

Quantification of group 5 grass pollen allergens in house dust

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Summary

Background It is widely known and accepted that grass pollen is a major outdoor cause of hay fever. However, it is of virtual importance for grass pollen allergic patients with symptoms all the year round to know the concentration of grass pollen allergens in their homes.

Objective The main objective of this study was to quantify the amount of grass pollen allergen in mass units ($\mu\text{g Phl p 5}$) in dust settled indoors and to detect the distribution of allergenic activity in different sampling locations of homes. Furthermore, we studied the seasonal fluctuation of allergen content in dust samples.

Methods We adapted the two site binding assay for detection of group 5 grass pollen allergens in samples from randomly selected homes in Hamburg ($n = 371$), Erfurt ($n = 396$), Hettstedt ($n = 353$), Zerbst ($n = 289$) and Bitterfeld ($n = 226$), Germany. Dust samples were collected from floor of living room (LR), bedroom (BR) or children's room (CR) and mattress (MA) during period of June 1995 to August 1998. The amount of the major grass group 5 allergens was detected in $\mu\text{g/g}$ dust.

Results Phl p 5 was detected in 67% of the samples analysed ($n = 4760$). The range was between undetectable ($< 0.03 \mu\text{g/g}$ dust) and $81 \mu\text{g/g}$ dust. Phl p 5 levels were significantly higher in the dust from LR (geometric mean $0.117 \mu\text{g/g}$ dust) or BR/CR floors (geometric mean $0.098 \mu\text{g/g}$ dust) than in mattresses (geometric mean $0.043 \mu\text{g/g}$ dust). We observed seasonal fluctuation of indoor Phl p 5 levels with peak in June but also annual differences. Thus Phl p 5 content indoors reflects also the different quantities of pollen counts of annual courses. During pollination period we found two times higher Phl p 5 levels ($0.172 \mu\text{g/g}$ dust, $P < 0.001$) than outside of grass pollination season ($0.095 \mu\text{g/g}$ dust). The indoor Phl p 5 levels outside of season seem to be independent of pollination before. We suppose that settled pollen grains or allergenic material from outdoor particles carried indoors via footwear and clothes accumulates in house dust.

Conclusion Although we not known how the allergens in settled dust are equilibrated with those in the air, the considerable high level of Phl p 5 in indoor dust even during periods when no grass pollen is present in the atmosphere may be an important cause of pollen-allergy symptoms outside of season.

Keywords: grass pollen allergen, quantification, settled dust, indoor allergen exposure, seasonal fluctuation

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Introduction

More than 20% of the population show symptoms of hay

fever or asthma [1]. Symptoms are triggered off grass pollen grains as well as pollen-derived particles appeared as potent allergens in the ambient air during summer [2,3]. Furthermore, some sensitive pollen-allergic patients report on allergenic symptoms not only during the pollen release but also both before and after the pollen period [4,5].

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Table 1. Numbers of homes by urban and rural community and participation at dust sampling

	Selected homes	Dust samples					
		First sampling			Second sampling		
		Living room	Bedroom	Mattress	Living room	Bedroom	Mattress
Hamburg	371	201	176	201	170	153	168
Erfurt	396	204	189	204	192	175	191
Zerbst	289	152	152	152	136	137	137
Bitterfeld	226	119	119	119	107	107	107
Hettstedt	353	183	183	183	170	170	170
Total	1635	859	819	859	775	742	773

Because pollen grains are considered to be a risk factor for grass pollen allergy generally outdoors the allergic subjects are advised to stay indoors during peak pollen periods. However, it is known very little about how much of grass pollen allergens reach the indoor environment and it appears possible that 'indoor pollen' could significantly contribute to allergen exposure. The occurrence of grass pollen allergens in indoor has not been studied in depth owing to lack of suitable sampling and analytical methods.

Indoor exposure to pollen allergens can be assessed by counting the numbers of pollen grains in house dust samples [6] or in air collected samples [7]. However, there are two problems. First, the procedure requires microscopic identification and is consequently slow. Second, the number of pollen is only indirectly related to the total grass pollen allergen level because empty pollen has released allergen molecules into the atmosphere. These allergens can interact with other sources of fine particles, e.g. indoor suspended particulate matter (SPM) [8].

Alternatively, grass pollen allergens can be measured quantitatively with specific immunoassays by means of monoclonal antibodies. Originally these assays were used to standardize grass pollen allergen extracts [9]. The clinical important grass allergens are group 1 recognized by 90–95% and group 5 allergens by 80–90% of all patients sensitive to grass pollen [10,11]. We adapted the two site binding assay of Phl p 5 for detection of group 5 grass pollen allergens in samples of house dust from randomly selected homes in different regions and seasons.

The present investigation thus has the following aims:

- to quantify grass pollen allergen in mass units per gram house dust;
- to study distribution of allergenic activity in different sampling locations of homes;
- to study seasonal differences of indoor levels of pollen allergens.

Material and methods

Homes

From June 1995 to August 1998, 4827 samples of settled dust were collected from 1635 homes of different German regions (Table 1). Hamburg is situated in the north of former West Germany about 150 km from the North Sea and 50 km from the Baltic Sea. Erfurt is located in a shallow basin in the south-west of the former East Germany. Both cities are administrative urban centres. The regions Zerbst, Hettstedt and Bitterfeld are three communities of the state of Sachsen-Anhalt. The homes were selected from population samples participated in the study about the influences of Indoor factors and Genetics in Asthma (INGA) [12]. The homes were visited twice at intervals of about 6 month.

Dust collection and processing

In each house, three dust samples were collected from 1 m² in a standardized manner using the same type of vacuum cleaner (Philips, Typ Flüsterjet Vitall 371, 1000 Watt, Hamburg, Germany) and the same device (collector and filter, ALK, Denmark). In general, the samples were obtained from living room (LR) and bedroom (BR) or children's room (CR) floors and mattresses (MA). The dust filters were weighed before vacuuming and after to analyse the settled dust gravimetrically. The dust filters were kept at –20 °C until they were extracted. The allergens were extracted from the sample dust plus filter paper with 0.125 M NH₄HCO₃ + 0.05% Tween 20 (v/v) for 2 h at RT under constant shaking, with an extraction ratio of 1:10–1:100 (w/v), depending on the amount of sampled dust. The solution was clarified by centrifugation at 1000 g. The extracts were stored frozen at –20 °C in separate aliquots until analysed to avoid repeated freezing and thawing.

Table 2. Comparison of indoor grass pollen allergen Phl p 5 concentrations between mattress dust samples and floor dust samples collected in living room and bedroom from Hamburg ($n = 371$), Erfurt ($n = 396$), Zerbst ($n = 289$), Bitterfeld ($n = 226$), and Hettstedt ($n = 353$)

	Concentration of indoor grass pollen allergen Phl p 5 ($\mu\text{g/g}$ dust)		
	Living room	Bedroom/children's room	Mattress
Valid n	1612	1541	1607
% below detection limit*	21.7	24.4	53.1
25 Percentile	0.056	0.032	<0.03
Median	0.151	0.123	<0.03
75 Percentile	0.275	0.219	0.120
Maximum	81.3	8.1	3.2

* <0.03 $\mu\text{g/g}$ dust.

Allergen analyses

The two site binding assay was performed as described previously [13] using two monoclonal antibodies against group 5 grass allergens. Phl p 5 concentrations were interpolated from the linear part of the standard curve and calculated in $\mu\text{g/g}$ dust. The interassay variation for repeated assays was <10% and the intra-assay variation was approximately <3%. The lower detection limits for Phl p 5 were 3 ng/mL.

Because there is considerable potential variability in the sampling and assay of allergens [14], the dust sampling were carried out by few trained fieldworkers throughout our study, and the dust samples were processed by the same technicians.

Pollen of *Phleum pratense* was purchased from Sächsisches Serumwerk GmbH, Dresden, Germany). The pollen grains were counted by means of a Cell Counter + Analyser System CASY 1 (SCHÄRFE, Reutlingen, Germany). The principle of measurement and evaluation of data was described in [15]. Each sample (5 mg pollen) was diluted 1 : 50 with CASYTON (SCHÄRFE). Every count/mL was automatically calculated from the arithmetic mean of three successive counts (0.4 mL each). The software for data evaluation (CASYSSTAT, SCHÄRFE) offers a fast graphical evaluation of the measurement parameters, for example, as diagrams of pollen grains diameter distributions and volume distributions.

The soluble proteins of 5 mg grass pollen were extracted and the Phl p 5 content quantified in the same way as the dust samples.

The local data for the grass pollen counts in Hamburg, Erfurt and Magdeburg (about 70 km away from the area of Zerbst, Bitterfeld and Hettstedt) were kindly provided by 'Stiftung Deutscher Polleninformationsdienst', Bad Lippspringe, Germany. The aeroallergy information service based on the volumetric sampling of airborne

pollen grains on adhesive tape of the Burkhard Pollen and Spore Trap.

Statistical analysis

Results of 4760 dust samples were available for statistical analyses. The ELISA results were transformed into decimal logarithms and analysed using SPSS (Version 6.12). For all Phl p 5 concentrations less than the detection limit, sample values were estimated by substituting one-half the detection limit (0.015 $\mu\text{g/g}$ dust). The Spearman rank-order correlation was used to test possible association between variables. The limit of significance chosen was 0.05. The levels of allergen at different times of the year were compared by Student's unpaired and paired t -test, respectively.

Results

Content of grass pollen allergen in dust samples

Dust sample weights varied widely from 0.019 (LR) to 11.95 g (MA). Likewise, the physical appearance of the samples varied greatly, ranging from small amounts of fine dust to large quantities of hair, fuzz, and debris. Therefore, we analysed the Phl p 5 content as concentration per unit weight and per area sampled. Highly statistically significant correlations were found between the amounts of grass allergen expressed as $\mu\text{g/g}$ dust and those expressed as $\mu\text{g}/\text{m}^2$ for mattress dust ($r_s = 0.942$, $P < 0.001$) and for floor dust ($r_s = 0.754$, $P < 0.001$).

Phl p 5 was detected in 67% of the samples analysed ($n = 4760$). The concentrations of grass pollen allergen in dust samples demonstrated large variations. The range for Phl p 5 was between undetectable (<0.03 $\mu\text{g/g}$ dust) and 81 $\mu\text{g/g}$ dust. (Table 2).

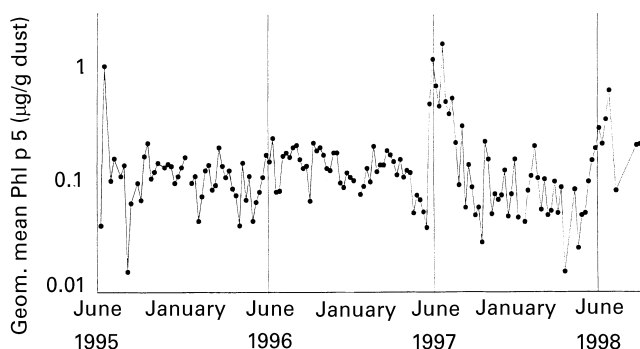


Fig. 1. Seasonal fluctuation of geometric mean grass pollen allergen concentration of Phl p 5 ($\mu\text{g/g}$ dust) analysed weekly from settled floor dust in living room ($n = 1612$).

In 148 of 1635 homes (9%) we were unable to detect grass pollen allergen to any location in just this moment sampling.

Distribution of Phl p 5 for different sampling location

Table 2 summarizes the levels of Phl p 5 for the three different sampling locations, in the living room, bedroom (BR/CR) and mattress. Regarding the grass pollen allergen distributions in the different sampling sites, generally Phl p 5 was more frequent on the floor.

Since in 868 homes the dust was sampled on the children's room floor instead of bedroom floor, we calculated separately the correlation for the living room and bedroom Phl p 5 concentrations or living room and children's room, respectively. Moderate correlations between living room and children's room allergen levels were found

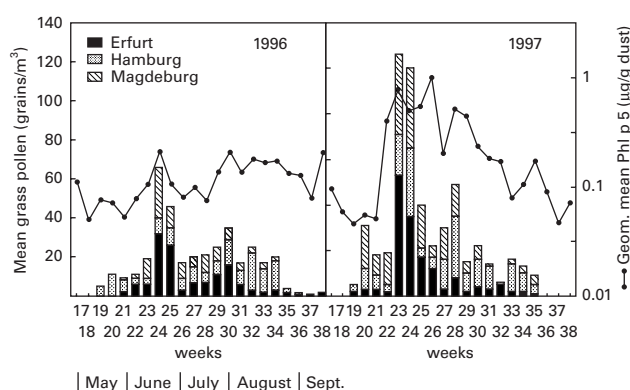


Fig. 2. Geometric mean of Phl p 5 levels ($\mu\text{g/g}$ dust) analysed weekly from indoor settled dust samples of living room and mean weekly airborne grass pollen counts in three regions of Germany during the summer 1996 and 1997. The pollen counts of Hamburg, Erfurt and Magdeburg (about 70 km away from the rural area of Zerbst, Bitterfeld and Hettstedt) were kindly provided by 'Stiftung Deutscher Polleninformationsdienst', Bad Lippspringe, Germany.

($r_s = 0.5274$) and weaker, but also statistically significant between living room and bedroom ($r_s = 0.3523$).

Because 78% of living room samples contained grass pollen allergen, only the living room Phl p 5 concentrations will be considered in the further analysis.

Seasonal differences of grass pollen allergen exposure

The sampling period was spread over four seasons: spring, summer, autumn and winter. Figure 1 encloses the weekly mean levels of indoor grass allergen from June 1995 to August 1998. We observed a seasonal fluctuation of indoor Phl p 5 levels but also annual differences. Peak levels were found in June as expected, but very weakly for 1996. In order to clarify this result, we compared the atmospheric grass pollen data (number of grains per m^3 , data were provided by aeroallergy information service) of three various stations in Germany during pollination period (weeks 17–39) of 1996 and 1997 (Fig. 2). The pollen counts were entered into the analysis as 7 day moving averages. The peak grass pollen period for 1997 (weeks 23 and 24) was exceptionally intensive compared to that for 1996 (weeks 24 and 25). Thus the mean allergenic activity of settled indoor dust reflects these different pollen counts of both years.

More interestingly is the fact that we detected also substantial amounts grass pollen allergen outside of grass pollination season. We defined the weeks 1–16 (January–April) and the weeks 40–52 (October–December) as 'winter' (nonpollination period in Germany). The summer time (grass pollination period) was defined for the months May to September (weeks 17–39). Because the dust sampling in the homes was repeated after 6 months we compared the allergen levels of 267 homes by Student's paired t -test at different times of the year. The mean values of Phl p 5 were about two times higher in summer ($0.172 \mu\text{g/g}$ dust, $P < 0.001$) than in 'winter' ($0.095 \mu\text{g/g}$ dust).

Furthermore, we compared the weekly mean Phl p 5 levels in settled floor dust of complete annual courses 1996 and 1997 with respect to summer and the next 'winter' data (Fig. 3). The indoor Phl p 5 concentration for summer 1996 sampling and the next 'winter' 1996/1997 was very similar with except of the upper and below end of the distribution. In contrast the frequency for summer 1997 shows a clear shift to higher Phl p 5 levels regarding winter 1997/98. Although the grass pollen quantities was different during the pollination season from 1996 to 1997, the Phl p 5 concentration indoors seems to be independent of the amounts of airborne grass pollen grains outdoors at the very least outside of grass pollination season. The mean Phl p 5 concentrations of sampling period winter 1997/98 ($0.072 \mu\text{g/g}$ dust, $n = 230$) were significantly lower as the winter 1996/97 ($0.123 \mu\text{g/g}$ dust, $n = 384$) although the pollen counts were higher in summer 1997 than 1996.

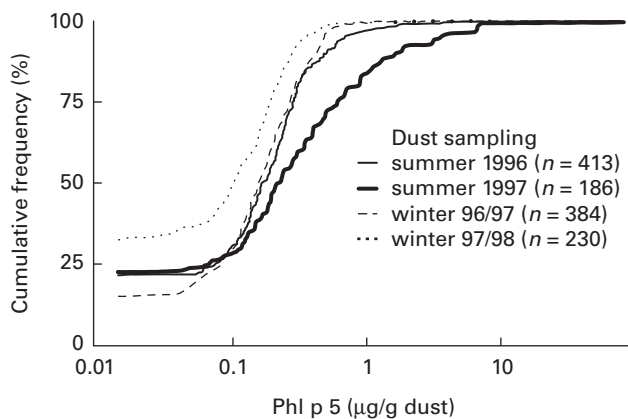


Fig. 3. Distribution of Phl p 5 concentrations ($\mu\text{g/g}$ dust) in settled dust samples of living room during the summer 1996 and 1997 (weeks 17–39) and the next nonpollination period 1996/97 (weeks 40–52 of 1996 and weeks 1–16 of 1997) and 1997/98 (weeks 40–52 of 1997 and weeks 1–16 of 1998).

Comparison of the number of pollen grains and Phl p 5 allergen content

To study the biological significance of Phl p 5 levels in house dust, we have also calculated the allergen content on a per pollen grain basis. We counted 2×10^5 grains in a range of 20–50 μm in diameter in 5 mg of *Phleum pratense* pollen by the Cell Counter + Analyser System CASY 1 and detected in this amount 8 μg Phl p 5. The content of Phl p 5 per pollen grain was consequently 0.04 ng. Schppi *et al.* [2] detected on average 0.05 ng of group 5 allergens per grass pollen grain. Because the lowest detection limit of our assay is 30 ng/g dust, we calculated on the basis of 0.04 ng Phl p 5 per pollen grain that the ELISA can identify the allergen equivalent of about 750 grains. The grass pollen grains in outdoor air (grains/ m^3 , see Fig. 2) reaches only rarely or never this high concentration. This means the indoor concentration of grass pollen allergens is extremely high not only in the pollen season but also outside of grass pollination season.

Discussion

House dust is a mixture consisting of a variety of potent allergens with different levels. The most important indoor allergens as, e.g. of mites, pets and cockroaches can be measured in absolute amounts (ng). To the best of our knowledge similar information concerning concentrations of indoor grass pollen allergen in house dust have not yet been published.

The fact that most people spend most of their time indoors makes the contribution of indoor grass pollen allergen exposure potentially significantly. The main objective of this study was to quantify the amount of grass pollen

allergen in mass units (ng or μg Phl p 5) in dust settled indoors. Although the relationships between dust and air concentration of airborne allergen particles are argued controversially [16,17], in large epidemiological studies, sampling settled dust is much more convenient than expensive air sampling.

Therefore, we adapted the two site binding assay of Phl p 5 [9–13] for a direct determination of grass allergen in extracts of a great number ($n = 4760$) of house dust. The monoclonal antibodies against Phl p 5 used in this study can detect the group 5 of different grass species, since antigenic and allergenic cross-reactivity has been shown between grass pollen allergens of group 5 [18,19]. A further important advantage of this method is, that it allows also quantification of allergen tacking to nonpollen particles, and that it therefore gives more detailed information on indoor exposure than the counts of pollen alone.

The monitoring of grass allergen levels in house dust demonstrated a consistent pattern. The mean values of the floor of living room was the highest, floor of children's room and bedroom was next, and mattresses were the lowest (Table 2). The explanation for larger amounts of grass allergen in the floors than in the mattress is mostly unknown, but socio-economic factors such as cleaning habits, old floors and frequently used rooms may be of importance. Moreover, grass allergen can be brought to the rooms on clothes, shoes etc. The higher correlation between living room and children's room compared with living room and bedroom seems partly to reflect a more efficient transport and dispersion of allergenic particles into the houses with children due to a more active way of life. Thus, one source of indoor grass pollen allergen could be the passive transport of settled outdoor dust [20]. Clothing that was previously hung outdoors to dry is another source.

Additionally grass allergen enter a home through open windows and doors in shape of the pollen itself or/and as allergenic aerosol [21]. The authors compared outdoor pollen levels of *Parietaria judaica* with allergenic activity measured both outdoors and indoors. Little difference was found between indoor and outdoor allergenic activity with the balcony open, but closing the balcony the indoor allergenic activity was then about one-third of that outdoors. We suppose that the level of indoor pollen allergen is dependent on a combination of factors that comprise season, pollution and climate, and characteristics of the home and occupants. But other important factors as ventilation in winter and summer, double glazing or geographical location could influence the allergen level indoors. These factors could explain possibly the lack of grass pollen allergen in 148 homes examined and are studied in further investigations.

The results of this study restrict to the seasonal and annual fluctuation of grass pollen allergen indoors. Other studies

reported of indoor pollen concentrations that can be quite high during the pollen production season [22]. Sterling *et al.* [7] showed that up to 67% of the indoor pollen concentrations can be explained by outdoor pollen concentrations. In contrast Holmquist *et al.* showed that grass pollen allergen carried by particles are present even in the absence of detectable pollen grains [23]. For instance, oak-pollen allergen was detected many months outside the pollen season [24]. Platts-Mills *et al.* [25] reported on a rapid fall in grass allergen that occurred in July and suggested that pollen that has settled on furniture is removed effective by cleaning. In most previous studies only a limited number of days during pollination have been used to collect samples for grass pollen allergen analyses [20–26]. In our study about three years were monitored. Our results show the presence of grass pollen allergen in house dust all the year round. The grass pollen allergen indoors does decrease after the peak pollen period (median 0.186 µg/g dust; maximum 81.3 µg/g dust) on a median of 0.134 µg/g dust and maximum of 4.9 µg/g dust in the ‘winter’ samplings. We suppose that not all sedimented grass pollen were removed after the grass pollen season. Grass pollen antigenic activity stayed high at 20 °C and relative humidities of 23–54% during the whole study period of one year [27].

Pollen counts tend to vary from year to year because of fluctuations in climatic factors [26]. Our results confirm this trend for the Phl p 5 content of different annual courses also indoors but only during pollination season. Sampling periods with high numbers of grass pollen grains outdoors (pollination 1997) were generally characterized by elevated Phl p 5 concentration indoors. It can therefore be postulated that the higher pollination rate 1997 was the crucial factor in the higher antigenic activities obtained in indoor dust. One important finding is the fact that the Phl p 5 content in the ‘winter’ season seems independent of previous grass pollination. For this reason we suggest, simple outdoor pollen counts may not reflect the total airborne allergen exposure [2]. Several studies [8,29] identified airborne grass pollen allergen in the atmosphere in a range of particle size from whole pollen grains (approx. 20–55 µm in diameter) to smaller size fractions <2.5 µm. It is possible that Phl p 5 may diffuse from intact settled pollen grains and the allergenic material may again become airborne, interacting with settled fine particles from other sources prior to resuspension. These particles infiltrate into the homes at any time and accumulate in house dust. Furthermore, it is possible that additional modifications of environmental factors outdoors and indoors might have influenced the allergenicity of pollen [30], but this was not the subject of this investigation.

The results of allergenic activity in homes are difficult to interpret because risk levels for grass allergens in dust capable of inducing symptoms have not yet been well

defined. In our study the median of allergen level reaches more than 0.1 µg/g dust in ‘winter’. These amounts of Phl p 5 are equivalent to about 2500 pollen grains of *Phleum pratense*. Thus the indoor level of grass allergen in one gram of house dust in summer and winter could be produce by several hundred to thousand pollen grains. Concentration of more than 50 grass pollen grains/m³ induced symptoms in pollinosis patients [26–31]. Thus about 2 ng/m³ of grass pollen allergen Phl p 5 (assuming 0.04 ng Phl p 5 per pollen grain) could induce symptoms. Assuming the relationship between the concentration of grass allergen per gram of dust and that per cubic metre of air is about 1000-fold, as described for cat allergen [32,33], we detected in floor dust of 21 living rooms, 19 bedrooms and 4 mattresses more than 2 µg Phl p 5. Whether grass allergen is airborne to the same extent as cat allergen remains to be investigated using an Anderson Cascade impactor.

Holmquist *et al.* [23] found that indoor air of school and office rooms carried substantial amounts (expressed as SQ units) of pollen allergens from birch and grass. There is still a considerable debate whether exposure should be assessed by measuring settled dust from allergen reservoirs or by sampling ambient air. Two different grass allergen-containing compartments exist in the outdoor and indoor environment: the pollen itself and the allergenic aerosol [2,21]. We do not know how high concentrations of antigens found in settled dust may rise into the air under different circumstance. However, the sedimentation is expected to be more important in indoor environment, where air currents are weaker and resuspension is less important than in outdoor environments. Although airborne levels might be more suitable for defining exposure to pollen allergens, levels of Phl p 5 in the living room floor could be used as a surrogate measure of personal exposure indoors in large epidemiological studies, if air samples are difficult to obtain.

Our observations may explain possibly continuing symptoms of grass pollen allergic subjects indoors during periods when little or no grass pollen is present in the atmosphere. One study is worth mentioning in this context [6]. The levels of tree pollen were significantly higher in the homes of tree pollen-allergic children than in the homes of patients without this sensitivity.

In conclusion, we used a rapid and very sensitive method for measurement of the total grass pollen allergenic activity in settled indoor dust. By the allergen measurements in absolute units (µg/g dust) it will be possible to compare the results of several studies. Our results show that the floor in homes may constitute a significant reservoir of grass pollen allergen. Moreover, the grass allergens seem to be one of the perennial allergens in German households like cat or mite allergens [34]. Thus this assay is an useful tool to study the allergen exposure in homes of grass pollen allergic patients with symptoms all the year round.

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