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Static and elevated pollen traps do not provide an accurate assessment of personal pollen exposure

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Summary
Background. *Volumetric pollen traps are commonly used to assess pollen exposure. These traps are well suited for estimating the regional mean airborne pollen concentration but are likely not to provide an accurate index of personal exposure. In this study, we tested the hypothesis that hair sampling may provide different pollen counts from those from pollen traps, especially when the pollen exposure is diverse.* ***Methods.*** *We compared pollen counts in hair washes to counts provided by stationary volumetric and gravimetric pollen traps in 2 different settings: urban with volunteers living in short distance from one another and from the static trap and suburban in which volunteers live in a scattered environment, quite far from the static trap.* ***Results.*** *Pollen counts in hair washes are in full agreement with trap counts for uniform pollen exposure. In contrast, for diverse pollen exposure, .individual pollen counts in hair washes vary strongly in quantity and taxa composition between individuals and dates. These results demonstrate that the pollen counts method (hair washes vs. stationary pollen traps) may lead to different absolute and relative contributions of taxa to the total pollen count.* ***Conclusions.*** *In a geographic area with a high diversity of environmental exposure to pollen, static pollen traps, in contrast to hair washes, do not provide a reliable estimate of this higher diversity.*

Introduction

Seasonal allergic rhinitis is a widespread disease that occurs around the world. According to International Study of Asthma and Allergies in Childhood (ISAAC), the 12-month prevalence of this disease ranges from 10 to 20% in developed countries (1). At a population level, exposure to types of pollen is measured by volumetric (2) pollen static traps. However, this technique is not accurate for personal measurements, because elevated static trap only provides a rough estimate of exposure over a large geographical area (3). Static traps are usually set up on the tops of buildings and are unlikely to sample the heaviest pollens, which remain close to the ground level. To address this issue, static gravimetric traps (4) have been developed that provide a better sampling of local pollens if they are located close to the ground. However, these traps are stationary and cannot account for the diversity of environmental exposure encountered by a given individual across time and locations. Because hair is a natural filter that is close to the respiratory tract, it has the potential to provide a much better estimate of personal exposure. Hair has already been used in the field of forensic medicine (5) to investigate murders and generate hypotheses about the places a victim had visited. It has also been used in other fields of environmental toxicology to assess cumulative exposure (6). In this study, we compared the results of the "Pollen Counts in Hair Washes" (PCHW) method to the counts provided by stationary volumetric and gravimetric pollen traps in 2 different settings (a large city and a suburban environment). The objective of the study was to know whether a static pollen trap can provide a reliable estimate of personal pollen exposure. The absolute quantities of pollen counts and the relative quantities of pollen from specific taxa were analyzed.

Methods

Study group

Twenty volunteers working in a hospital setting were selected from 2 urban and suburban areas with contrasting environmental conditions in southern France. They were 13 females and 7 males, 15 to 58 years old with a mean age of 33 +/- 9 years. They were outdoors for a mean (S.D.) weekly duration of 17.7 +/- 4.2 hours. The first set comprised 11 people living in the same district of the city of Marseille (these individuals were identified M1 to M11). Due to the restricted area (the max distance from their homes to the static traps was less than 1 km), we hypothesized that these individuals experienced similar individual pollen exposures. Furthermore, they all worked at the University hospital, where the Hirst trap is located. They all lived less than 1 km away from the hospital. A second set of 9 people was chosen from the Valence area (identified as V1 to V9) and who lived up to 20 km from the city center; these individuals lived in diverse environmental conditions, and they most likely experienced different pollen exposures (**figure 1**).
All volunteers were asked to wash their hair once a week on 5 occasions, from 4th February to 9th March 2008 in Marseille and from 11th February to 9th March 2008 in Valence. These time periods correspond to the pollination of *Cupressaceae,* the major pollinating taxon in southern France, which accounts for most of the total annual pollen count.

Pollen Counts

Pollen counts in traps
At both sites, atmospheric pollen was counted using the volumetric spore-trap Hirst method (Hirst, 1952). These 2 volumetric spore-traps (located on the tops of buildings) were the 2 permanent pollen traps used in Marseille and Valence, respectively, by the National Aerobiology Monitoring Network (RNSA). Results were expressed as average of 7 daily slides. Another type of static trap was used, namely the Cour trap. The Cour trap is not usually used as a gravimetric trap, but it was used in this study on a horizontal holder, without any suction. This type of receptor is frequently used in agronomy and pollen allergy studies (Katelaria et al; 2004), allowing the sedimentation of pollen through horizontal filter composed of hydrophilic gauze with a 400 cm^2 sampling surface. Cour traps were installed for the study in Marseille and 10 km away from Valence (**figure 1**). They were situated 1.2 meters above the ground in order to trap nearby pollens. Results were expressed as the total pollen caught along a week.

Pollen counts in hair washes
Volunteers were instructed how to wash their hair. It was mentioned that they should employ a sustained and careful massaging of the scalp. Furthermore, hair characteristics were collected and taken into account in the data analysis
Participants were asked not to wash their hair in between the experimental washings. One 1.5-liter bottle is sufficient to wash hair (2 liters for long hair). A plastic mineral water bottle or a well-cleaned soft drink bottle can be used, and tepid water is preferable. A personal preliminary observation has shown that cold tap water does not contain any pollen. The hair was wetted with 0.5 liters before shampooing, and 1 liter was used to completely rinse the hair after shampooing. The same brand of shampoo was used for all hair washes. The entire 1.5 liters was collected in a basin which had been previously cleaned using clear water, poured into the bottle and transported as soon as possible to the laboratory. If immediate transport was impossible, a thymol crystal was added to prevent contamination or microbial growth. The liquid was centrifuged, and the pellet was treated under heat overnight in a 20% potash solution, then for 10 minutes in 30% HCl, then acetolyzed and stained by fuchsine, and the pellet was suspended again in 80% glycerin and analyzed with a microscope on a microscope slide at 400X or 630 X magnification. Pollens were not altered by hair washing as there was no significant pollen fragmentation following the above-mentioned treatment (7).
The results were expressed as the number of pollen grains/m^3 (Burkard trap), the number of pollen grains/cm^2 (Cour trap), or the number of pollen (grains/wash) for each taxon and overall, averaged over the week, as is commonly done by aerobiologic networks. Hair washing occurred once a week, at the same time of day in the late afternoon. Personal hair characteristics of each participant were recorded: length (long vs. short), texture (curly vs. stiff), and thickness (thick vs. fine)

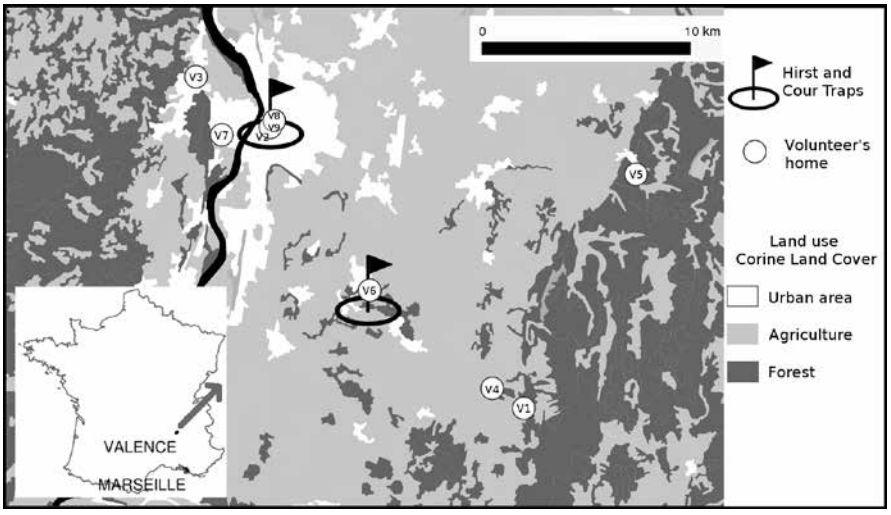
Diaries

Volunteers were asked to record in a diary whether they moved to places where they could get exposed to unusual vegetation

Statistical analysis

Pollen data correspond to averages for an entire week. Week 1 to week 5 refer to an experimental period, not to a calendar date.
At both sites, Pearson correlation coefficients were computed between the PCHW mean values from all volunteers and the pollen counts from the Hirst or Cour traps to estimate the concordance among pollen counts over the whole study period. The distribution of the variables was normal, allowing to use such a statistics.
The variability of PCHW within and among volunteers was graphically illustrated using boxplots.
The effect of "individual" (volunteer) and "date" (week within pollination period) on the prediction of pollen counts on hair washes (PCHW) from the Hirst pollen trap counts was analyzed using the following generalized linear model (8):

Figure 1 *- Location of volunteers' houses and pollen receptors involved in the assessment of individual pollen exposure in Valence vicinity using the "Pollen Counts in Hair Washes" (PCHW) method. Volunteers from the Valence sample experimented heterogeneous environmental conditions, as indicated by land use data from Corine Land Cover. In Marseille all volunteers lived in a restricted urban area.*



Log (PCHW) ~ log (Burkard) + date + individual (I)

Due to overdispersion, the quasi-Poisson distribution was used as the link function.

We estimated the effect of the "pollen count method" (Hirst, Cour or PCHW) and the effect of personal pollen exposure on the relative contribution of *Cupressaceae* to the total pollen counts using the following generalized linear model:

*Cupressaceae Rate ~ trap-type * date + individual (in PCHW)* (II)

Due to overdispersion, we selected the quasi-binomial distribution as the link function.

In Valence, 2 other taxa (*Alnus* and *Fraxinus*) also produce substantial amounts of pollen grains. Here, the pollen frequency patterns were analyzed using a multinomial logit model. The pollen counts from *Cupressaceae*, *Alnus*, *Fraxinus* and the cumulative pollen counts from all other taxa were taken as the 4 categorical variables and analyzed by the following model with *Cupressaceae* as the baseline:

$$\log\left(\frac{\pi_t}{\pi_c}\right) = \beta_{0t} + \text{trap} - \text{type} + \text{date} \quad \text{(III)}$$

where π_t is the probability of collecting pollen from taxon t (excluding *Cupressaceae*) and π_c is the probability of collecting pollen from *Cupressaceae.*

The significance of the effects of trap-type and date were tested using submodels. The differences in the models' deviances were compared to the chi-square distribution.

All statistical analyses were performed using the R statistical software (R Foundation for Statistical Computing, Vienna, 7) with the glm and multinom function from the net package (9). Analysis of mean pollen concentrations according to hair characteristics was performed using an analysis of variance (ANOVA), with 3 controlled variables.

Results

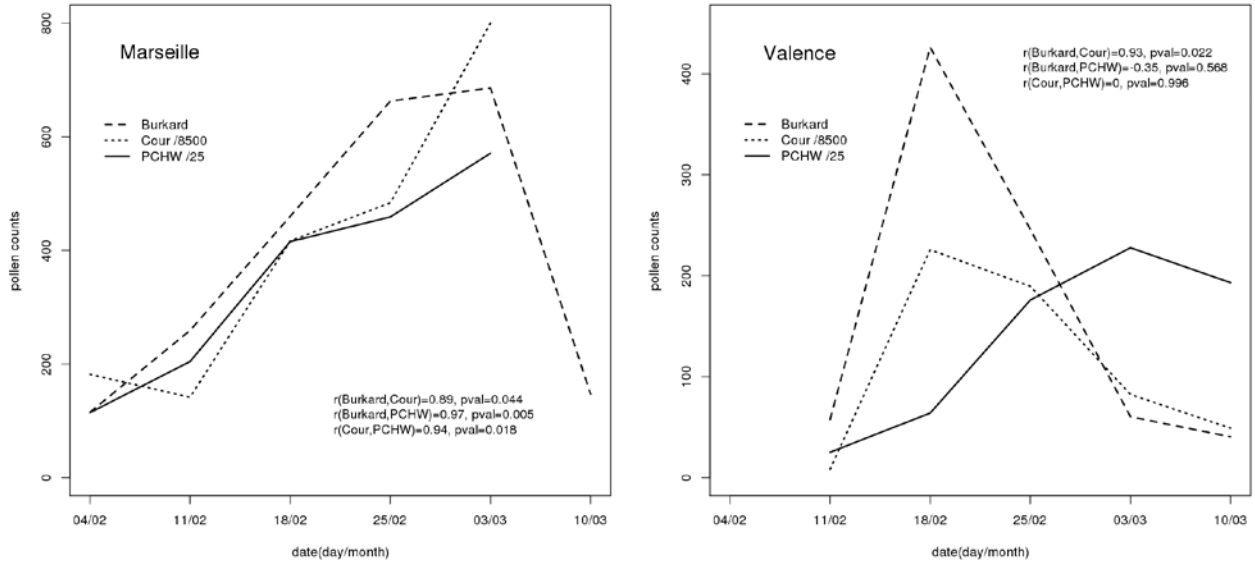
Correlation between pollen trap counts and PCHW

Pollen trap concentrations over the 5-week study period confirmed that this period corresponded to the time of *Cupressaceae* pollination (**figure 2**). In Marseille, *Cupressaceae* pollen counts from the 2 stationary pollen traps (Burkard and Cour) increased weekly from week 1 to week 5, while in Valence the maximum pollination occurred in weeks 3 and 4. At both sites, the 2 stationary traps were in good qualitative accordance with each other. The mean PCHW was in good accordance with the pollen trap assessments in Marseille, but the correlation between these methods was very poor in Valence. The *Cupressaceae* pollen peak derived from the mean PCHW values occurred two weeks after the pollen peaks from the 2 pollen traps.

The individual *Cupressaceae* PCHW exhibited uniform patterns among volunteers in Marseille, but very different trends were observed in Valence. Similar results were observed for the total pollen concentrations from all species. The boxplot of the individual PCHW illustrates the large diversity of pollen exposure amongst volunteers in Valence relative to Marseille (**figure 3**).

In Marseille, the curve displaying individual PCHW for total pollen counts was parallel to the one obtained by Hirst and

Figure 2 *- Cupressaceae pollination dynamics in February and March 2008 in Marseille and Valence, estimated through pollen counts from the volumetric Hirst pollen trap, the gravimetric Cour trap, and the new hair washes method (PCHW).*



Cour traps (**figure 2**) and did not vary significantly according to either the individual or the time (**table 1**).

Diary analysis

In Valence, the PCHW was not related to pollen trap counts, but instead varied significantly between individuals. This individual-based effect is mainly due to the very high pollen counts measured with two volunteers (V5 and V6) at weeks 5 and 6. More than one quarter of these high pollen counts were due to *Buxus* pollen, and the same analysis for *Cupressaceae* pollen counts led to a slightly reduced individual effect (p value = 0.03). Furthermore, hair washes performed by a volunteer who lived in an area heavily contaminated by *Ambrosia* found that those pollens represented

Figure 3 *- Variability of individual pollen counts, including all taxa, in hair (PCHW) in Marseille (white boxes) and Valence (grey boxes), over the entire study period.*

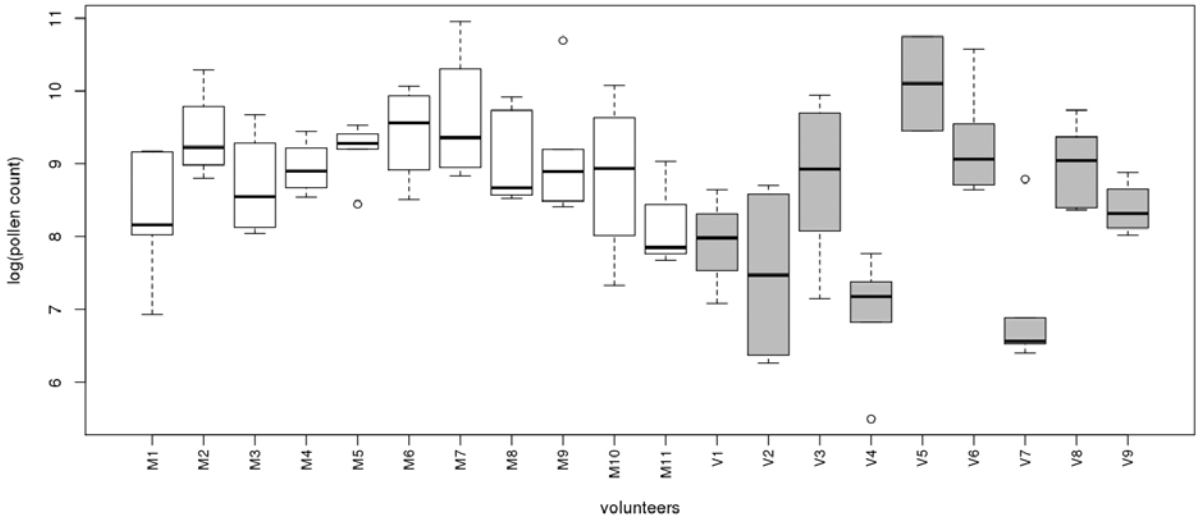


Table 1 *- Analysis of the PCHW values according to Hirst trap counts, individual (volunteer) and date of sampling in Marseille (a) and Valence (b) using the generalized linear model (I). Df: degree of freedom.*

(a)	Df	Deviance	Resid.Df	Resid.Dev	F	Pr(>F)
NULL			46	31892		
log(Hirst)	1	5334.4	45	26558	8.68	0,00607[2]
date	4	1725.0	41	24833	0.70	0.596
Individual	10	7550.2	31	17283	1,23	0.312
(b)						
NULL			38	14453		
log(Hirst)	1	271.8	37	14181	1.14	0.296
date	4	1989.1	33	12192	2.09	0.113
Individual	8	6076.2	25	6116	3.19	0.012[1]

[1]p value < 5%, [2]p value < 1%

43% of total, although the study was done in wintertime. A female participant who lived in the maquis had a level of *Buxus* pollens that was 25.9% of total. For an individual living in an area with many mimosa trees, those pollens represented 12.6% of total in her hair washes. Lastly, an individual who was exposed to lime trees had a 3% content of this pollen, whereas this percentage was less than 0.1% for the other washes from the same individual.

Contribution of Cupressaceae to the total pollen count

Analysis of the proportion of *Cupressaceae* pollen within the total counts confirmed the stronger pollination dynamics in Valence than in Marseille, where the proportion of *Cupressaceae* pollen was consistently high (90% versus 60% in Valence). The variability of the *Cupressaceae* pollen contribution among the count methods appears to be moderate, despite the fact that the proportion of *Cupressaceae* assessed by PCHW in Marseille was less than the proportion determined by the 2 stationary traps. In Valence, the Hirst trap that was installed in the city center produced a lower estimate than those of the Cour trap or the PCHW method.
According to the results obtained using model II, the *Cupressaceae* pollen rate was significantly affected by the date at which hair washes were performed, and the pollen count method at both sites (**table 2**). In Valence, the interaction between these two factors was also highly significant, and was mainly due to the high proportion of *Cupressaceae* pollen collected in week 2 (18 to 25 February) in the 2 stationary traps (79% and 75% in the Hirst and Cour traps, respectively, versus 27 to 47% in PCHW). Removing the week 2 counts produced results that were quite different: the *Cupressaceae* pollen contribution no longer depended on the trap type, and the interaction between the trap type and the date was reduced to a level similar to that observed in Marseille. At both sites, the variability of the *Cupressaceae* pollen contribution among volunteers was low.

Multi-species pollen pattern in Valence

According to the multinomial analysis (model III), the trap type and the date of pollen collection significantly affected the relative quantities of pollen from the different taxa.
When assessed over the 5-week period, the *Alnus* / *Cupressaceae* pollen ratio was 2.15 time higher for PCWH than for Hirst traps. Contrasting results were observed for the *Fraxinus* / *Cupressaceae* pollen ratio (0.39 times lower). Removing week 2 from the multinomial analysis decreased the PCWH *Fraxinus* / *Cupressaceae* pollen ratio to one quarter of the value derived from the Hirst trap. In contrast, for *Alnus* and the other taxa, the ratios obtained with the PCWH and Hirst traps were similar. PCHW indicated a higher proportion of *Alnus* pollen (and pollen from other species) for week 2 only, while the proportion of *Fraxinus* pollen indicated by the Hirst trap was always higher than those of PCWH.

Contribution of hair characteristics

The data in **table 3** demonstrates that, although mean PCHW values were higher in subjects with long and curly hair, the differences were not statistically significant.

Discussion

To the best of our knowledge, this is the first study comparing hair washes to static pollen traps as methods for pollen sampling.

Table 2 *- Analysis of the Cupressaceae pollen contribution to the total pollen counts in Marseille (a) and Valence (b1 and b2): effects of pollen count methods (Hirst, Cour and PCHW), individual and date. GLM (II).*

(a)	Df	Deviance	Resid. Df	Resid. Dev	F	Pr (> F)
NULL			56	7041.2		
trap-type	2	2244.48	54	4796.7	24.04	1.329e-07[3]
date	4	1904.20	50	2892.5	11.47	6.873e-06[3]
trap-type[1] date	8	807.50	42	2085.0	2.43	0.035[1]
individual (in PCHW)	10	894.38	32	1190.6	2.16	0.048[1]
(b1, all weeks)						
NULL			48	6840.5		
trap-type	2	848.17	46	5992.4	16.97	1.924e-05[3]
date	4	2146.90	42	3845.5	21.48	6.307e-08[3]
trap-type[1] date	8	2766.21	34	1079.2	13.84	1.334e-07[3]
individual (in PCHW)	8	403.89	26	675.4	2.02	0.084
(b2, without week from 18 to 25 February)						
NULL			38	3427.5		
trap-type	2	189.08	36	3238.4	3.22	0.063
date	3	1738.47	33	1500.0	19.76	4.595e-06[3]
trap-type[1] date	6	526.52	27	973.4	2.99	0.03[1]
individual (in PCHW)	8	403.45	19	570.0	1.72	0.1581

[1]p value < 5%, [2]p value < 1%, [3]p value < 0.1%

Table 3 *- Mean (+/- S.D.) PCWH according to hair characteristics.*

	Mean	Standard deviation	F	p
Long hair	41,932	45,671		
			1.19	0.19
Short hair	19,822	47,352		
Curly hair	41,993	56,959		
			3.79	0.06
Stiff hair	9,405	11,543		
Thick hair	28,711	15,155		
			0.05	0.95
Fine hair	29,867	14,811		

The limitation of the study was that it did not take into account all anthropogenic variables such as jobs and daily activities.

Pollens are not altered by hair washing. There are indeed other instruments dedicated to evaluate personal pollen exposure, such as nose filters or individual portable air samplers, although they are not convenient for the former and they only allow for a very limited sampling of airflow for the latter.

The data from Marseille showed that the pollen counts from hair washes were highly correlated to the pollen counts of the stationary traps. For individuals who were living in the same neighborhood and who were subjected to similar pollen exposures, the variability in the PCHW results could not be explained by inter-individual variability over the 5-week study period. Rather, this variability was dependent on the relationship between the time of sampling and the local concentration of pollens.

In Valence, participants were selected from a large area that was located 20 km from the city center. The pollen counts from the hair washes exhibited large qualitative and quantitative diversity and, over the 5-week study period, had a non-significant correlation with the pollen counts from the static traps located in the city center. There was a large intra-individual variation due to daily activities, especially walking in a forested area where huge quantities of *Buxus* and *Quercus* pollen can be deposited in the hair.

Thus in Marseille, both static traps and hair washes were representative of exposure whereas, in Valence, static traps did not provide a valid estimate of exposure because volunteers move

and travel back and forth between their homes and the city center. This is so because pollen counts from hair washes include on the one hand pollen of large size, like *Mimosaceae*, which cannot be collected by a static trap positioned on a roof, and on the other hand pollen released in places such as forests or open spaces. Such large-sized pollens are likely to contribute to clinical symptoms, provided that the patient is sensitized to them.

The number of pollens collected by hair washes can indeed be influenced by many uncontrolled factors. This is part of the inter-individual variation which was taken into account in the statistical analyses. Differences obtained under those circumstances were all the more significant as the inter-individual, as well as the intra-individual variations, tend to bias the association towards the null value.

To assess background pollen concentrations, we used Hirst traps and Cour traps. The French National aerobiological network, which provided us the pollen counts, exclusively uses Hirst traps. Rotorod is not used in France. Cour traps located at 1.2 m above the ground were dedicated to assessment of nearby exposure. A gravimetric trap does not by itself provide a better sampling of local pollens, but it does so if it is located close to the ground.

In Marseille, the proportion of *Cupressaceae* pollen in the total pollen count when determined by PCHW was significantly lower than the estimates based on the Hirst trap. This result suggests that a significant portion of the *Cupressaceae* pollen came from distant sources, such as domestic hedges in periurban areas or windbreaks in agricultural areas.

Analysis of the proportion of *Cupressaceae* pollen in Valence revealed unexpectedly high values for week 2 from the two stationary traps. It appears that the large amount of *Cupressaceae* pollen came from regions where this taxon was heavily pollinating. In the south of France, *Cupressaceae* are mostly found in the lower part of the Rhône Valley and in coastal plains. Pollination usually occurs from mid-February to late March and follows south-to-north dynamics that are controlled by daily temperatures. Valence is located in the northern part of the *Cupressaceae* area, and pollination in this area generally occurs 2 to 3 weeks later than in Marseille.

Analysis of the wind directions and intensities that occurred during the 5-week study period revealed that there were 3 days with southerly winds during week 2:2 of these days had moderately intense winds, and one of these days had strong winds. Thus, the high quantity of Cupressaceae pollen came from the southern part of the Rhône Valley and was transported there by the wind.

Interestingly, it should be noted that this peak was observed in the 2 stationary pollen traps but not in PCHW. Consequently, this long-distance pollen transportation was also likely responsible for the low correlation between the pollen counts from the 2 traps and those from PCHW. Additional experiments should be conducted to determine if pollen transported over long distances always contributes little to individual exposures.

According to our results, the pollen trap type may also affect the determination of the relative contributions of pollen from different taxa. In Valence, the differences observed in week 2 were due to the large *Cupressaceae* pollen contribution. The data from the 4 other weeks revealed that the Hirst trap indicated a higher proportion of *Fraxinus* pollen. This result must be confirmed by considering the location of the pollen sources relative to the locations of the pollen traps, as well as the morphological and aerodynamic characteristics of pollen from the different taxa.

In conclusion, our study provides evidence that in a geographical area with a high diversity of environmental exposure to pollen, pollen counts from static traps do not provide a valid estimate of the diversity of pollen exposure. Pollen counting in hair samples cannot currently be recommended as a suitable technique for assessing personal exposure because its precision and reproducibility has not yet been established. The results presented here are of clear interest to practicing allergist as they highlight the limitations of pollen counts provided by static pollen traps, thus underscoring the necessity to design new devices for assessing personal pollen exposure.

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