Variations and origin of the atmospheric pollen of *Cannabis* detected in the province of Tetouan (NW Morocco): 2008–2010

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HIGHLIGHTS

► The incidence of Cannabis pollen in the atmosphere of Tetouan (Morocco) is studied.
► The data obtained could serve as an indicator of the cultivation of this species.
► The Cannabis pollen levels could be clinically important for allergic patients.

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abstract

Cannabis, also called marihuana or hemp, is a wind-pollinated plant that produces hundreds of flowers on large inflorescences. It is also one of the oldest psychoactive plants known to humanity. Morocco has become one of the main producers of *Cannabis* resin (hashish), primarily supplying the European market. The aim of this paper is to ascertain whether the atmospheric monitoring of *Cannabis* pollen can play a role, from a criminological point of view, in the surveillance of *Cannabis* cultivation in the area of Tetouan (NW Morocco) as well as to estimate pollen emission so that the sensitive population can be warned about the allergic diseases that its pollen can cause.

Aerobiological samplings were made with the aid of a Hirst type volumetric trap (Hirst, 1952), which worked uninterruptedly during a 3-year period (2008–2010) according to the methodology proposed by the Spanish Aerobiology Network, the REA.

*Cannabis* pollen was present in the atmosphere of Tetouan mainly from early April to late August, a period in which about 95% of the annual counts were registered. The highest levels were detected in June and July, with concentrations more or less evenly distributed throughout the day with slight increases of 5% between 12:00 and 16:00 h. The strong association between skin test reactivity, respiratory symptoms, and pollination period found by other authors, together with the levels registered, suggests that *Cannabis* pollen could be a clinically important aeroallergen for sensitive patients. On the other hand, the data obtained could serve as an indicator of the cultivation activity of this species and should be taken into account by the state authorities since they provide strong evidence of the existence of *Cannabis* crops in the region of Tetouan.

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1. Introduction

Marihuana, *Cannabis*, or hemp is one of the older psychoactive plants known to humanity. Some botanists recognize three species belonging to the genus *Cannabis*: *Cannabis sativa* L., *Cannabis indica* Lam. and *Cannabis ruderalis* Janish. (Grinspoon and Bakalar, 1993) while, for others, the last two are subspecies of *C. sativa*. Whatever the case, the first mentioned species is the most abundant in northern Morocco. It is a wind-pollinated plant that produces hundreds of flowers on large inflorescences, a single flower producing about 350,000 pollen grains (Faegri et al., 1989).

Morocco has become one of the main producers of *Cannabis* resin (hashish), supplying primarily the European market. In the past twenty years, *Cannabis* cultivation has spread from the traditional areas in the central Rif, where it had been grown since the fifteenth century, to new areas. This expansion is often at the expense of forested areas, as well as of the best arable and irrigated land, thus contributing to soil erosion and disappearance of licit agriculture. In 2003, the first survey conducted by the Government of Morocco and the United Nations Office on Drugs and Crime (UNODC) attempted to measure the size of the phenomenon. This study estimated *Cannabis* cultivation at about 134,000 ha in a total area of 14,000 km², covering five provinces, with
86% of the Cannabis cultivated in three of them: Chefchaouen (50%), Taounate (19%) and Al Hoceima (17%), while the provinces of Larache (9%) and Tetouan (5%) only played a secondary role in Cannabis cultivation (UNODC, 2003).

In subsequent years, the UNODC surveys revealed a decline in cultivation to 72,500 ha in 2006 (UNODC, 2006) and to 60,000 ha in 2008 (UNODC, 2008), a reduction of 55% compared with 2003, as indicated by the Moroccan Interior Ministry. The most important decreases were in the provinces of Al Hoceima (−54%) and Taounate (−43%) while a smaller fall was noted in the province of Larache (−1%). These decreases are attributed to outreach activities by the local administration. However, Cannabis cultivation increased significantly in the provinces of Tetouan (+19%) and Chefchaouen (+13%).

The aim of this paper is to ascertain whether the atmospheric monitoring of Cannabis pollen can play a role, from a criminological point of view, in the surveillance of Cannabis cultivation, not only in the area of Tetouan but also at a global level, in other areas wherein Cannabis is cultivated. In this way, we can either confirm that the drug control policy has put an end to the cultivation of Cannabis or, if not, the geographical origin of the crops can be identified. The importance of studying Cannabis pollen dispersion in the air of Tetouan is not only to help control the illicit cultivation of Cannabis, but also to estimate pollen emission in order to warn the sensitive population about the allergic diseases that its pollen can cause (Sáenz, 1978; Lewis et al., 1983), as occurs in Pakistan (Sadiq et al., 2007), India (Singh and Kumar, 2003) and several regions of North America, where hemp is widely grown (Maloney and Brodley, 1940; MacQuiddy, 1955; Freeman, 1983; Stokes et al., 2000).

2. Material and methods

The city of Tetouan is located in the eastern sector of the Tingitane Peninsula (NW Morocco, 35°34′N; 5°22′W), in the Thermomediterranean belt, at an altitude of 65 m a.s.l. (Fig. 1). Climatic conditions are predominantly Mediterranean warm subhumid (Renbad, 1982), with an average annual rainfall of 728 mm, and annual mean temperature of 18.2 °C. It is situated between two mountain ranges, one located to the northwest (the Haouz-Ghorghiz) and the other one to the southeast (the Rif range), which determines that the prevailing winds are those blowing from the northeast (the coastal zone) and southeast (inland) (Fig. 2).

Aerobiological samplings were made with the aid of a Hirst type volumetric trap (Hirst, 1952), a seven-day recorder model by Burkard™, situated on the flat roof of the Department of Biology of the Faculty of Science (University Abdelmalek Essaâdi), 15 m above ground level, in an open area with no nearby buildings that could obstruct the free air circulation. The pollen trap was uninterruptedly kept operational from 1 January 2008 to 31 December 2010, aspirating a flow of 10 l/min. Pollen counts were made by means of a light microscope, 4 longitudinal sweeps per slide being made, using a ×40 objective (0.45 mm microscopic field), according to the methodology proposed by the Spanish Aerobiology Network, the REA (Galán et al., 2007). Samples were always counted by the same operator, the pollen concentrations being expressed as the number of pollen grains/m³ of air. The mean daily values were used for elaborating tables and figures.

The main pollen season (MPS) was calculated as described by Andersen (1991). This season represented 95% of the annual total, beginning on the first day in which the cumulative daily count reached 2.5% of the annual figure (calculated from the 1st of January), and finishing on the day in which 97.5% of the annual total had been reached. In order to establish the intradiurnal variations, only rain-free days when the mean daily pollen concentration equalled or exceeded the mean for the MPS were taken. For calculating the Intradiurnal Distribution Index (IDI), the methodology proposed by Trigo et al. (1997) was followed. This index ranges from 0 to 1, depending on the difference between the minimum and the maximum daily concentration values. The graph shows the 2-hour cumulative percentages.

Finally, in order to identify the influence of the main meteorological parameters on pollen concentrations, a statistical analysis was performed using Spearman’s correlation test. The meteorological data were provided by an automatic meteorological station located in the Physics Department of the Faculty of Sciences, where the pollen trap was installed. To better study the influence of these meteorological parameters on pollen concentrations, the MPS was divided into pre-peak and post-peak periods in the case of temperatures, since temperatures tend to increase as the MPS progresses, as do pollen concentrations during the pre-peak period. However, during the post-peak period, the pollen counts tend to decrease, while temperatures continue to rise. The pre-peak was defined as the period running from the beginning of the pollen season to the peak day. On the contrary the post-peak is the period that goes from the peak-day to the end of the pollen season.

The meteorological parameters included in the analysis were: air temperature (medium, maximum and minimum daily values), relative humidity (mean, maximum and minimum daily values), daily rainfall, wind speed (mean and maximum daily values), daily wind percentage from the four quadrants (NE, SE, SW and NW) and percentage of calms.

Finally, in order to statistically compare the results obtained for pollen in the different years of study, a Kruskal–Wallis rank classification was made. This is a useful tool when there are more than two independent samples, to know if they come from the same population or from populations with the same average values. As the test result was significant for $p \leq 0.05$, we made an a posteriori test to look for the reasons for the significance found. For this, we used the Mann–Whitney U-test. These tests are a good alternative to parametric test such as ANOVA or t-test, when the data (as in our case) do not present normal distribution (Martín Andrés and Luna Castillo, 1995; Sokal and Rohlf, 1995; Siegel and Castellán, 1995; Toro et al., 1998). The same statistical analysis was carried out in the case of mean temperature and rainfall, the two main meteorological parameters that affect pollen concentrations. The normality was checked by means of the test of Kolmogorov–Smirnov.

3. Results

3.1. Seasonal distribution

Cannabis pollen presented an annual pollen index (annual sum of the daily means) of 1862, on average, during the studied period. It is the 5th pollen type in the order of abundance in the atmosphere of Tetouan, representing 4% of the annual total. The highest value (2841) was registered in 2008, while in 2009 and 2010 this annual index showed values of 1436 and 1237, respectively, with a decrease
of between 50% and 47% over the first year of study. This pollen type is present in the atmosphere of Tetouan mainly from early April to late August, a period in which about 95% of the annual counts were registered, the highest concentrations being detected in June and July, months in which the maximum peaks occur, followed by May. The highest monthly pollen index was detected in June 2008 with a value of 1175 (Figs. 3, 4).

During the seasonal period, several peaks of different intensities appeared, a first peak of high intensity being detected in early June with daily mean concentrations of 92, 51 and 53 pollen grains/m³ in 2008, 2009 and 2010, respectively. However, although only slightly higher, the maximum peaks occurred later, in late June in 2008 and 2009 and in mid July in 2010, this last observation representing a delay of approximately a month compared with the first two years of the study (Figs. 3, 4).

The length of the main pollen season (MPS) was 155 days on average, with a maximum difference of 45 days registered between the last two years of samplings. The date of the beginning of the MPS varied from late March (2009) to late April (2008 and 2010), with a maximum difference of 32 days. It always ended in September, with a maximum variation of 23 days (Table 1).

The duration of the pre- and post-peak periods was similar in 2009, but in 2008 the post-peak was 25 days longer, while in 2010 it was 37 days shorter than the pre-peak period. However, the quantities of pollen registered during the pre-peak periods were always higher than those registered during the post-peaks.

![Wind rose for the period 2008–2010.](image)

![Annual (A) and monthly (B) pollen indices reached by Cannabis pollen in Tetouan during the years 2008–2010.](image)

![Seasonal behaviour of the daily mean concentrations of Cannabis during the years of study (2008–2010) and the 5-day running media (line).](image)
3.2. Intradiurnal variations

The intradiurnal patterns showed by this pollen type was similar in all the years studied, with a very low intradiurnal distribution index, IDI (Trigo et al., 1997), of 0.07 on average. This shows that concentrations were more or less evenly distributed throughout the day with small increases, reaching 13% between 10:00 and 16:00 h. The lowest percentages (about 4%) were registered at dawn (Fig. 5).

3.3. Correlations with meteorological parameters

In general, we obtained positive and highly significant correlation coefficients between pollen concentrations and temperatures (mean, maximum and minimum) in almost all the cases, for a level of confidence of $p \leq 0.001$. In contrast, the coefficients were negative in the case of relative humidity and rainfall (Table 2).

Winds did not show a very clear pattern of behaviour because they were not homogeneous during the years studied. However, in the case of wind direction, it can be observed how for the two dominant winds (northeast and southwest, 1st and 3rd quadrants, respectively), the coefficients were always negative for the former and positive for the latter during the three years of the study, although not always significantly so. Anyway, the coefficients were significant when the data of the studied period, as a whole, were taken into account. This association between pollen concentrations and wind direction also presented negative values in the case of winds blowing from the 2nd and 4th quadrants (Table 2).

3.4. Statistical analysis

The results of the Kruskal–Wallis test showed significant differences regarding pollen concentrations ($\chi^2 = 18.927, p = 0.000$), as well as rainfall ($\chi^2 = 8.664, p = 0.013$). However, there were no significant differences regarding mean temperature ($\chi^2 = 2.950, p = 0.229$). The a posteriori Mann–Whitney U-test carried out for pollen, showed significant differences between all the years for $p \leq 0.05$, 2008 vs. 2010 being the pair of years that showed the most significant differences ($Z = −4.283, p = 0.000$), followed by 2009 vs. 2010 ($Z = −2.479, p = 0.013$) and 2008 vs. 2009 ($Z = −2.068, p = 0.039$). In the case of rainfall, only the years 2008 vs. 2009 did not present significant differences ($Z = −0.080, p = 0.936$) (Table 3).

4. Discussion

The relationship between man and hemp goes far back into history. Its cultivation was reported in classical times, and there are many varieties, with concomitant variations in morphology. *Cannabis* presents trizonoporate, sometimes tetrazonoporate, pollen grains, which have been studied by several authors, including Migal (1969), Makino and Melhem (1973) and Punt and Malotaux (1984) from a morphological point of view. Migal (1969) found that monoecious varieties produced pollen grains significantly smaller (27 μm on average) than those of the normal dioecious plants (33 μm), the pollen of which were also more uniform in size and number of pores. Both mentioned forms of *Cannabis* pollen were recorded during the analysis of the aerobiological samples from Tetouan.

4.1. Seasonal behaviour

From an aerobiological point of view, *Cannabis* pollen presents a very long pollination period that lasts from April to September, with a very similar seasonal distribution during the years studied, including two peaks of maximum intensity, one in early June and the other in late June (Fig. 4). These two peaks are due to the sequential flowering of the rain-fed and irrigated crops of *Cannabis*, which implies a gap in the pheno-ology of the two cropping patterns and, therefore, a longer period of

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**Table 1**

Main parameters of *Cannabis* main pollen season in Tetouan during the studied period (2008–2010).

<table>
<thead>
<tr>
<th>Year</th>
<th>MPS (95%)</th>
<th>Peak days</th>
<th>Pre-peak</th>
<th>Post-peak</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Date start/end</td>
<td>Length (days)</td>
<td>Total grains</td>
<td>Date</td>
</tr>
<tr>
<td>2008</td>
<td>15/04–26/09</td>
<td>165</td>
<td>2701</td>
<td>11/06</td>
</tr>
<tr>
<td>2009</td>
<td>28/03–15/09</td>
<td>172</td>
<td>1367</td>
<td>10/06</td>
</tr>
<tr>
<td>2010</td>
<td>29/04–03/09</td>
<td>127</td>
<td>1237</td>
<td>01/06</td>
</tr>
</tbody>
</table>

*a* Peak data used in the establishment of the pre-peak and post-peak periods.

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**Fig. 5.** Intradiurnal distribution pattern of airborne *Cannabis* pollen during the years 2008–2010.
Table 2
Spearman's correlation coefficients obtained between the daily pollen concentrations of Cannabis pollen and the main meteorological parameters during the period 2008–2010.

<table>
<thead>
<tr>
<th></th>
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<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>T max. °C</td>
<td>0.768**</td>
<td>0.224†</td>
<td>0.694***</td>
<td>0.306**</td>
<td>0.534***</td>
<td>0.375**</td>
<td>0.849***</td>
<td>0.034</td>
</tr>
<tr>
<td>T mean °C</td>
<td>0.801†</td>
<td>0.239†</td>
<td>0.684</td>
<td>0.259†</td>
<td>0.557***</td>
<td>0.346†</td>
<td>0.872</td>
<td>−0.053</td>
</tr>
<tr>
<td>T min. °C</td>
<td>0.0665***</td>
<td>0.072</td>
<td>0.596</td>
<td>−0.049</td>
<td>0.401***</td>
<td>−0.050</td>
<td>0.808</td>
<td>−0.208</td>
</tr>
</tbody>
</table>

| 2008           | (n = 70)     | (n = 95)      | (n = 85)     | (n = 87)      | (n = 82)     | (n = 45)      | (n = 77)          | (n = 78)          |
| R. humidity (maximum) | −0.176*       | −0.259***      | −0.159       | −0.367**      | −0.367**     | −0.376**      | −0.376**          |                  |
| R. humidity (mean)  | −0.224**      | −0.278***      | −0.227**     | −0.227**      | −0.237***    | −0.376**      | −0.376**          |                  |
| Rainfall (mm)     | −0.086        | −0.076        | −0.105       | −0.174†       | −0.174†      | −0.174†       | −0.174†           |                  |
| Wind speed (maximum) | 0.009        | 0.114         | 0.162        | 0.090         | 0.090        | 0.090         | 0.090             |                  |
| Wind speed (mean) | −0.011        | −0.146†       | −0.061       | −0.244†       | −0.244†      | −0.244†       | −0.244†           |                  |
| % wind 1st quadrant| −0.089        | −0.122        | −0.042       | −0.069        | −0.069       | −0.069       | −0.069            |                  |
| % wind 2nd quadrant| −0.104        | −0.122        | −0.042       | −0.069        | −0.069       | −0.069       | −0.069            |                  |
| % wind 3rd quadrant| 0.132         | 0.183**       | 0.137        | 0.210†        | 0.210†       | 0.210†       | 0.210†            |                  |
| % wind 4th quadrant| −0.060        | 0.042         | −0.067       | −0.170†       | −0.170†      | −0.170†      | −0.170†           |                  |
| % calm           | −0.025        | 0.235**       | −0.065       | 0.036         | 0.036        | 0.036        | 0.036             |                  |

* p ≤ 0.05.
** p ≤ 0.01.
*** p ≤ 0.001.

In bold, significant values.
4.2. Intradiurnal variation

Apart from our results, we have not found studies related to intradiurnal variations of Cannabis pollen. In general, the behaviour followed by this pollen type throughout the 24 h of the day was very similar in the three years of study, with slight increases around midday. These increases could have been favoured by the daily maximum temperatures that are reached at almost the same time, which would facilitate anther dehiscence and pollen release (Trigo et al., 1997). This influence of temperature on pollen concentrations has been highlighted previously in different localities, in both herbaceous and tree species, by authors including Suarez-Cervera and Seoane-Camba (1983), Galán et al. (1991), Bricchi et al. (1992), Recio (1995), Alcazar (1995) and Jato et al. (2002).

The values of the intradiurnal distribution index (Trigo et al., 1997) are relatively low, meaning that Cannabis pollen concentrations are more or less evenly distributed in the atmosphere of Tetouan throughout the day.

A long pollen season combined with the almost continuous presence of pollen in the atmosphere throughout the 24 h of the day could be a clinical problem for people allergic to Cannabis pollen, since this pollen type has been cited as an aeroallergen by several authors, for example, Maloney and Brodkey (1940), Sáenz (1978), Lewis et al. (1983) and Sadiq et al. (2007). In addition, Zhatov (1983) reported that the viability of pollen Cannabis could exceed 7 days.

In Nebraska, Stokes et al. (2000) found a strong association between skin prick test reactivity (61%), respiratory symptoms (73%), and Cannabis pollen period, suggesting that this pollen type could be a clinically important aerolleneggen to sensitive patients. Torre et al. (2007), in north Italy, found several patients that presented rhinitis and/or asthma symptoms in the summer months without showing positive skin tests for the pollen types present in the atmosphere at this season. When they tried to determine the pollination pattern of pollens that are not usually tested (because no company produces the corresponding extract), the authors found a significant percentage of Cannabaceae from the end of July to mid-September, as mentioned above. Unfortunately, although the potential allergenicity of Cannabis pollen has been reported in the United States and supported by positive skin prick tests in patients with rhinitis or asthma during the pollen seasons, the clinical significance of Cannabis pollen as an aeroallergen in Europe is still undefined.

4.3. Correlations with meteorological parameters

On the basis of the results obtained (Table 2), the meteorological parameters that best explain the daily fluctuations of Cannabis concentration in the atmosphere of Tetouan are temperatures and relative humidity. Increases in temperature favours anthesis processes and anther dehiscence, increasing pollen concentrations. The correlation coefficients obtained between pollen concentrations and temperatures were, in general, positive, and higher and more significant during the pre-peak periods. In the post-peak period we also obtained positive and significant values because during this period there are still slight peaks despite the general tendency for pollen to decrease. Relative humidity has the opposite effect, hindering anther dehiscence and favouring the agglutination of atmospheric particles, which reduces their buoyancy in the atmosphere (Emberlin, 1994). Rainfall also has a negative effect on pollen concentrations, because of its washing effect in the atmosphere (Belmonte and Roure, 1985; Keyman et al., 1989; Recio et al., 1997; Fornaciari et al., 1997). However, although all the coefficients obtained were negative, this coefficient was significant only for the complete period (2008–2010). This is probably due to the lower number of rainy days that occurred during the MPS (19 days as average between April and September, both inclusive) and, consequently, to the lower number of data used in the statistical analysis for the single years.

Regarding winds (speed and direction), most coefficients were not significant, probably because the crops are grown in sheltered areas, away from the city and protected by the orography of the territory. However, as mentioned in the introductory section, because Tetouan is situated between two mountain ranges, the prevailing winds are those blowing from NE, 1st quadrant, and SW, 3rd quadrant (Fig. 2). This is the reason why the correlation coefficients between pollen concentrations and winds blowing from the 2nd (SE) and 4th quadrants (NW) were always negative or not significant, since the mountainous barriers are high enough to hinder the pollen reaching the sampler. But, in the case of the prevailing winds, if we take into account the 3-year period, the value of the coefficient was negative and significant for winds blowing from the northeast (1st quadrant), which, coming from the sea, tend to lower the pollen concentrations in general. On the contrary the coefficient was positive and significant for winds blowing from the southwest (3rd quadrant). Hence, we think that much of the pollen collected could have come from the southwest, transported by the winds blowing from that quadrant.

All this means that, despite the campaigns carried out by the authorities to reduce the crops, illegal Cannabis cultivation continues in the region, since it is a business that moves a great amount of money, disappearing from some areas and appearing in others, because of the fear of being arrested by the police.

We think the data obtained can be regarded as a good indicator of the cultivation activity of this species and should be taken into account by the Moroccan authorities since they constitute strong evidence of the existence of Cannabis crops in the region of Tetouan. We also consider that the methodology here applied in detection and quantification of Cannabis pollen in the atmosphere can be used everywhere to control hemp cultivation and may help to determine the areas of origin.

Finally, the results of the statistical analysis (Table 3), showed significant differences between the pollen data of the three years studied as well as the rainfalls. For this, we think that the different regimes of rainfalls of the years studied, together with the deterrent campaigns led by local authorities are the main causes of the differences found, as commented above, since mean temperature and winds showed a similar behaviour during the years studied.

5. Conclusions

Cannabis pollen is present in the atmosphere of Tetouan mainly from early April to late August, a period in which about 95% of the annual counts were registered, the highest levels being detected in June and July.

Based on the data described by other authors, the levels of Cannabis pollen registered could be clinically important for sensitive patients. The data obtained are a good indicator of the cultivation activity of this species and should be taken into account by the state authorities since they constitute a strong evidence of the existence of Cannabis crops in the region of Tetouan.

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