ORIGINAL ARTICLE

Soy Aeroallergens in Thoracic Fraction Particles (PM_{10})

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■ **Abstract**

Background: The aerodynamic diameter of biological particles determines their ability to penetrate the human respiratory system.
Objective: To assess the content of allergens less than 10 µm in diameter in the particle fraction of airborne dust in order to improve control of exposure to harmful soybean aeroallergens.
Methods: In this study, 98 pairs of particulate matter measuring less than 10 µm in diameter (PM_{10}) and total suspended particulate (TSP) filters were collected in parallel and analyzed for soy aeroallergens by the inhibition enzyme-linked immunosorbent assay.
Results: The median levels found were 6 and 22.5 U/m^3 for PM_{10} and TSP filters, respectively. A good correlation was found between soy aeroallergen content in PM_{10} and TSP filters. The median proportion of soy aeroallergen content in PM_{10} filters versus TSP filters was 28.6%, and varied widely across different days.
Conclusions: Due to this wide variation between days, it seems that soy aeroallergen content in TSP filters is not a good surrogate of soy allergen content in PM_{10} filters. Further clinical studies should be conducted to assess differences in the health impact of soy allergen content in PM_{10} filters and TSP filters.

Key words: Air monitoring. Exposure assessment. Particle size. Asthma due to soy.

■ **Resumen**

Introducción: El diámetro aerodinámico de las partículas biológicas determina su habilidad para penetrar en el aparato respiratorio.
Objetivo: Determinar el contenido de alérgenos de diámetro inferior a 10 µm en la materia particulada del aire, con el fin de mejorar el control de la exposición a los aeroalérgenos nocivos de la soja.
Métodos: En este estudio, se muestrearon en paralelo 98 pares de filtros, siendo uno de materia particulada de diámetro <10 µm (PM_{10}) y el otro de partículas suspendidas totales (PST). Mediante un método de ELISA de inhibición se analizó la concentración de aeroalérgenos de soja en todos los filtros.
Resultados: La mediana de los niveles encontrados fue de 6 y 22,5 U/m^3 para los filtros PM_{10} y PST, respectivamente. Se encontró una buena correlación entre el contenido de aeroalérgenos de soja en los filtros PM_{10} y PST. La proporción mediana de aeroalérgenos de soja en los filtros PM_{10} frente a PST fue de 28,6% y varió ampliamente entre los diferentes días.
Conclusiones: Debido a la amplia variación de la proporción de aeroalérgenos de soja en los filtros PM_{10} frente a PST entre días, parece que el contenido de alérgeno de soja en los filtros PST no es un buen indicador del contenido de dichos alérgenos en los filtros PM_{10}. Por ello, deben ser realizados más ensayos clínicos con el fin de evaluar si el contenido de alérgenos de soja en los filtros PM_{10} y PST tienen diferente impacto sobre la salud.

Palabras clave: Control ambiental. Evaluación de la exposición. Tamaño de partícula. Asma por soja.

Introduction

Asthma outbreaks due to soybean dust inhalation have been described in several cities around the world [1-3]. In the city of Barcelona, Spain, a new scheme was recently adopted to control the emission and dispersion of soybean dust into the atmosphere during unloading operations [4,5]. This scheme includes the daily monitoring of soy aeroallergen levels in total suspended particulate (TSP) filters.

The aerodynamic diameter of biological particles determines the ability of these particles to penetrate the human respiratory system, and thus may have an impact on their toxicity or effects on health [6]. Larger particles may deposit in the upper airways (nose, throat), but particles with an aerodynamic diameter of less than 10 μm, which are often referred to as PM_{10} or the thoracic fraction, can penetrate into the thoracic part of the airways, where they may cause health problems [7]. Hence, to improve the control of exposure to harmful soybean allergens it is important to assess the content of allergens with a diameter smaller than 10 μm (PM_{10}) in the particle fraction of airborne dust.

PM_{10} particles may also act as carriers of allergens into the airways [8]. Gly m 1, an acidic glycosylated allergen, is the main allergen responsible for soy asthma epidemics [9]. It has been hypothesized that the binding of a protein allergen to diesel exhaust particles is influenced by the charge of the allergen and/or glycosylation [8,10]. Thus, Gly m 1 seems to be a perfect candidate to bind to particulate matter and as a consequence increase its allergenicity.

It is well known that emission activities and meteorological conditions play an important role in airborne pollutant concentrations [11]. Weather conditions such as rain, humidity, wind speed and direction, temperature, and amount of sunshine may have both direct and indirect effects on bioaerosols [12]. The aims of the present study were to determine the load of soy allergens in thoracic particles in the air near soy-related plants at the Port of Barcelona, compare this with soy aeroallergen load in TSP filters, and investigate the correlation of those levels with emission activities (unloading of soy at the port) and meteorological conditions.

Material and Methods

Air sampling was performed in Barcelona from 17 April to 1 August 2009 (107 days). It was conducted in parallel over a 24-hour period, with 2 samplers located at the port, approximately 800 m to the north of the soy processing station closest to the city and 500 m to the south of the nearest dwellings [5]. One sampler was fitted with TSP filters and the other with PM_{10} filters. Only days on which TSP and PM_{10} filters were available were included in the analysis.

The sampler used routinely for the daily monitoring of soy aeroallergens in Barcelona was used to collect the TSP filters [5,13]. This is a large-volume automated air sampler (CAV-A/HF, MCV, SA) loaded with glass microfiber filters with a pore size of 1 μm, (Whatman International, Ltd.) working at a flow rate of 55 m^3/h.

PM_{10} samples were collected using the CAV-A (MCV) sampler equipped with the head PM1025CAV (MCV) working at a flow rate of 30 m^3/h. Glass microfiber filters with a 15-cm diameter, grade GF/A (Whatman), were used for sample collection.

Proteins were extracted from the PM_{10} and TSP filters into 2 mL and 5 mL of elution buffer (phosphate buffered saline 0.2%, bovine serum albumin 0.1%, Tween-20), respectively, following a method described elsewhere [13]. Soy aeroallergens were quantified using the inhibition enzyme-linked immunosorbent assay [13] with an undiluted pool of sera from soy-allergic patients as detection antibody. To decide which values were valid and which were not in each plate, the plate-individual limit of detection (LOD), defined as half of the extrapolated concentration of the least concentrate standard curve point, was calculated. A LOD of 300 U/mL was assigned to samples with undetectable levels, as this figure has been reported in previous studies [13] and is currently used for the daily monitoring of soy aeroallergen levels in the city of Barcelona. The lower LOD for a typical 24-hour air sample of 150 m^3 was considered to be 10 U/m^3 for TSP filters and 4 U/m^3 for PM_{10} filters.

The data acquisition system used to obtain daily meteorological data was composed of a Campbell Scientific CR1000 data-logger equipped with a variety of sensors to determine the following variables: average and maximum wind speed, wind direction, mean temperature, maximum relative humidity, pressure, rainfall, and direct solar radiation.

All statistical analyses were performed using GraphPad Prism version 4.01 for Windows (GraphPad Software), except for the regression model. Samples with undetectable levels were assigned the lower LOD value. The percentage of detectable samples, and the median and range of soy aeroallergen levels in TSP and PM_{10} filters were calculated. The percentage of soy load in PM_{10} filters with respect to TSP filters was calculated and plotted against the geometric mean. The correlations between airborne soy allergen levels in TSP and PM_{10} filters, as well as their relationship with meteorological parameters, were analyzed using the Spearman rank correlation coefficient (r_s). The effect of soy unloading at the port and soy aeroallergen levels were analyzed with the Mann Whitney U test. A regression model was built by forward selection of meteorological parameters and unloading data to explore the functional relationship between these parameters and soy aeroallergen levels from the 2 filters. The regression model was constructed using STATA software.

Results

A total of 196 valid samples were collected, forming 98 pairs of valid TSP and PM_{10} samples, and analyzed for soybean allergens. The median levels were 6 (range, 4-80) and 22.5 (range, 10-400) U/m^3 for PM_{10} and TSP filters, respectively (Table 1). Within those samples, detectable levels were found in 72 (73.5%) of the PM_{10} filters and in 80 (81.6%) of the TSP filters (Table 1). The mean LOD was 316.61 U/mL, with levels ranging from 295.4 to 401.01 U/mL. These limits were used to calculate the detection limit in U/m^3 for the TSP and

Table 1. Descriptive Statistics for Soy Aeroallergen Levels in Total Suspended Particulate (TSP) and PM_{10} Filters for all Samples and for Samples With Detectable Levels[a]

	All Samples					Detectable Samples				
Filter	No.	Min	Max	Median	IQR	No.	Min	Max	Median	IQR
PM_{10}	98	4	80	6	4-11	72	4	80	8	6-13
TSP	98	10	400	22.5	13-52	80	10	400	27	19-56.5

Abbreviations: IQR, interquartile range; Max, maximum; Min, minimum, PM_{10}, particles with a diameter <10 μm.
[a]Nondetectable samples were assigned the value of the lower limit of detection.

PM_{10} filters using a mean sampled volume of 150m^3. Mean calculated detection limits were 10.55 U/m^3 (range, 9.85-13.37 U/m^3) for TSP filters and 4.22 U/m^3 (range, 3.94-5.35 U/m^3) for PM_{10} filters.

The temporal variation in soy allergen concentrations in TSP and PM_{10} filters is shown in Figure 1. The variation in soy aeroallergen levels during the study period was similar in the 2 filters (Figure 1), with parallel levels found in both filters on most days.

Analysis by Spearman rank correlation of soybean aeroallergen levels for pairwise filters calculated for all samples (n=98) and for samples with detectable levels for both filters (n=70) revealed a high correlation (r_s=0.769, *P*<.0001 and r_s=0.748, *P*<.0001 respectively). The median value of soy aeroallergen levels in PM_{10} filters was around 28.6% of TSP filter levels, although this percentage varied widely (6.8%-100%, Figure 2A). When only detectable samples were analyzed, the median value was 27.1% (range, 6.8%-100%, Figure 2B). In both cases, greater dispersion was seen for lower levels of soy aeroallergen.

Soy unloading operations at the port during the day were used as a surrogate of higher soy aeroallergen emissions. The difference in the medians of soy aeroallergen levels on days with and without unloading was not statistically significant (Figure 3). However, high levels of soy aeroallergens were only recorded on unloading days (Figure 3). High levels for TSP filters were considered when levels were above the empirical threshold value of 160 U/m^3, which is used for the daily surveillance of soy aeroallergens levels in the city of Barcelona [5]. For PM_{10} filters, the threshold value was roughly estimated to be 46 U/m^3, which is 29% of the TSP threshold value.

Median values and interquartile ranges of meteorological parameters are shown in Table 2. The correlation coefficients between average daily soy allergen concentrations in the PM_{10} or TSP fraction and average daily meteorological parameters

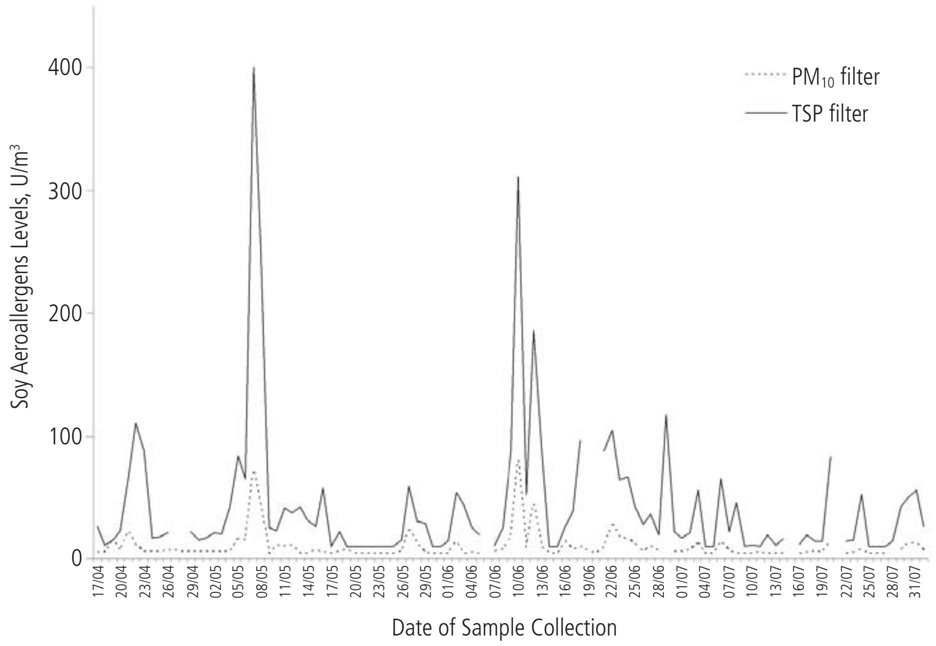

Figure 1. Time-course plot of soy aeroallergen levels in TSP and PM_{10} filters. TSP indicates total suspended particulate; PM_{10}, particles with a diameter <10 μm.

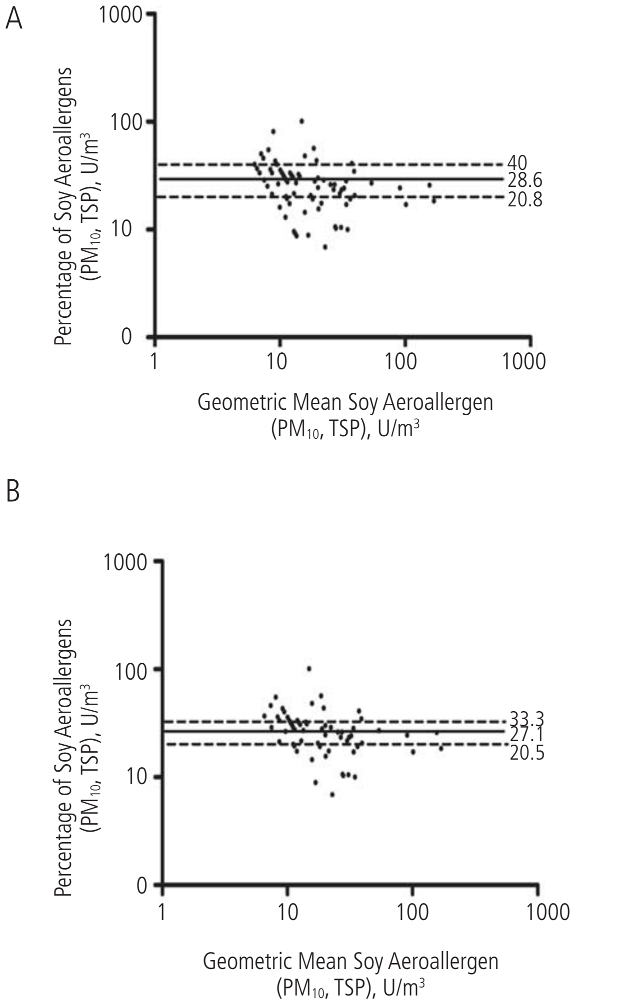

Figure 2. Relative difference (ratio) in percentage plotted against the geometric mean of soy aeroallergen levels analyzed in TSP and PM_{10} filters. A, All samples. B, Only samples with detectable levels of soy aeroallergens in both filters. The solid line represents the median value of the PM_{10}/TSP ratio, and the dashed lines represent the 25th and 75th percentiles. TSP indicates total suspended particulate; PM_{10}, particles with a diameter <10 µm.

are also shown in Table 2. No analysis was performed for rainfall, as it only rained on 8 days out of 98 (8.2%). No correlation was found for wind speed or temperature. However, wind direction, maximum relative humidity, and solar radiation were correlated with soy aeroallergen concentrations in both fractions. Wind direction also correlated with the ratio of soy load in PM_{10} with respect to TSP (r= -0.339, *P*=.001). Conversely, when the association between soy aeroallergen levels, meteorological parameters, and soy unloading data was analyzed using a regression model, only average wind speed and soy unloading explained part of the variance (albeit only 15%) in soy aeroallergen levels in TSP filters. In PM_{10} filters, 13% of the variance was explained by average wind speed and pressure (Table 3). Finally, maximum wind speed explained only 9% of the variance in PM_{10}/TSP levels (Table 3).

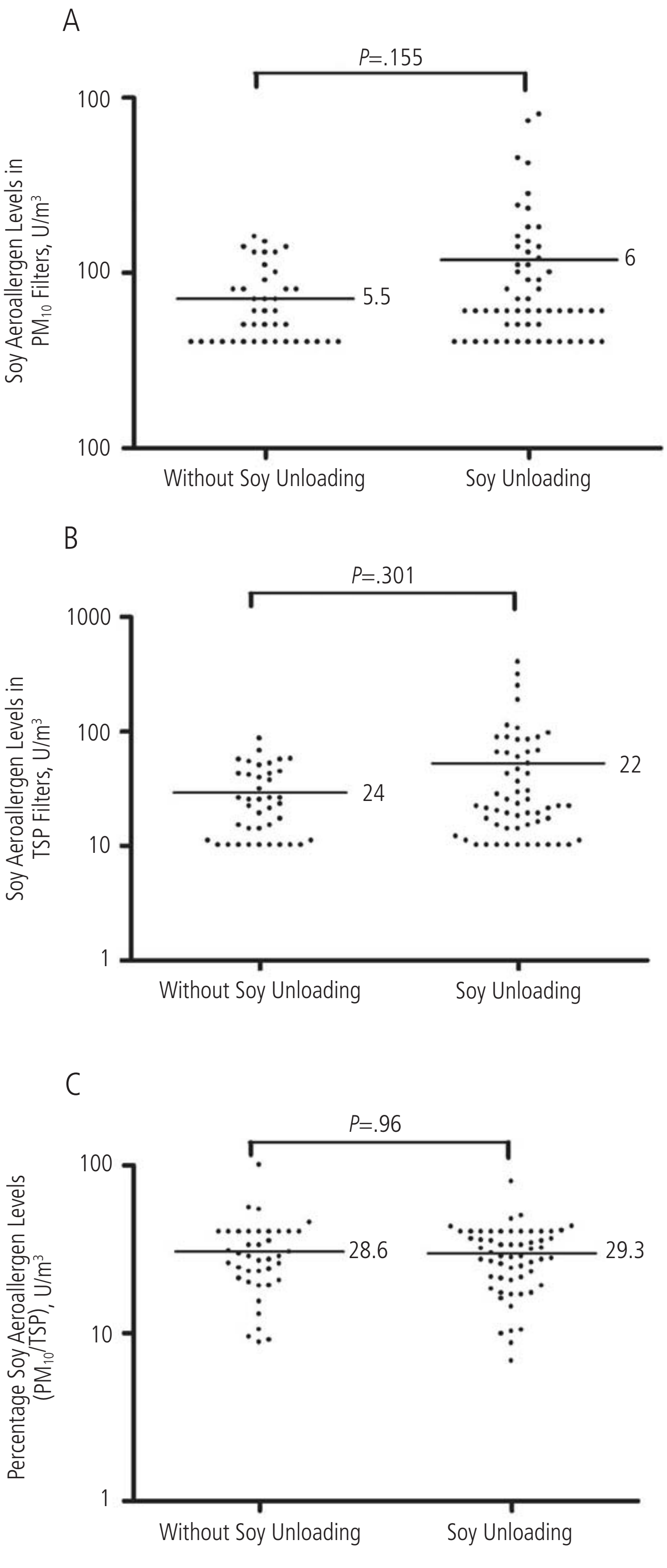

Figure 3. Soy aeroallergen levels on days when soy was unloaded compared with days without unloading. A, PM_{10} filters. B, TSP filters. C, PM_{10}/TSP ratio (percentages). TSP indicates total suspended particulate; PM_{10} particles with a diameter <10 µm.

Table 2. Descriptive Statistics for Meteorological Variables and Correlation With Soy Aeroallergen Levels in Total Suspended Particulate (TSP) and PM_{10} Filters, and Percentage Ratio of Soy Aeroallergen levels in PM_{10} Filters to Soy Aeroallergen Levels in TSP Filters

Meteorological Variables	25th Percentile	Median	75th Percentile	TSP		PM_{10}		%PM_{10}/TSP	
				rs	*P* Value	rs	*P* Value	rs	*P* Value
Average wind speed, m/s	2.77	3.51	3.96	0.110	.282	0.079	.438	–0.053	.607
Maximum wind speed, m/s	3.84	4.86	5.60	0.152	.135	0.090	.380	–0.098	.338
Wind direction, °	123.5	189.5	222.5	0.503	<.0001[a]	0.419	<.0001[a]	–0.339	.001[a]
Temperature, ºC	18.2	21.7	24.1	–0.032	.754	–0.056	.582	–0.042	.681
Maximum relative humidity, %	83.13	89.58	94.59	–0.345	.001[a]	–0.382	.0001[a]	0.127	.214
Pressure, hPa	1012	1015	1018	0.168	.097	0.279	.005[a]	0.021	.841
Direct solar radiation, W/m^2	246.8	304.5	324.3	0.226	.025[a]	0.260	.010[a]	–0.142	.164

Abbreviation: PM_{10}, particles with a diameter <10 µm.
[a]Statistically significant with a value of $P \leq .05$.

Table 3. Linear Regression Models to Study the Relationship Between Soy Aeroallergen Levels in Total Suspended Particulate (TSP) and PM_{10} Filters and Percentage of PM_{10}/TSP and Meteorological and Soy Unloading Data[a]

Independent Variables	Soy Aeroallergen Levels in TSP Filters (R^2=.1528; P=.0018; n=79)			Independent Variables	Soy Aeroallergen Levels in PM_{10} Filters (R^2=.1344; P=.0036; n=81)			Independent Variables	% PM_{10}/TSP (R2=0.0953; P=.0063; n=77)		
	Coefficient	*t*	*P* Value		Coefficient	*t*	*P* Value		Coefficient	*t*	*P* Value
Average wind speed, m/s	25.820	2.670	.009	Average wind speed, m/s	4.930	2.700	.009	Maximum wind speed, m/s	–4.230	–2.810	.006
Soy unloading	28.450	2.060	.043	Pressure, hPa	0.800	2.350	.021	Intercept	48.510	6.850	.000
Intercept	–50.070	–1.580	.118	Intercept	–818.290	–2.360	.021				

Abbreviation: PM_{10}, particles with a diameter <10 µm.
[a]Column headings indicate the dependent variable.

Discussion

The soy aeroallergen concentrations obtained during the study period showed that, on most days, the soy allergen load in the PM_{10} fraction was lower than but parallel to the load in whole airborne dust. Notwithstanding, a significant proportion (29%) of soy allergens were associated with thoracic particles (<10 µm). However, this proportion varied widely on different days, despite the good correlation found between soy aeroallergen content in PM_{10} and TSP filters.

Regarding the significant proportion of soy allergens found in the PM_{10} fraction, similar results have been reported for indoor allergens. Custovic et al [14], for example, found that a small but significant proportion of airborne Der p 2 (20.6%) was carried on small particles (1.1-4.7 µm) [14]. The same research group also observed that in houses with dogs, a significant proportion (approximately 20%) of airborne Can f 1 was associated with small particles (<4.7 µm) [15] and that around 23% of airborne Fel d 1, the main cat allergen, was carried on small particles (<4.7 µm) [16]. Regarding outdoor allergens, many fungal spores are well within the respirable size range, and pollen allergens have been shown to be present in outdoor air on particles smaller than intact pollen [17]. For instance, Schappi et al [18] recovered birch pollen allergen 1.2 ng Bet v 1/m^3 in particles of less than 7.5 µm.

To our knowledge, soy aeroallergen levels in samples of different particle sizes have not been compared to date; in general, these levels have been assessed using TSP filters in both outdoor air [4,13] and at industrial sites [19]. Health risks associated with airborne particles are influenced by particle size [20]. Measurement of bioaerosols in TSP filters may provide information on human exposure. However, TSP filter analysis embraces allergen-bearing particles of a wide range of sizes. Consequently, it may not be sufficiently specific to relate exposure to health risks, as it would be not known how deep into the respiratory tract the particles penetrate. Thus, size-resolved sampling of the total aerosol may be necessary in order to properly assess the health risks associated with inhalation of airborne particles [20].

In our study, the median proportion of soy allergen carried on particles of less than 10 μm in diameter was 28.6%, but this figure varied greatly between days, with values ranging from 6.8% to 100%, especially on days when soy levels were low. Thus, the proportion of soy allergen that can reach the lower respiratory tract would also vary substantially from one day to another. The reason for the differences in the soy allergen load between PM_{10} and TSP filters between days is unclear. Our correlation results suggest that parameters related to the dissemination of the allergen, such as wind direction, may be involved. A strong correlation between wind direction and the particle size distribution of polycyclic aromatic hydrocarbons (PAH) was observed by Schnelle-Kreis et al [21]. However, those authors also observed that the variability of PAH concentrations detected with 1 wind direction was as high as the variability from one direction to another [21]. In our study, regression analysis showed that only maximum wind speed significantly explained a small part of the variance (9%). One possible explanation for the inverse correlation between maximum wind speed and the percentage of soy aeroallergens on PM_{10}/TSP could be that the high allergen values were detected on days when strong winds were blowing in the direction of the samplers, which would facilitate the transport of larger particles and thus prevent them from depositing before reaching the samplers.

Nevertheless, meteorological factors do not seem to fully account for differences in the ratio of soy allergen content between PM_{10} and TSP filters, even though these differences may play an important role in exposure assessment. In a study of the association between birch antigen particles in different size classes and meteorological conditions, Pehkonen et al [22] showed that it would be difficult to develop methods for predicting airborne antigen concentrations on the basis of meteorological factors. Those authors found that the only size class which clearly reacted to meteorological changes was the largest one (>7.2 μm), which contains intact pollen grains; small size classes, which are allergologically more significant, seem to be relatively independent of meteorological changes [22]. However, Jamriska et al [23] found that in the 15- to 880-nm size range, total particle concentration was dominated by emission (ie, traffic flowrate) and wind velocity and to a lesser extent by temperature and relative humidity, but they also observed that not all particle sizes had the same response to meteorological conditions. For instance, relative humidity appeared to influence particles in the 50- to 150-nm size range, whereas temperature significantly influenced particles in the 15- to 30-nm range.

Discrepancies between soy allergen load in PM_{10} and TSP filters on different days could also be due to limitations of the study. For instance, we did not analyze sampler efficacy, and it is possible that this was modified during outfitting with a size-selective inlet, as might have had occurred with the sampler used to collect the PM_{10} filters.

Conclusions

In this study the median proportion of soy aeroallergen content in PM_{10} filters was 28.6%. This proportion varied widely between days, despite the good correlation found between soy aeroallergen content in PM_{10} and TSP filters. What is more, differences in the ratio of soy allergen content between PM_{10} and TSP filters could not be fully explained by meteorological factors. Thus, soy aeroallergen content in TSP filters is not a good surrogate of soy allergen content in PM_{10} filters. However, further studies are needed to establish the size distribution of soy aeroallergens and to determine the source of the variation in this distribution on different days. Finally, clinical studies should be conducted to assess differences in the health impact of soy allergen content in PM_{10} filters and TSP.

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