

Short communication

Predictors of group 5 grass-pollen allergens in settled house dust: comparison between pollination and nonpollination seasons

Background: Some sensitive pollen-allergic patients may experience perennial symptoms, and for these patients it is vitally important to know the concentration of grass-pollen allergens in their homes. The main objective of this study was to detect predictors associated with high grass allergen concentration in settled house dust during and outside the grass pollination season.

Methods: We detected group 5 grass-pollen allergens in dust samples from randomly selected homes of three counties in the region of Sachsen-Anhalt: Hettstedt ($n = 183$), Zerbst ($n = 152$), and Bitterfeld ($n = 119$). Dust samples were collected from the floors of living rooms (LR) and children's rooms (CR) from April 1996 to March 1998.

Results: Phl p 5 was detected in settled house dust in 91% of the homes. The range was between undetectable ($< 30 \text{ ng/m}^2$) and $4300 \text{ ng Phl p 5/m}^2$ sampling area during grass pollination, and $1300 \text{ ng Phl p 5/m}^2$ outside the pollination season, respectively. We observed a partially higher concentration of indoor allergenic activity several weeks after the grass-pollen peak. A positive association was found between Phl p 5 levels and total dust amounts throughout the year. Thus, we assume that grass allergens accumulate in settled house dust. During pollination, the Phl p 5 levels are 1.8 times higher in homes where vacuuming of carpets takes place only once a week or less than in those vacuumed daily. Furthermore, higher concentrations of Phl p 5 in settled floor dust can be expected in homes located within suburban areas (mean ratio [MR] 1.74) and prefabricated slab buildings (MR = 1.54).

Conclusions: Quite high levels of Phl p 5 accumulate in settled house dust even during periods when no grass pollen is present in the atmosphere. The removal of dust by frequent cleaning reduces the indoor exposure to grass allergens. Such information is essential to train patients in allergen avoidance.

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Hay fever symptoms may be triggered by natural exposure to atmospheric grass pollen during the summer in sensitive subjects; therefore, the pollen counts provide a guide to hay fever sufferers. But some pollen-allergic patients with classical symptoms during the flowering period can experience symptoms also outside the pollen season (1, 2). The obvious (though not the only) explanation lies in the presence of fine, allergen-containing particles released from grass-pollen grains in humid air (3–5). Therefore, even if grass-pollen grains are not found intact, the allergen concentration may be high and can be analyzed only by specific immunoassays, as by determination of group 5 grass-pollen allergens (3, 6, 7).

As most people spend most of their time indoors, a considerable amount of attention has focused in recent

years on human exposure to allergens of dust mite, cockroach, cat, and dog. However, little is known about the concentrations of grass-pollen allergen in residential environments. Convincing evidence has been found that pollen allergens are present in settled dust indoors (8, 9). A recent study demonstrated the presence of a major allergen of *Phleum pratense*, Phl p 5, in house dust of different German regions all year round (7). The median allergen level reached more than $0.1 \mu\text{g Phl p 5 per gram}$ house dust outside the pollination season, which is equivalent to about 2500 pollen grains. Thus, the advice of clinicians to patients with hay fever to stay indoors during the pollination season is not sufficient. There are only a few studies on grass-allergen levels in settled house dust, most of which were too small to analyze the effects of indoor conditions, such as the characteristics

of homes and occupants. In this study, we examined which factors are associated with indoor grass-allergen concentration during and outside the grass pollination season in randomly selected homes of three counties in the state of Sachsen-Anhalt.

Material and methods

Selection of homes

We selected 454 homes from population samples participating in the cross-sectional survey on long-term effects of ambient air pollution on children's health in Germany in three areas of Sachsen-Anhalt, in 1992–93 and 1995–96 (10). These three counties are geographically close to each other, but differ greatly in environmental pollution.

Dust samples were collected in each case from living-room (LR) and children's room (CR) floors in 119 dwellings in Bitterfeld, 183 dwellings in Hettstedt, and 152 dwellings in Zerbst between April 1996 and March 1998. The selected homes were visited by trained study personnel. A screening questionnaire was completed, which included questions on building and housing characteristics, ventilation, and cleaning habits, as well as living conditions. Indoor climate (temperature and relative humidity) was measured for a period of 7 days after dust sampling. Study design and methods used have been detailed elsewhere (11). Two homes with missing information on home characteristics were excluded from the analysis.

Dust collection and processing

In each apartment, dust samples were taken by vacuuming of 1 m² for 2 min from the LR and CR floors according to a standardized protocol (12). Dust was collected on cellulose filters with ALK sampling devices (ALK allergen mouthpiece, Hørsholm, Denmark) and stored at –20°C until extraction. The method of extraction has been detailed previously (7).

Allergen analyses

The two-site binding assay was performed as described previously with two monoclonal antibodies which recognize group 5 allergens of different grass species (13). Phl p 5 concentrations were interpolated from the linear part of the standard curve and calculated in µg/g dust and µg/m², respectively. The interassay variation for repeated assays was <10%, and the intra-assay variation was approximately <3%. The lower limit of detection (LOD) for Phl p 5 was 3 ng/ml.

Data analysis

Because Phl p 5 levels expressed as ng/g dust and as ng/m² are best described by a log-normal distribution, Phl p 5 concentrations were decimal log (lg) transformed. Mean concentrations were therefore expressed as geometric means (GM) with 95% confidence intervals (CI). Allergen levels below LOD were estimated at one-half of the LOD. For the estimate of allergen levels on the floor, Phl p 5 measurements in the LR and CR were calculated as an average of these two locations.

Nonparametric methods were used to analyze the relationship between Phl p 5 levels, housing characteristics, and occupant behavior. The Spearman rank-order correlation coefficient was used for continuous variables, and the Wilcoxon test and the Kruskal-Wallis test were used for categorical variables.

The season of dust collection was categorized as the grass-pollination season (May–September; weeks 17–39) or nonpollination periods (October–April; weeks 40–52 and 1–16). From October to April, grass pollen was completely absent, according to the aeroallergy information service. The local data for the grass-pollen counts within Magdeburg (about 70 km from Zerbst, Hettstedt, and

Bitterfeld) were kindly provided by Stiftung Deutscher Polleninformationsdienst, Bad Lippspringe, Germany, and were based on the volumetric sampling of airborne pollen grains on adhesive tape of the Burkhard pollen and spore trap.

Multiple linear regression analyses were performed, including housing and occupant conditions that were found to have at least a marginally significant association ($P < 0.10$) with Phl p 5 levels in the nonparametric analyses or for reasons of the contents. Associations between Phl p 5 levels and explanatory factors were presented as mean ratios (MR), which are the ratios of the GM in the respective category compared with a reference category. Statistical significance was set at the conventional 5% level. All analyses were performed with SPSS (Version 10.0.5).

Results

Phl p 5 concentrations, season, housing characteristics, and occupant behavior

Phl p 5 concentrations in floor dust were available for 451 of the 452 homes. In 40 of 451 homes (8.9%), we were unable to detect grass-pollen allergen in LR and CR floor dust at the moment of sampling. GM, geometric standard deviation (GSD), and percentiles are presented in Table 1 to give a more detailed description of the distribution of the Phl p 5 levels. Dust samples taken during the grass pollination (46% of homes) contained 235 ng Phl p 5 /m² with a range of <LOD to 4300 ng Phl p 5 /m². The remaining samples collected outside the pollination season had GM of 97 ng Phl p 5 /m² with a range of <LOD to 1300 ng Phl p 5 /m². The contents of Phl p 5 in settled house dust sampled during pollination correlated moderately with the mean weekly airborne grass-pollen counts outside ($r_s = 0.326$, $P < 0.0001$; $n = 209$). The peak of grass-pollen antigens in settled dust indoors was not simultaneous with the pollen peak outside (Fig. 1). The antigenicity increased slowly, peaking several weeks later than the pollen count.

The Phl p 5 concentrations from floor dust stratified by pollination season, selected housing characteristics, house location, and occupant characteristics are shown in Table 2. Phl p 5 levels were associated in any case by pollination season, place of residence, type of building, and indoor climate, as well as individual occupant characteristics, such as number of persons living in the apartment, cleaning habits (amounts of dust), and ventilation. The association of absolute humidity with the Phl p 5 levels probably reflected the seasonality of indoor temperature and humidity during summer and winter.

Other categorical variables such as story of the dwelling, size of the dwelling, type of carpets, presence of pets, vicinity of the house, or location of the living-room window seem not to be significantly associated with the grass-allergen levels in bivariate analyses. Associations between the level of parental education or sensitization to grass pollen and Phl p 5 concentration

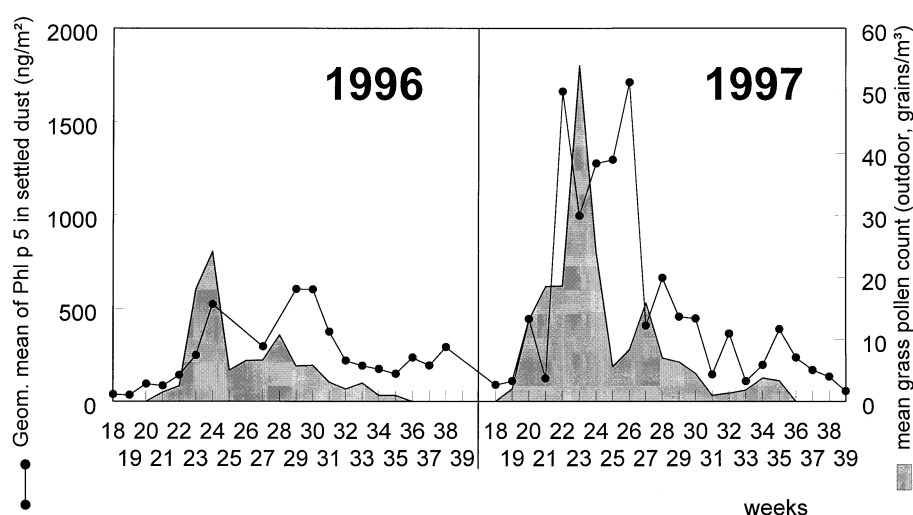


Figure 1. Geometric mean of Phl p 5 levels (ng/m^2) analyzed weekly from floor dust and mean weekly airborne grass-pollen counts during summers of 1996 and 1997. Pollen counts within Magdeburg (about 70 km away from Zerbst, Bitterfeld, and Hettstedt) were kindly provided by Stiftung Deutscher Polleninformationsdienst, Bad Lippspringe, Germany.

were not found. Consequently, we found no indication for avoidance measures in homes occupied by children with hay fever or allergic sensitization.

Multiple linear regression analysis

Because many variables were found to be related to indoor Phl p 5 concentration, multiple linear regression analyses were performed, including the a priori variables place of residence and sampling season, as well as terms for house and occupant characteristics. In order to estimate the influence of microenvironment on concentrations of Phl p 5 in settled house dust, characteristics such as the vicinity of the house and location of living-room windows were also included in the models. The regression analysis explained 29.5% of the variance of Phl p 5 in house dust (single results not shown).

To determine the differences between the sampling

during pollination and nonpollination periods with respect to the influence of different housing characteristics and occupant behaviors on the indoor Phl p 5 levels, we used stratified regression models including the same factors for each period separately. The results of the analyses for indoor Phl p 5 concentration are summarized in Table 3. The model for the pollination season ($R^2=0.317$) yielded stronger correlation between observed and predicted indoor Phl p 5 concentrations than the one for the nonpollination season ($R^2=0.163$). Only the total amount of dust was positively associated with the indoor Phl p 5 levels in both seasons. The association between Phl p 5 and the other potential confounding variables as predictors disappeared outside the pollination season.

In contrast, the variables related to outdoor characteristics such as place of residence or vicinity of the house were contributed more during the pollination

Table 1. Concentration of Phl p 5 in settled house dust samples expressed per square meter and per gram of dust

	Indoor Phl p 5 concentration (ng/m^2)		
	Living-room floor	Children's room floor	Floor (average)
N	451	449	451
GM (GSD)	121.2 (3.8)	135.9 (3.7)	146.5 (3.2)
25th percentile	65.0	75.0	82.0
Median	145.0	159.0	147.0
75th percentile	265.0	292.0	283.0
Maximum	5756	6080	4276
Indoor Phl p 5 concentration (ng/g dust)			
N	451	449	451
GM (GSD)	127.3 (3.9)	120.9 (3.5)	145.7 (3.1)
25th percentile	60.3	74.2	82.7
Median	158.5	137.4	159.3
75th percentile	281.8	254.8	255.5
Maximum	6918	6119	4664

GM: geometric mean; GSD: geometric standard deviation.

Table 2. Concentration of Phl p 5 (ng/m²) in floor-dust samples. Geometric means and 95% confidence intervals related to potential influencing factors

	<i>n</i>	GM	95% CI	<i>P</i> value
Place of residence				
Hettstedt	183	156	(131; 187)	0.0369
Zerbst	151	155	(131; 183)	
Bitterfeld	117	123	(99; 153)	
Sampling period				
Nonpollination	242	97	(87; 110)	<0.0001
Pollination of grasses	209	235	(199; 278)	
Building characteristics				
Type of house				
Concrete slab ¹	105	183	(143; 233)	0.0435
Other kinds of house	343	135	(120; 152)	
Location of house				
Village	76	140	(107; 182)	0.3614
Suburban area	272	156	(135; 180)	
City center/industrial area	103	129	(105; 158)	
Housing and occupant characteristics				
Absolute humidity in LR				
<8.1 g/kg (median)	219	120	(104; 140)	<0.0001
≥8.1 g/kg	219	178	(152; 208)	
Number of persons living in apartment				
2–3 persons	151	118	(97; 144)	0.0144
≥4 persons	299	163	(144; 186)	
Dust amount (g/m ²)				
<0.5 g	34	68	(50; 92)	<0.0001
0.5–<1 g	170	124	(105; 147)	
≥1 g	247	182	(157; 211)	
Ventilation during sampling period				
Rarely	151	105	(90; 123)	<0.0001
Average	149	144	(119; 174)	
Frequently	151	207	(170; 253)	
Parental education ²				
Up to 8 years	15	142	(71; 283)	0.7085
10 and 12 years	235	151	(130; 175)	
More than 12 years	194	138	(117; 163)	
Sensitization to grasses ³				
No	298	144	(126; 165)	0.4690
Yes	139	150	(126; 179)	
Hay fever				
No	425	145	(130; 162)	0.2799
Yes	24	168	(100; 282)	

¹ Prefabricated slab houses and central heating.² Highest school education of father or mother.³ Tested by skin prick test or RAST.

LR: living room; GM: geometric mean; CI: confidence interval.

season. After adjustment for potential predictors, Phl p 5 levels were higher in Bitterfeld than in Hettstedt and Zerbst during the pollination period. Higher concentrations of Phl p 5 in settled floor dust could be expected also in homes located in suburban areas (MR = 1.76) and in concrete slab houses (MR = 1.54). Furthermore, associations between cleaning habits and Phl p 5 concentrations were found. Homes where vacuuming of carpets took place only once a week or less were characterized by about 1.8 higher Phl p 5 concentrations than those vacuumed daily. Moreover, with increasing quantity of dust sampled from floors under standard conditions, the Phl p 5 concentration rose 1.9 times. After adjustment, the potential predictors, such

as average or frequent ventilation or whether the LR windows faced a side street or backyard, led to a nonsignificant increase of indoor Phl p 5 levels.

Discussion

Pollen grains are generally considered to be a risk factor for respiratory symptoms mainly outdoors and during pollination. Pollen grains, as well as antigenic particles, may settle down quickly, both outdoors and especially indoors, where air movements are slow. Previous studies have compared grass-allergen concentrations in indoor and outdoor air. The indoor air allergen concentration varied from 2% to 16% (14) and from 40% to 60% (15) of the corresponding concentrations in the outdoor air at the time of sampling. In the observations presented here, no special attempt was made to measure the atmospheric pollen-allergen levels simultaneously. In this study, predictors of grass-pollen allergen in settled house dust collected all the year were studied for the first time.

Of course, our results confirmed that allergen concentrations reach their highest levels in house dust in coincidence with the pollination season. Moreover, other variables such as place of residence, vicinity of the house, type of building, and cleaning habits have a significant influence on the Phl p 5 level, but only during pollination periods. The positive association between type of building (prefabricated slab buildings) and Phl p 5 levels is particularly interesting. Heinrich et al. (10) found a higher odds ratio of pollen sensitization among children who lived in the new types of buildings.

During the pollen season, the adjusted Phl p 5 concentrations were 54% lower in Hettstedt and 44% lower in Zerbst (both rural areas) than in Bitterfeld (urban area). This finding is difficult to explain. Since our three studied counties share similar climatic conditions, we cannot exclude the possibility that air pollutants have a bearing on pollen. A previous study showed that there is an overall higher concentration of pollen grains in polluted urban areas than in rural regions, independent of weather conditions (16). The interaction between pollen and pollutants could lead to local pollen preactivation, to modulation of allergen release, to generation of allergenic aerosols in a humid atmosphere, and to adsorption of pollen proteins to airborne particles. In contrast, Behrendt et al. (5) demonstrated sulfur dioxide-induced reduction of Phl p 5 availability in pollen, and speculated that the bioavailability of grass-pollen allergens is grossly reduced in regions polluted with sulfur dioxide as compared to nonpolluted regions.

The amount of dust per area sampled has a high impact on allergen concentration in both pollination and nonpollination seasons. Therefore, settled house dust may be regarded as a long-term reservoir of grass-

Table 3. Mean ratio of Phl p 5 concentrations on floor for grass pollination season (May–September) and nonpollination season (October–April), as determined by multiple linear regression (pollination: $n=198$, $R^2=0.317$; nonpollination: $n=234$, $R^2=0.163$)

Factor	<i>n</i>	Phl p 5 (ng/m ²) in settled house dust during						
		pollination			<i>P</i> value	nonpollination		
		MR	95% CI			MR	95% CI	<i>P</i> value
Place of residence								
Bitterfeld	19	1				1		
Hettstedt	98	0.47	(0.27; 0.84)	0.0108		1.01	(0.75; 1.35)	0.9708
Zerbst	81	0.52	(0.30; 0.90)	0.0206		1.19	(0.87; 1.62)	0.2692
Type of house								
Other kinds of house	154	1				1		
Concrete slab ¹	44	1.54	(1.05; 2.27)	0.0285		1.16	(0.87; 1.54)	0.3252
Location of house								
City center/industrial area	40	1				1		
Village	41	1.07	(0.65; 1.77)	0.7965		1.10	(0.71; 1.69)	0.6707
Suburban area	117	1.74	(1.16; 2.62)	0.0087		1.05	(0.79; 1.40)	0.7280
Absolute humidity in LR								
< 8.1 g/kg (median)	47	1				1		
≥ 8.1 g/kg	151	0.91	(0.68; 1.40)	0.9071		0.93	(0.70; 1.23)	0.6159
Vacuuming of floor								
Daily	32	1				1		
More often per week	124	1.81	(1.16; 2.82)	0.0096		0.90	(0.61; 1.33)	0.6079
Seldom	42	1.72	(1.02; 2.91)	0.0432		1.31	(0.85; 2.02)	0.2281
Ventilation during sampling period								
Rarely	21	1				1		
Average	62	1.44	(0.84; 2.46)	0.1876		1.12	(0.87; 1.45)	0.3868
Frequently	115	1.36	(0.82; 2.26)	0.2397		0.89	(0.62; 1.30)	0.5612
Windows in LR face toward								
Main street	29	1				1		
Side street	87	1.49	(0.95; 2.34)	0.0824		0.86	(0.62; 1.20)	0.3766
Park or backyard	82	1.39	(0.88; 2.21)	0.1614		0.80	(0.57; 1.13)	0.2146
Number of persons living in apartment								
2–3 persons	58	1				1		
≥ 4 persons	140	1.18	(0.84; 1.65)	0.3326		1.12	(0.88; 1.42)	0.3557
² Amount of dust per g/m ²	198	1.91	(1.52; 2.40)	< 0.0001		1.41	(1.23; 1.62)	< 0.0001

¹ Prefabricated slab houses and central heating.

² Change per increase of 1 g dust/m².

pollen allergens, and the removal of dust by frequent cleaning is needed to reduce the allergen level. Thus, our results support the earlier claim (8) that the essential source of indoor grass-pollen allergens, apart from pollen itself, is mainly allergen-loaded dust carried indoors by passive transport or open windows. Furthermore, the highest concentrations of indoor allergens were found especially several weeks after the pollen peak. This is in line with data from Finland (8, 9). When the antigenic activity of settled house dust remains high for weeks or months after pollination, allergic subjects may easily have late symptoms as when cleaning dusty surfaces. It is difficult to judge whether the level of exposure might be of clinical significance. A concentration of more than 50 grass-pollen grains/m³ was found to induce symptoms in pollinosis patients (17). These amounts of grass pollen are equivalent to about 2 ng Phl p 5 (7). We found >30 ng/m² Phl p 5 (i.e., >750 pollen grains/m²) in 89% of homes outside the pollination season (maximum 1300 ng/m²) and in 93% of homes during the pollination season (maximum 4300 ng/m²), respectively. If exposure to pollen aller-

gens is prolonged, allergic inflammation is always detectable (18) and the “disease window” of sensitized patients is also prolonged. In addition, the possible synergistic effect of pollen allergen and viral respiratory infections is particularly interesting. Calhoun et al. (19) reported a lowering of the sensitivity threshold and an increase in the specific bronchial reaction to ragweed pollen after experimental infection with rhinovirus 16. Up to now, the grass-pollen allergen has rarely been investigated as a cause of “winter” respiratory symptoms, but this was not the subject of this investigation.

In this context, it is worth mentioning that grass pollen has been found at high levels in dust from the houses of grass-pollen-sensitive patients treated for asthma (20), and the levels of tree pollen were also significantly higher in the homes of tree pollen-allergic children than in the homes of patients without this sensitivity (21).

In conclusion, Phl p 5 accumulates in settled house dust even outside the pollen season. Therefore, the levels of grass-pollen allergens in dust are probably high enough to provoke allergic symptoms. To reduce the

indoor exposure to grass allergens, one must remove dust by frequent cleaning. Our findings provide important complementary information to the pollen

count and will enable clinicians to estimate the quite different risks for asthma and hay fever patients sensitive to grass allergens.

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