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Quantification of Birch and Grass Pollen Allergens in Indoor Air

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Abstract Birch and grass pollen grains as well as pollen-derived small particles appear as potent allergens in the outdoor air during spring and summer. The occurrence of pollen allergens in indoor air, however, has not been studied in depth due to lack of suitable sampling and analytical methods. Herein, a recently reported "direct on sampling filter estimation" (DOSAFE) technique (Acevedo et al., 1998) has been validated for quantification of pollen allergens in indoor air using two school rooms and two office rooms as experimental models. Using DOSAFE and polyclonal antibodies against water extracts of pollen from *Betula pendula* and *Phleum pratense L*, we found that indoor air of school and office rooms carried substantial amounts of pollen allergens, expressed as SQ units, predominantly occurring as particles with smaller diameters than the pollen grains. In one school room the indoor air birch pollen allergen concentrations increased from 242 to 403 SQ units/m^3 over the sampling period although the corresponding outdoor air concentrations decreased from 350 to 90 SQ units/m^3. Electrostatic air cleaning in one office room reduced its grass pollen allergen concentrations by more than 95% to 0.02–0.34 SQ units/m^3 as compared to the control room.

Key words Indoor air; Pollen allergens; Air sampling; Small particles; Quantification; Birch; Grass; School rooms; Office rooms.

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Introduction

The protein content of birch (*Betula*) and grass (*Phleum*) pollen grains might be released into the ambient air as antigenic particles with diameters down to 0.1 μm (Spieksma et al., 1990; Spieksma et al., 1991; Rantio et al., 1994; Pehkonen et al., 1994; Yli-Panula, 1997). The degree of dispersion is influenced by different meteorological conditions (Rantio et al., 1994; Schäppi et al., 1997). When in contact with rainwater, the grass pollen grains might rupture with subsequent discharge of their particulate content (Suphioglu et al., 1992). The result of this process is that outdoor and indoor air can carry a complex and heterogeneous mixture of native and empty pollen grains together with small-size pollen-derived protein particles. However, as the sedimentation rates of the different pollen grains and particles vary from meter per seconds to days, weather-dependent fractionation processes can also occur, leading to enrichment of the small particles in the air even in the absence of detectable pollen grains.

As the antigenic birch and grass pollen proteins are potent allergens, it is of virtual importance to analyze and control their concentrations in house rooms. Convincing evidence has accumulated that birch and grass pollen allergens are present in settled dust in urban and rural homes (Yli-Panula, 1997; Yli-Panula et al., 1995).

However, information about the corresponding concentrations of inhaleable allergens in the indoor air are needed, as it is not known how the allergens in settled dust are equilibrated with those in the air (Yli-Panula et al., 1997). Hitherto, quantification of pollen allergens in indoor air has been hampered by the lack of a simple and sensitive method of analysis. Indoor air *Parietaria judaica* allergens have been sampled by means of glass-fiber filters, trapping particles with diameters larger than 3 μm, and analyzed after elution from the filters (Dámato et al., 1996).

Recently, a new method for the direct quantification of airborne pollen allergens has been developed (Acevedo et al., 1998). This "direct on sampling filter estimation" (DOSAFE) technique utilizes porous polytetrafluoroethylene filters which efficiently adsorb the relevant antigenic proteins from an arbitrary volume of air. Subsequent treatment of the filters with specific

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antibodies followed by chemiluminescent reagents allow quantification of the antigens representing the allergens. The purpose of the present study is to verify the usefullness of this convenient method for the determination of the concentration of birch and grass pollen allergens in indoor air in school and office rooms. The results are reported herein.

Experimental Procedure

Material

Water extracts of pollen from birch (*Betula pendula*) and timothy grass (*Phleum pratense L*) for skin prick testing (Soluprick SQ®) each containing 100,000 SQ units/ml (manufacturer's specification) were purchased from ALK A/S (Hørsholm, Denmark) and used as reference allergens for the standard curves. The birch and grass pollen extracts contained 23 µg *Bet v 1* and 25 µg *Phl 5* protein/ml as major allergens, respectively, according to the manufacturer.

Polytetrafluoroethylene (PTFE, Teflon™) filters, 50 mm in diameter with 0.2 µm nominal pore size and transluscent polycarbonatefilter holders with 6.5 mm air inlet diameter were obtained from Sartorius (Göttingen, Germany). All other materials and chemicals were the same as described by Acevedo et al., 1998.

Reciprotor vacuum pumps for air sampling were purchased from Lectrostatic (Skara, Sweden) and an ENLIL 600 ozone-free electrostatic air-cleaner was obtained from Svensk Luftrening AB (Stockholm Sweden). The cleaning capacity was 600 m^3 of air/h. Air-volume metering instruments were of the bellow gas meter type and were calibrated against a Gallus 2000 G 1,6 from Euromekanik AB (Göteborg, Sweden). For sampling of outdoor air allergenes, a device was used which was built up by a polycarbonate filter holder attatched to a brass metal tube which was freely movable on a gas-tight ball bearing. On the other side of the bearing an air outlet was fixed and connected to the vacuum pump. The movable brass tube carrying the filter holder was equipped with a large wing of aluminum sheet which brought the filter holder into a position straight against the wind direction. A ventilated wooden box with the inner sides coated with foamed plastic was used to muffle the noise from each pump used for air-sampling in the school rooms. The residual noise created from the sampling device did not disturb the room occupants. Sampling of pollen and allergens was performed in 1997 in May and June-July for the birch and grass species, respectively.

Methods

The DOSAFE technique was used as described in detail by Acevedo et al., 1998 using a Luminoskan luminometer from Labsystems OY (Helsinki, Finland).

After completed sampling each filter was collected and stored for 6 months in a sealed polystyrene plastic box at −30°C prior to analysis.

Briefly, the Teflon™ sampling filters were treated with isopropanol for fixation of allergens followed by washing steps, reaction with primary antibody specific to the target allergen, treatment with secondary antibody coupled to alkaline phosphatase and finally with reagents for luminescens reaction after relevant washing steps.

For each set of sampling filters to be analyzed, fifteen different dilutions of the reference antigen and a blank were pipetted onto a separate filter at defined positions to produce a standard curve. This was used for evaluation of the amount of pollen antigens on the sampling filters. Values for standards and samples represent single determinations. The standard curve ranged from 0.064 to 200 SQ units and 0.032 to 100 SQ units for the birch and grass pollen major antigens, respectively. The corresponding ranges for the calculated coefficients of variation for the curves produced at the analyses were 0.974 to 0.988 and 0.933 to 0.981. The between-days method precision for these ranges is below 20% (Acevedo et al., 1998). After luminometry the sampling filters were stained using the BCIP/NBT reagents from Bio-Rad laboratories (Hercules, CA, USA).

Quantification of birch and grass pollen grains in outdoor air was performed using a Burkard Seven Day Pollen trap from Burkard Manufacturing Ltd. (Rickmansworth, Hertfordshire, UK (Hirst, 1952)). The pollen counts were kindly made by the Palynological Laboratory at the Swedish Museum of Natural History in Stockholm, Sweden. Meteorological parameters were recorded each day between 6 p.m. and 6 p.m. for 24-h periods which started the day before the pollen allergen sampling day. This was performed by the Swedish Meteorological and Hydrological Institute at Bromma-Stockholm, Sweden (59°35'N and 17°95'E).

Study Design

School Rooms

The study rooms were chosen from a school in a northern suburb of Stockholm, Sweden about 10 km from the city.

Room No. 1

This schoolroom with the dimensions of 7.5×6.0×3.3 m (l×w×h) was occupied during the air sampling by a

teacher and 11 disabled school children, some of whom were sitting in wheelchairs. Each lesson lasted for about 60 min followed by a 10 min break. The room was ventilated through natural ventilation with an exhaust air opening over the entrance door. During the day, the windows of the room were open for short times. Each evening, the floor of the room was cleaned by sweeping it with an oil-moistened mop. The air-sampling filter holder with the Teflon™ filter was placed about 1 m above the floor and close to the wall opposite the windows. The muffled vacuum pump was running from the morning to the end of the school-day. The air-flow rate through the Teflon™ sampling filter was about 11 l per min for 7.5 h.

Room No. 2

This school room had the same dimensions as room No. 1 but was used by 30 normally motorially active children and one teacher. The times for the lessons and breaks were the same as above. This room was mechanically ventilated by controlled intake and waste air. Windows were opened occasionally. Each evening, the floor of the room was cleaned by sweeping it with an oil-moistened mop. The air sampling filter holder with the Teflon™ filter and muffled vacuum pump was positioned as above and run from the morning to the end of the school day. The air-flow rate through the sampling filter was about 12 l per min for 7 h.

Outdoors

The outdoor air-sampling device, with the Teflon™ filter fitted in a holder automatically orientating itself against the wind direction (see Materials section), was placed outdoors on a roof of a building in the close vicinity of the school rooms. The sampler was positioned about 4 m above the ground and was protected from rain. The sampling-rate was about 8 l of air per min for 7 h and the pump was run in parallel with those of room Nos. 1 and 2. After completed sampling the filters were handled and stored as described above. The Burkard trap sampling of the birch pollen grains was performed for 24 h, about 10 m above the ground, 4 km from the study school.

Office rooms

The rooms for this study were selected in the Institute for Working Life, Solna Sweden.

Room No. 3

This office room had the dimensions 3.93×2.72×2.95 m (l×w×h). It was only furnished with a small wooden desk for the handling of sampling filters and study protocol. No person except the experimentalist had access to the room during the air sampling. The normal ventilation of the room was eliminated by closing the intake and waste air openings. The filter holder with the Teflon™ filter was placed about 0.8 m above the floor close to the wall with the window. This was left open for one hour in the early morning prior to air sampling which started after that the window had been closed. The air-flow rate through the sampling filter was about 13 l per min for 7 h.

Room No. 4

This room was adjacent to room No. 3 and had the dimensions 3.93×3.55×2.93 m (l×w×h). The other parameters and experimental conditions for this room were the same as those described for room No. 3 above, but the present room also harboured an ENLIL 600 air-cleaner (see Materials section). The sampling filter holder with the Teflon™ filter was placed 0.8 m above the floor in the outlet air stream from the air-cleaner. The air-flow rate through the sampling filter was about 16 l per min for 8 h.

Outdoors

Parallel with the indoor air sampling of allergens in rooms No. 3 and No. 4 samples of outdoor air grass pollen allergens were collected on Teflon™ filters using the same device as described above for the outdoor air sampling of birch pollen allergens. In addition a Burkard pollen trap placed beneath this device was operated. Both instruments were placed on a roof adjacent to the experimental rooms, about 10 m above the ground on the same level as the rooms. The air inlet of the Burkard trap and that of the movable filter holder of the allergen sampling device both simultaneously orientated themselves against the wind direction at sampling. The air flow through the sampling filter was about 18 l per min for 8 h. The pollen grains collected on the Burkard trap tape were counted for the recorded time period and defined as Burkard local pollen concentration. The Burkard officially reported 24-h grass pollen concentrations were determined 5 km from the place of this study. This trap was positioned on the roof of a building 59°30'N and 18°03'E.

Results and Discussion

Birch Pollen Allergens in the Air of the School Rooms

Outdoors

During the 2-week air-sampling period in May 1997 there was essentially no rain in the sampling region. However, from the second week, May 20th, the recorded daily outdoor minimum temperatures were un-

Table 1 Concentration values for outdoor birch pollen obtained with the Burkard trap and for outdoor and indoor air birch pollenallergens for the school rooms, determined by the DOSAFE technique, related to meteorological conditions in May 1997

Day[1]	Burkard[2]	Outdoors	Room 1	Room 2	Temperature °C[3]			Rel. air humidity %[3]			Precipitation[3,4]
	pollen n/m³	allergen as SQ units/m³×filter			max	min	average	max	min	average	mm
May 13	484	740	59		18.1	5.0	13.0	97	31	69	0.5
May 14	412	439	74		17.7	8.4	13.7	81	38	57	0
May 16	288	35	21		17.5	7.0	12.5	75	28	51	0
May 20	24	44	63	242	8.9	2.2	6.2	78	42	56	0
May 22	45	350	302	267	12.3	1.8	7.3	84	22	49	0
May 23	22	145	60	338	10.7	0.9	6.8	77	24	45	0
May 26	6	90	63	403	13.3	3.8	9.4	89	50	73	0.5

[1] Sampling time ca. 8:00 a.m. to 4:30 p.m.
[2] Estimated average outdoors for a 24-h period
[3] Outdoors 6:00 p.m. to 6:00 p.m. for a 24-h period
[4] The 12th had 1.1 mm

usually low (Table 1). The first days of the first sampling week started with high concentrations of Burkard trap birch pollen, which gradually decreased until the 8th day. The outdoor air allergen concentration followed this pattern but decreased more rapidly as depicted in Fig. 1. From day 8, the outdoor birch pollen grain concentration was low with a peak on May 22nd with a value about 5% of that of the first day, May 13th. The allergen concentration in outdoor air during this week was also lower than that of the first week and also reached its highest concentration on the 22nd to about 30% of that of the first day (Fig. 1).

Indoors

Room No. 1

The indoor air allergen concentrations in room No. 1, harbouring the disabled children, varied from about 5 to 50% of those of the corresponding outdoor air samples during the first four sampling days. On day 8, May 20th, the indoor air allergen concentration was 25% higher than that of the outdoor air. However, the amounts of indoor allergens for the different days were of the same order of magnitude, between about 20 to 70 SQ units per m³ of air (Table 1 and Fig. 2). On day 10, May 22nd, the indoor air allergen concentration was almost as high as that of the outdoor air, 302 and 350 SQ units per m³, respectively, which showed coinciding peaks on that day as discussed above. On the following two days the indoor allergen concentrations decreased to values similar to those of the first days of the sampling period accompanied by a decrease in outdoor air allergen concentration (Table 1 and Fig. 2).

Room No. 2

The indoor allergen concentrations of room No. 2, occupied by the normally motorially active children were determined the second week of the study. This room

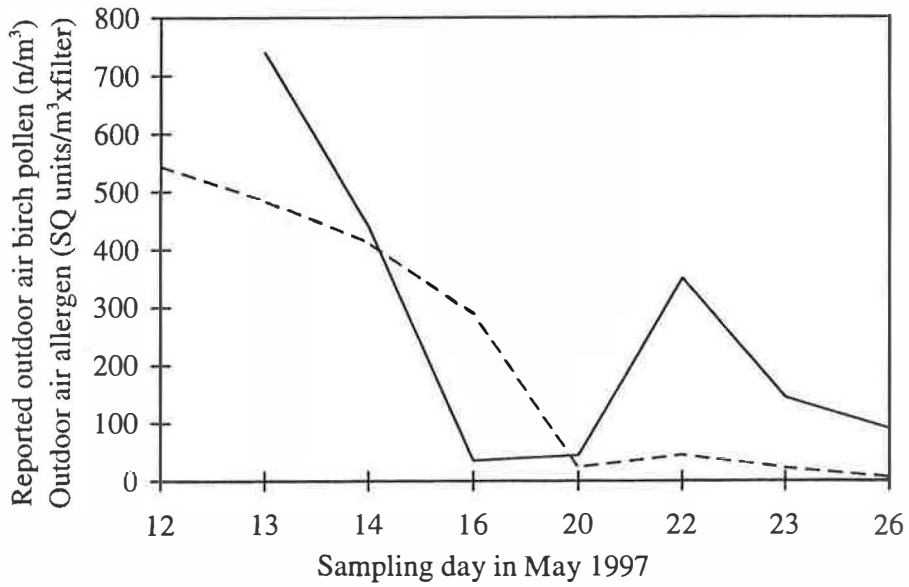

Fig. 1 Concentrations of outdoor air birch pollen obtained with the Burkard trap, reported as average values from 24-h sampling periods (dashed line) and of outdoor air birch pollen allergens determined by the DOSAFE technique (solid line) in May 1997 at the time for sampling of indoor air allergens in the school rooms

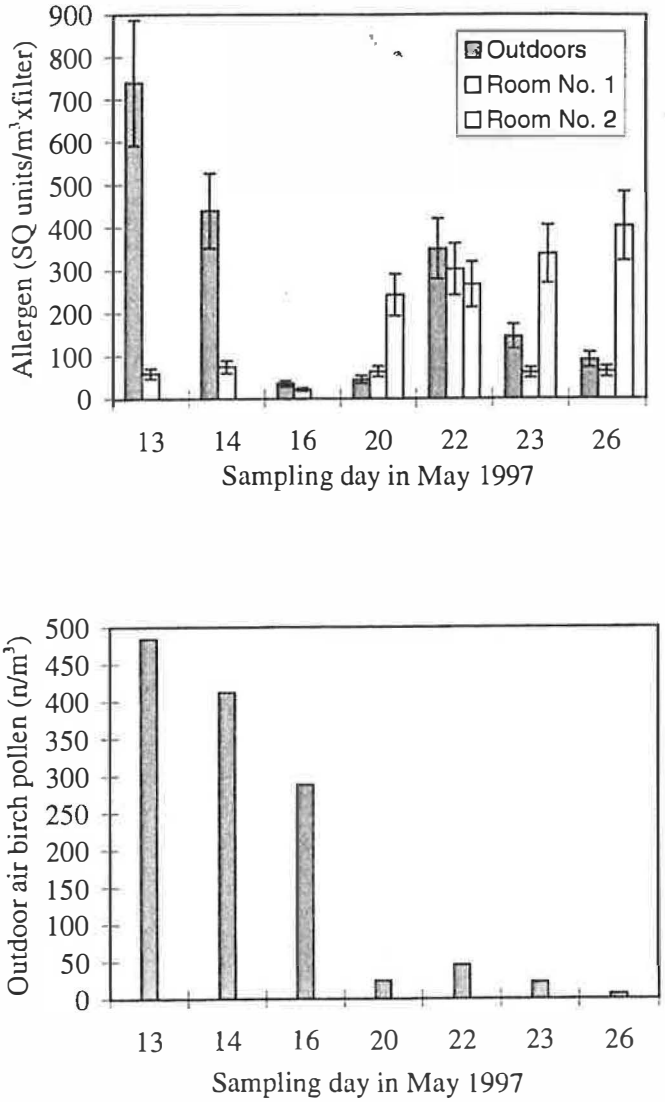

Fig. 2 Bar charts showing the concentrations of outdoor air birch pollen obtained with the Burkard trap, reported as average values from 24-h sampling periods (lower panel) and of outdoor air birch pollen allergens quantified by the DOSAFE technique as well as of corresponding indoor air pollen allergen concentrations (upper panel) using the latter method in the two different school rooms in May 1997

demonstrated high and increasing concentrations of pollen allergens, from about 30 to 50% of the highest value for outdoor air of the first day, May 13th of the sampling period. These figures correspond to an increase of indoor air concentration of pollen allergens from 242 to 403 SQ units per m^3 of air. (Table 1 and Fig. 2). During all days of this week, except day 10, May 22nd, the concentrations of pollen allergens in indoor air of room No. 2 were strongly elevated, about two to five times as compared to the corresponding outdoor air concentrations. On the 22nd, showing a outdoor air allergen concentration peak, the indoor air allergen concentration was about 80% of that of the outdoor air.

Stained filters from samples of indoor air generally showed the presence of a small particulate fraction of pollen allergens as exemplified with filters from May 22nd which showed that the outdoor air filter had the highest fraction of large spots originating from large particles and pollen grains. (Fig. 3). The present results indicate that in the week following the appearance of high concentrations of birch pollen grains in outdoor air, a lasting or increasing allergen concentrations might be manifest in indoor air carrying predominantly small allergenic particles. This is in agreement with previous findings for allergens in settled dust (Yli-Panula, 1997; Yli-Panula et al., 1995). It has been suggested that the allergens might be transported into the indoor environment on clothing, shoes and hair (Ekebom et al., 1996; Pehkonen, et al., 1993). The higher concentrations of pollen allergens in room No. 2 occupied by the motorially active children, as compared to room No. 1, seem thus partly to reflect a more efficient transport and dispersion of allergenic particles into the room No. 2 due to a more active way of life.

The results also demonstrate that the DOSAFE technique is efficient and practicable for quantification of indoor air birch pollen allergens occurring in levels down to a few SQ units per m^3 of air.

Grass pollen allergens in the air of the office rooms.

Outdoors

During the 2-week sampling period in June-July 1997, rain was falling during the first five days. The smallest amounts of precipitation were recorded on day 2, June

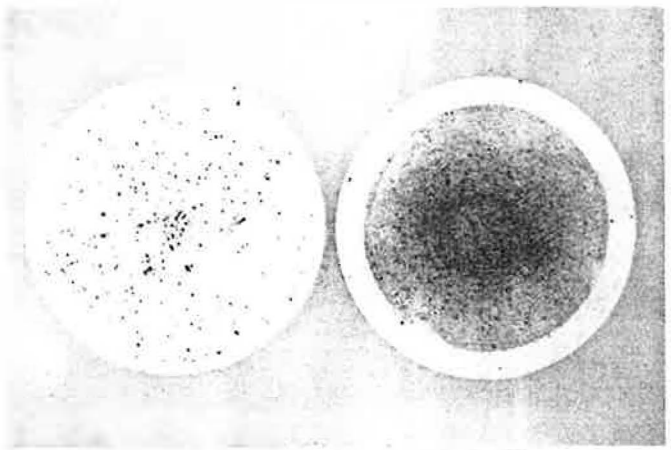

Fig. 3 Photographs of specifically stained Teflon™ sampling filters from analysis of outdoor and indoor air birch pollen allergens by the DOSAFE technique. The samples were those collected on the 22nd of May 1997 at the study of the school rooms. The filters to the left and right represent the outdoor and indoor air samples, respectively. The sampling conditions are as described in the Material and Methods sections. Note the fine-granularly stained area of small allergenic particles between the spots of larger ones on the filter from the indoor air

Table 2 Concentration values for reported and local outdoor air grass pollen obtained with the Burkard trap and for outdoor and indoor air grass pollen allergens determined by the DOSAFE technique for the two different office rooms, related to meteorological conditions in June-July 1997

Day[1]	Burkard pollen n/m^3		Outdoors	Room 3	Room 4[5]	Temperature °C[3]			Rel. air humidity %[3]			Precipitation[3,4]
	local[1]	reported[2]	allergen as AQ units/m^3×filter			max	min	average	max	min	average	mm
June 23	14	24	345	4.3	0.11	19.6	12.2	15.6	94	51	75	13.3
June 24	13	14	111	6.4	0.11	18.6	12.5	15.5	89	61	75	0.5
June 25	4	6	177	3.6	0.34	17.4	11.6	14.2	94	67	80	3
June 26	0	5	233	5.2	0.16	16.7	13.1	14.5	94	65	84	5.7
June 27	15	20	22	0.4	0.02	19.3	8.5	14.6	93	46	65	5.5
June 30	13	38	20	1.2	0.02	25.6	14.2	20.1	92	68	78	0
July 1	11	49	143	4.0	0.08	26.7	18.5	23.0	89	62	75	0
July 2	11	31	115	4.9	0.06	27.0	19.7	23.1	85	49	71	0
July 3	3	18	87	3.3	0.05	23.8	15.4	19.9	85	44	65	0
July 4	4	15	40	6.4	0.04	25.4	17.5	20.9	81	48	64	0

[1] Sampling time ca. 8:00 a.m. to 4:30 p.m.
[2] Estimated average outdoors for a 24-h period
[3] Outdoors 6:00 p.m. to 6:00 p.m. for a 24-h period
[4] The 22nd had 11 mm.
[5] Containing the ENLIL air cleaner

24th, and day 3, June 25th (Table 2 and Fig. 4). The concentration curve for outdoor air Burkard reported grass pollen grains demonstrated one maximum corresponding to medium amounts of pollen on June 23rd followed by a minimum with low amounts on June 26th and a second maximum with high amounts on the 1st of July. The concentration curve for Burkard local grass pollen grains, determined for the daily sampling period, showed a similar profile but with lower values than those of Burkard reported. However, the second peak of the local concentration curve was blunter (Fig. 4) than the corresponding peak of the reported one. By contrast, the concentrations of outdoor air grass pollen allergens followed a curve showing two peaks which were delayed about three days as compared to those produced by the grass pollen grain

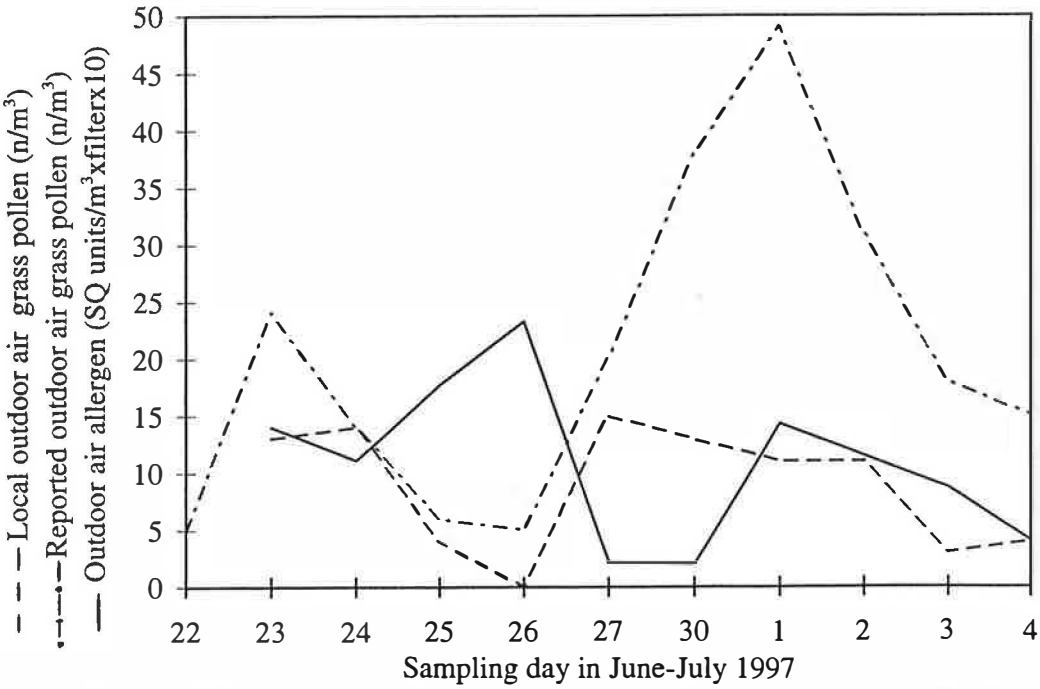

Fig. 4 Concentrations of Burkard officially reported and local outdoor air grass pollen grains and grass pollen allergens as quantified by the DOSAFE technique in June-July 1997 at the time for sampling of indoor air allergens in the office rooms Nos. 3 and 4. The Burkard officially reported and local pollen concentration values were obtained from 24 h and 8 h of sampling, respectively. Note that the appearance of elevated pollen allergen concentrations is delayed about 3 days after the maximum of the pollen grain concentration on June 23rd

concentrations (Fig. 4). It seem reasonable to assume that this lag is the result of the light rainfall preceding the recorded pollen allergen concentration maxima. During rain the pollen grains might be washed down to the ground and when moist, rupture to release allergenic particles. These might then become airborne after periods of dry weather (Suphioglu et al., 1992; Rantio-Lehtimäki, et al., 1994; Schäppi, et al., 1997). Fig. 4 also indicates that the airborne grass pollen allergens seem to decrease during rainy days. On the last four days of the sampling period, which were dry, the grass pollen allergen concentration curve followed those of the pollen grains.

Indoors

Room No. 3

This office room which was ventilated in the morning by keeping a window open for 1 h, showed indoor air pollen allergen concentration values that varied between 0.4 to 6.4 SQ units per m^3 corresponding to about 2 to 16% of the corresponding concentrations of the outdoor air at the time of sampling (Table 2).

Room No. 4

This office room, containing the electrostatic air cleaner, was also ventilated for 1 h in parallel with room No. 3 by keeping a window open. Very low concentrations of indoor air grass pollen allergens in the range of 0.02 to 0.34 SQ units could be recorded indicating an efficient air-cleaning process (Table 2). As compared to the room without air cleaner only on average about 2% pollen allergen remained in the indoor air in the room with the cleaner.

Thus, the allergenic activity in SQ units in the indoor air is directly related to particles which can be eliminated by a suitable air-cleaner. Staining of filters showed that small particles predominantly were present in indoor air samples, appearing as very small, nearly not perceivable spots (Fig. 3). Stained sampling filters from the room with the air cleaner demonstrated a completely white background between a few almost invisible small stained spots (not shown here). The results demonstrate that the DOSAFE technique for quantification of indoor air grass pollen allergen particles can determine extremely low concentration levels without interference from the background. This technique thus represents a simple and convenient means for monitoring the quality of indoor air in respect of occurrence of pollen allergen particles.

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