild response at (hourly mean) concentrations of 180-240 µg/m$^3$, a moderate response at 240-360 µg/m$^3$ and a severe response above 360 µg/m$^3$ [28, 29]. The review concludes that a considerable amount of new scientific information on the adverse effects on health of particulate matter, O$_3$ and NO$_2$, observed at levels commonly present in Europe, has been published in recent years [28]. This new evidence supports the scientific conclusions of the WHO air quality guidelines, last updated in 2005, and indicates that the effects in some cases occur at air pollution concentrations lower than those serving to establish these guidelines [29].

Diesel combustion is also an important factor to take into account in the composition of the pollution particles, since at present 70% of all particles and 90% of those <5 µm (respirable particles) are produced from its combustion, inducing important biological factors, such as increasing Th2 response [30, 31].

As the rising frequency of obstructive respiratory diseases during recent years, in particular allergic asthma, can be partially explained by changes in the environment,
with the increasing presence in the atmosphere of chemical triggers (particulate matter and gaseous components such as NO₂ and O₃) and biologic triggers (aeroallergens), measures need to be taken to mitigate the future impact of climate change and global warming [32]. Over the last 50 years, the earth’s temperature has risen markedly, likely because of growing concentrations of anthropogenic greenhouse gas [32]. For this reason, it is important to emphasize to patients that climate change is increasing exposure to allergens and suggest what they can do to minimize their exposures to reduce allergy and asthma symptom, such as checking pollen levels frequently. In Spain, patients can sign up for free alerts on the Spanish Society of Allergy and Clinical Immunology aerobiology network. For patients with asthma, it is important to check ozone levels.

Conclusions:

The effect of the temperature and pollution (mainly O₃, even at lower atmospheric concentrations than the established guidelines about its effects on health), could contributed to the observed higher seasonal allergic rhinitis symptom score in 2009.

We highlight the need to continue research into the impact of these changes, and into strategies and policies to reduce greenhouse gas emissions and air pollution.
References:


Table 1. Descriptive statistics of the main variables studied during both studied periods of time: 23 March to 31 December in 1996 and 2009.

<table>
<thead>
<tr>
<th>Studied Variables</th>
<th>1996</th>
<th></th>
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<td></td>
<td>N</td>
<td>Missing</td>
<td>Mean± Standard Deviation Median(Minimum-Maximum)</td>
<td>N</td>
<td>Missing</td>
<td>Mean± Standard Deviation Median(Minimum-Maximum)</td>
</tr>
<tr>
<td>Symptoms* (score)</td>
<td>284</td>
<td>81</td>
<td>1.45±1.74 1.00(0.00-8.00)</td>
<td>284</td>
<td>81</td>
<td>2.33±1.34 1.74(0.61-6.61)</td>
</tr>
<tr>
<td>Grass pollen counts* (grains/m³)</td>
<td>284</td>
<td>81</td>
<td>21.8±57.2 2.00(0.00-552.0)</td>
<td>284</td>
<td>81</td>
<td>8.28±17.9 1.00(0.00-121.0)</td>
</tr>
<tr>
<td>SO₂* (µg/m³)</td>
<td>284</td>
<td>81</td>
<td>17.7±9.25 15.0(6.00-61.0)</td>
<td>284</td>
<td>81</td>
<td>9.87±3.42 9.00(6.00-21.0)</td>
</tr>
<tr>
<td>CO* (µg/m³)</td>
<td>284</td>
<td>81</td>
<td>13.7±6.71 12.0(5.00-40.0)</td>
<td>284</td>
<td>81</td>
<td>0.40±0.13 0.37(0.19-1.02)</td>
</tr>
<tr>
<td>NO₂* (µg/m³)</td>
<td>284</td>
<td>81</td>
<td>64.2±17.0 61.5(32.0-117.0)</td>
<td>282</td>
<td>83</td>
<td>52.6±14.0 51.0(17.0-96.0)</td>
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<td>PM10* (µ/m³)</td>
<td>284</td>
<td>81</td>
<td>35.2±8.75 34.0(19.0-61.0)</td>
<td>128</td>
<td>237</td>
<td>30.0±10.7 29.0(10.0-60.0)</td>
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<td>O₃* (µg/m³)</td>
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<td>81</td>
<td>33.7±15.1 34.0(3.00-71.0)</td>
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<td>81</td>
<td>43.6±17.9 45.0(8.00-82.0)</td>
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<td>Temperature (ºC)</td>
<td>284</td>
<td>81</td>
<td>18.1±6.64 17.8(2.50-31.7)</td>
<td>284</td>
<td>81</td>
<td>18.0±7.31 18.1(0.00-29.8)</td>
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<tr>
<td>Humidity* (%)</td>
<td>284</td>
<td>81</td>
<td>59.0±13.6 55.0(37.0-95.0)</td>
<td>284</td>
<td>81</td>
<td>34.1±19.8 29.0(8.00-95.0)</td>
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<tr>
<td>Wind speed* (m/s)</td>
<td>284</td>
<td>81</td>
<td>16.8±6.06 16.0(7.00-39.0)</td>
<td>284</td>
<td>81</td>
<td>14.8±8.70 13.0(0.00-51.0)</td>
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<tr>
<td>Rainfall* (L/m²)</td>
<td>284</td>
<td>81</td>
<td>0.87±2.99 0.00(0.00-24.0)</td>
<td>284</td>
<td>81</td>
<td>8.17±26.7 0.00(0.00-209.0)</td>
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</tbody>
</table>

* Significant differences: p-Value = 0.000, nonparametric paired samples Wilcoxon test.
Rainfall

Plots of the main variables studied during both time periods 23 March to 31 December in 1996 and 2009.

Rainfall* 1996 vs 2009 (L/m²)

Humidity 1996 vs 2009 (%)

Symptom score*/++ (points) vs grass pollen counts* (grains/m³)

Symptoms vs pollen grains 1996

Symptoms vs pollen grains 2009

Temperature

Ozone

Ozone* 1996 vs 2009 (µg/m³)

Temperature* 2009 vs 1996 (ºC)
Date

Fig 1: *Daily measurement of all variables. ++ Mean seasonal allergic rhinitis symptoms score recorded by 25 in 1996 and 23 patients in 2009.
Figure 2. CATPCA 96

CATPCA analysis explains 66.4% of the variance

<table>
<thead>
<tr>
<th></th>
<th>Symptoms</th>
<th>Grass pollen counts</th>
<th>PM10</th>
<th>O3</th>
<th>Temperature</th>
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<tbody>
<tr>
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<td>0.179</td>
<td>0.278</td>
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<tr>
<td>Grass pollen counts</td>
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<td>0.146</td>
<td>0.222</td>
<td>0.281</td>
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<tr>
<td>PM10</td>
<td>0.179</td>
<td>0.146</td>
<td>1.000</td>
<td>0.097</td>
<td>0.334</td>
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<tr>
<td>O3</td>
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<td>0.222</td>
<td>0.097</td>
<td>1.000</td>
<td>0.626</td>
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<tr>
<td>Temperature</td>
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<td>0.281</td>
<td>0.334</td>
<td>0.626</td>
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</table>
Fig 3. CATPCA 2009

**Component Loadings**

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<th>Dimension 2</th>
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<tr>
<td>Grass pollen counts</td>
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<tr>
<td>PM10</td>
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<td></td>
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<td>Temperature</td>
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**Correlations Transformed Variables**

<table>
<thead>
<tr>
<th></th>
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<th>Grass pollen counts</th>
<th>PM10</th>
<th>O3</th>
<th>Temperature</th>
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<tbody>
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<td>0.118</td>
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<tr>
<td>Grass pollen counts</td>
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<td>1.000</td>
<td>0.186</td>
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<tr>
<td>PM10</td>
<td>0.118</td>
<td>0.186</td>
<td>1.000</td>
<td>0.056</td>
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<tr>
<td>O3</td>
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<td>0.259</td>
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<td>1.000</td>
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<tr>
<td>Temperature</td>
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<td>0.527</td>
<td>1.000</td>
</tr>
</tbody>
</table>

CATPCA analysis explains 70.5% of the variance.