

Analysis of allergens in ambient aerosols: Comparison of areas subjected to different levels of air pollution

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Abstract

Recent studies describe interactions of pollen surfaces with aerosol particles; pollen surfaces undergo morphological changes and the release of allergens and allergenic fragments from the pollen can be enhanced. Thus allergens from pollen can be found in particle size fractions much smaller than undamaged pollen ($< 5 \mu\text{m}$). This may explain allergic reactions in parts of the lungs which cannot be reached by undamaged pollen. In Switzerland the birch tree (*betula verrucosa*) major allergen Bet v 1 and the grass (*phleum pratense*) pollen major allergen Phl p 5 are of particular relevance for inducing pollinosis. In this study aerosols of different aerodynamic diameters were sampled by Andersen-Impactors over 18 months. Sampling areas are subjected to different levels of air pollution (Zürich, Switzerland, urban; Payerne, Switzerland, rural; Davos, Switzerland, alpine). Samples were scanned by electron microscopy and submitted to specific allergen assays (ELISA) for birch pollen major allergen Bet v 1 and grass pollen major allergen Phl p 5 respectively. Particle and major allergen concentrations were highest in Zürich, followed by Payerne and, significantly lower, Davos. Scanning electron microscopy investigations showed interactions of aerosols with pollen surfaces in Zürich and Payerne. The presence of Bet v 1 in smaller aerosol fractions was demonstrated in Zürich and Payerne some weeks before and after birch pollen was counted.

Keywords: Aerosols; Pollen; Particle size fractions; Bet v 1; Phl p 5

1. Introduction

The prevalence of allergic diseases has increased over recent years (Wüthrich, 1989; D'Amato et al., 1991). In Switzerland, 10–20% of the population suffer from allergic diseases, mainly hay fever. The most recent epidemiological studies have shown prevalence rates of 11.1% for allergic rhinitis (hay fever) alone (Wüthrich et al., 1995). The concentrations of most important air pollutants have also increased over recent years. So it is a matter of interest whether the increasing prevalence of allergic diseases is connected to the increase in air pollution.

Some studies have shown that allergens from pollen can be found in particle size fractions much smaller than undamaged pollen (smaller than $5 \mu\text{m}$) (Spieksma et al., 1990; Suphioglu et al., 1992) and pollen fragments or released allergens may account for this observation. Several fragmentation or release mechanisms are postulated (Ruffin et al., 1986; Pehkonen and Rantio-Lehtimäki, 1994), for example enhanced allergen release by interaction with airborne particulated matter (Becker et al., 1990). This may explain allergic reactions in parts of the lungs which cannot be reached by undamaged pollen because of their aerodynamic diameter.

So called major allergens amongst the allergy relevant proteins are responsible for inducing most pollen allergies. In Switzerland the birch tree (*betula verrucosa*) major allergen Bet v 1 and the grass (*phleum pratense*)

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pollen major allergen Phl p 5 are of particular relevance (Wüthrich et al., 1995). Therefore, determination of the concentrations of these two major allergens is of great interest.

This investigation aimed to answer the following questions: Can interactions of pollen surfaces with aerosol particles be proved? and what specific major allergen concentrations from birch trees and grass can be found in the different particle size fractions?

Aerosol samples originating from areas subjected to different levels of air pollution, especially of airborne particulated matter, were to be compared (Monn et al., 1994).

Aerosols of different aerodynamic diameters were continuously sampled by Andersen-Impactors during one and a half y at a height of about 10 m above ground. The two week sampling period chosen represents a compromise between a high resolution in time and accumulation of material of an adequate quantity for analysis. Sampling areas are subjected to different levels of air pollution:

- Zürich, Switzerland, urban (annual mean concentrations 1994: NO₂ [nitrogen dioxide] 40 µg/m³; SO₂ [sulfur dioxide] 11 µg/m³; O₃ [ozone] 35 µg/m³)
- Payerne, Switzerland, rural (annual mean concentrations 1994: NO₂ 16 µg/m³; SO₂ 2 µg/m³; O₃ 48 µg/m³)
- Davos, (Seewaldhorn) Switzerland, alpine (annual mean concentrations 1994: NO₂ 4.7 µg/m³; SO₂ 1.2 µg/m³; O₃ 68 µg/m³) (BUWAL, 1995).

Samples were weighed and examined qualitatively by scanning electron microscopy. They were submitted to specific allergen assays (Enzyme Linked Immuno Sorbent Assay, ELISA) for birch pollen major allergen Bet v 1 and grass pollen major allergen Phl p 5 respectively.

This experimental design made it possible to characterize the temporal progress of the particulated mass, Bet v 1 and Phl p 5 concentrations in different particle size fractions at the three sampling sites.

At the sampling sites pollen was routinely counted and differentiated according to species (Swiss National Pollen Observation Program, NAPOL). It was therefore possible to compare the total Bet v 1 and Phl p 5 concentrations with the corresponding sum of birch and grass pollen counts. Air pollutant concentrations as well as meteorologic parameters were also routinely determined.

2. Materials and methods

2.1. Sampling of particles, especially of pollen and pollen fragments

Sampling was carried out by means of Andersen Cascade Impactors (Andersen Inc., Atlanta, GA). An

air stream (28.5 lpm) is lead through stages with opening diameters decreasing from stage to stage. The air stream hits a sampling medium (glass fibre filter with a radius of 40.5 mm) positioned beneath every stage. So different size fractions of particles are successively deposited at various stages: The first stage collects particles with an aerodynamic diameter of above 9 µm; the following stages collect particle size fractions of 9–5.8 µm, 5.8–4.7 µm, 4.7–3.3 µm, 3.3–2.1 µm, 2.1–1.1 µm, 1.1–0.65 µm, 0.65–0.43 µm and <0.43 µm. In this way the behaviour of particles in the human respiratory tract is hereby simulated: large particles are deposited in nose, mouth or trachea, smaller particles are able to penetrate into progressively deeper compartments of the lung.

Particle mass concentrations were determined gravimetrically. Glass fibre filters were dried for 24 h, conditioned for 24 h (at room temperature and 52% relative humidity) and weighed (Mettler AT 261 Deltarange) before and after exposure.

Parallel sampling showed that this procedure allowed a mass concentration determination with a precision of ± 10%.

2.2. Qualitative analysis of the sampled aerosols, especially of the sampled pollen

Small samples of the exposed filters were coated with a gold-palladium layer and examined qualitatively under a scanning electron microscope (Hitachi field-emission SEM S-700). Per sampling site, 24 samples were examined during the whole sampling period.

2.3. Determination of immunologically relevant antigens Bet v 1 and Phl p 5

Monoclonal antibody based two-site binding assays were used to standardize allergen extracts in terms of quantification of single allergens (Chapman, 1988). Details of this assay were kindly provided by Allergopharma Joachim Ganzer KG, Reinbek/Hamburg, Germany, to quantify Bet v 1 and Phl p 5, major allergens of birch pollen, and *phleum pratense* pollen, respectively. The assays were adapted to quantify the allergens directly after extraction from particulate filters.

The filters from two successive stages were pooled in order to get higher concentrations. The resulting pools contained particle fractions of >9 µm, 9–4.7 µm, 4.7–2.1 µm, 2.1–0.65 µm, and <0.65 µm. The filters were placed in a petri dish containing 0.125 M NH₄HCO₃ and 0.1% BSA and stirred overnight on a orbital shaker at 4°C. The resulting extracts were lyophilized under centrifugation for 3 h (Univapo Concentrator Centrifuge 150 AS Autospin, UniEquip Martinsried/München, Germany) and stored at –80°C

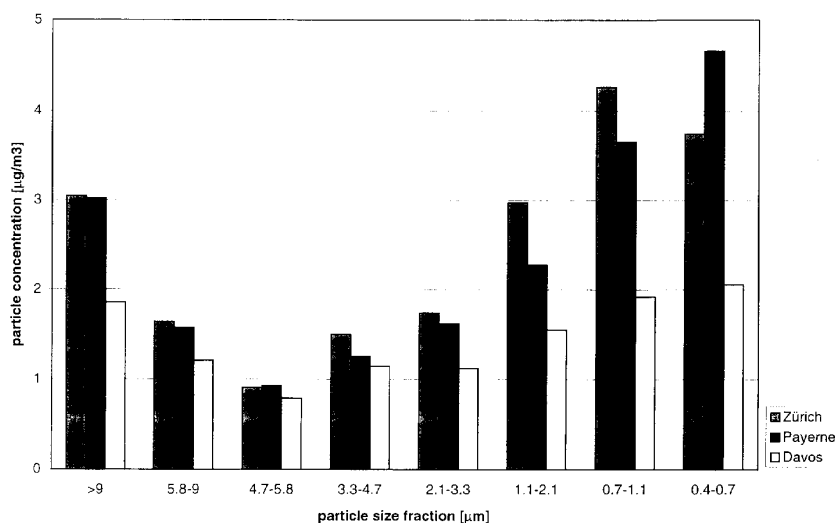


Fig. 1. Mean particle concentrations in the different size fractions ($n = 36$ per sampling site); values in $\mu\text{g}/\text{m}^3$.

until further analysis. This procedure produced extracts of high concentration.

Quantification of Bet v 1: 60% of the lyophilisate from every sample was dissolved in 150 μl phosphate buffered saline, containing 0.05% v/v Tween-20 (TPBS) and 0.01% Thimerosal. 100 μl of purified monoclonal antibodies 3B4F11D6 against Bet v 1 at 10 $\mu\text{g}/\text{ml}$ in a sodium carbonate buffer were added to each well of a microtitre plate and incubated overnight at 4°C (coating). All subsequent incubations were performed at room temperature. After blocking with TPBS/Thio and two subsequent washes, 100 μl of Bet v 1 standard solution (concentration range 1–1000 ng/ml) and the samples were added to the wells. After 1 h of incubation the plates were washed three times and incubated with 100 μl of biotinylated monoclonal antibodies 2E10G6G7 1:1000. Again after 1 h of incubation the plates were washed three times. Streptavidin-peroxidase (100 μl) (Sigma, Deisenhofen, Germany) was added and incubated for 30 min. The Bet v 1 ELISA was developed with *o*-phenylenediamine as the substrate. The resulting absorbance was quantified by means of a photospectrometer at 492 nm (Titertek Multiscan MCC/340).

Quantification of Phl p 5: 40% of the lyophilisate from every sample was dissolved in 100 μl TPBS. The subsequent steps of Phl p 5 quantification method is described (Fahlbusch et al., 1993).

Repeated submission of the same sample to allergen quantification showed that this procedure allowed a precision of $\pm 10\%$. In cases the Bet v 1 and Phl p 5 concentrations were in the lower range of the standard curve, the precision of the determination was reduced to $\pm 15\%$.

2.4. Pollen counts

At the sampling site pollen of about 40 species were routinely collected with a Burkard Pollen Trap (Leuschner and Boehm, 1979). They were trapped on a vaseline covered tape on a clock-driven drum. The collected pollen on 20% of the tape were counted and differentiated according to species by means of a light microscope; the values were multiplied by five to obtain the final concentration of pollen grains (Swiss National Pollen Observation Program, NAPOL).

3. Results

3.1. Concentrations of particles in different size fractions

The total concentration of suspended particles (mean values January 1994 until June 1995; $n = 36$ per sampling site) was 25 $\mu\text{g}/\text{m}^3$ in Zürich, 22 $\mu\text{g}/\text{m}^3$ in Payerne and 13 $\mu\text{g}/\text{m}^3$ in Davos. Particle concentrations at different stages showed characteristic patterns with maximum mass concentrations for particles with aerodynamic diameters of about 1 μm (accumulation range) and of $> 9 \mu\text{m}$. Fig. 1 shows the mean particle concentrations in different size fractions for the three sampling sites.

3.2. Qualitative analysis of the sampled aerosols, especially of the sampled pollen

Scanning electron microscopy investigations showed interactions of aerosols with pollen surfaces in Zürich and Payerne. Fig. 2 shows a pollen sampled in Zürich in April 1994.



Fig. 2. *Cladosporium* spore interacting with particles (2500 x); sampled in Zürich in April 1994.

In Davos particle-pollen interactions could not be found. Because of the small scale of the analysis conclusive quantitative evidence for differences between samples from variously polluted regions could not be obtained.

3.3. Major allergen *Bet v 1* and *Phl p 5* concentrations in different size fractions

The *Bet v 1* concentrations were highest in Zürich, the *Phl p 5* concentrations were highest in Payerne, corresponding to the amount of birch and grass pollen. In Zürich and Payerne *Bet v 1* and *Phl p 5* were identified in all particle size ranges, including the smallest fractions. Sampling periods with high birch and grass pollen quantities were generally characterized

by elevated *Bet v 1* and *Phl p 5* concentrations in the aerosol fraction with the largest aerodynamic diameter. In Davos *Bet v 1* and *Phl p 5* concentrations were significantly lower and confined to the largest particle size class.

Fig. 3 shows the mean *Bet v 1*, Fig. 4 the mean *Phl p 5* concentrations (January 1994 to June 1995, $n = 36$ per sampling site) in different size fractions for the three sampling sites.

3.4. Temporal progress of the *Bet v 1* and *Phl p 5* concentrations

In Zürich and Payerne *Bet v 1* was detected, especially in the lower particle size ranges, before and after the birch pollen was counted. Fig. 5 shows the total *Bet v 1* concentrations and the corresponding birch pollen counts in Zürich (January 1994 until June 1995). In Davos the existence of *Bet v 1* was confined to the period in which birch pollen was counted.

The existence of *Phl p 5* was demonstrated at all three sampling sites only during the time that grass pollen was counted. Fig. 6 shows the total *Phl p 5* concentrations and the corresponding grass pollen counts in Zürich (January 1994 to June 1995).

3.5. Comparison of *Bet v 1* and *Phl p 5* concentrations with birch and grass pollen counts

The two-week means of total *Bet v 1* and *Phl p 5* concentrations were compared with the corresponding sum of birch and grass pollen counts. The spearman correlation coefficient r_s for birch pollen and *Bet v 1* is 0.58 ($n = 60$; $P < 0.0001$); for grass pollen and *Phl p 5* r_s is 0.81 ($n = 60$; $P < 0.0001$).

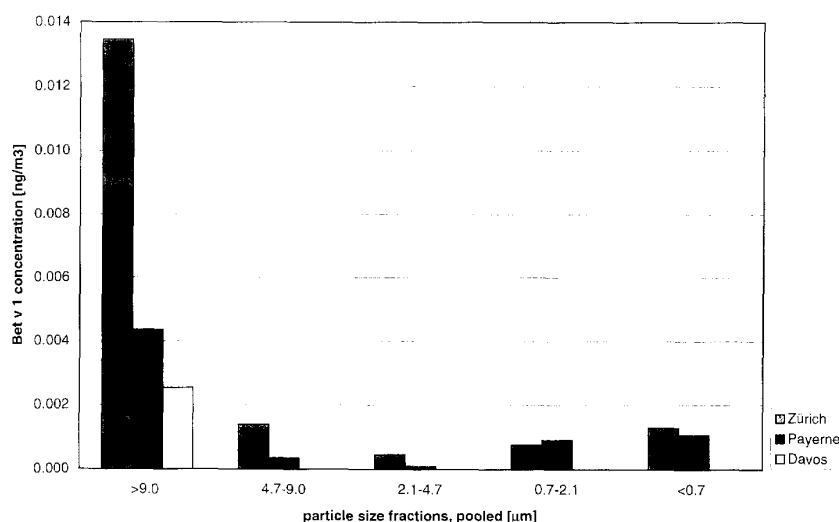


Fig. 3. Mean *Bet v 1* concentrations [ng/m³] in the different pooled size fractions ($n = 36$ per sampling site).

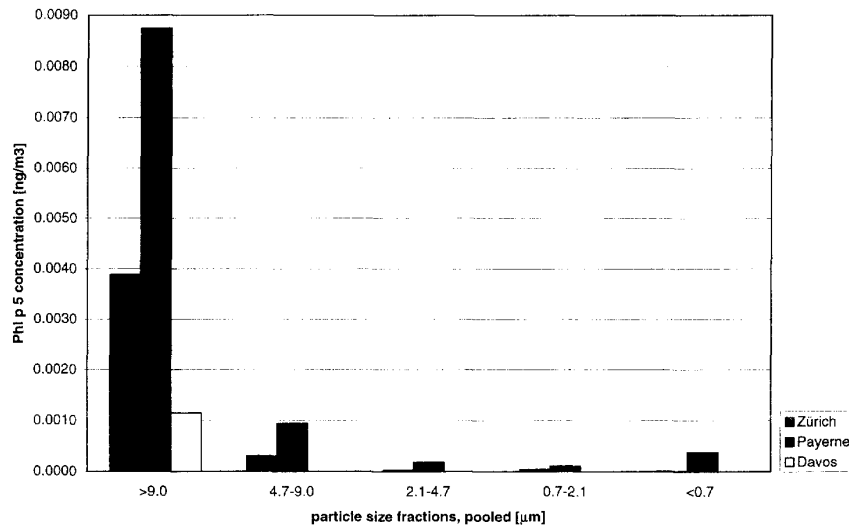


Fig. 4. Mean Phl p 5 concentrations [ng/m³] in the different pooled size fractions (n = 36 per sampling site).

4. Conclusions

An important finding in Zürich and Payerne was that particles interacted with pollen surfaces. In Davos, the sampling site with the lowest air pollution, pollen-aerosol interactions could not be found.

The Bet v 1 concentrations were highest in Zürich, the sampling site with the largest amount of birch pollen; the Phl p 5 concentrations were highest in Payerne, the sampling site with the largest amount of grass pollen. In Davos, the sampling site with the smallest amount of birch and grass pollen, Bet v 1 and Phl p 5 concentrations were lowest.

In Zürich and Payerne a certain portion of the total Bet v 1 and Phl p 5 was detected in the particle size

fractions which are able to penetrate into deep compartments of the lung. This may explain allergic reactions in parts of the lungs which cannot be reached by undamaged pollen. In Davos Bet v 1 and Phl p 5 were found only in the largest particle size fraction. This may indicate that the pollen fragmentation and allergen release probability, for example due to pollen-particle interactions, is lower in areas with low air pollution.

Another relevant aspect was the existence of Bet v 1 in smaller aerosol fractions before and after birch pollen was counted. This antigenic material may originate from birch pollen fragments which cannot be detected by conventional pollen counting. It may also originate from bark, leaves and buds of birch trees (Fountain et al., 1992) and may explain allergic symptoms before

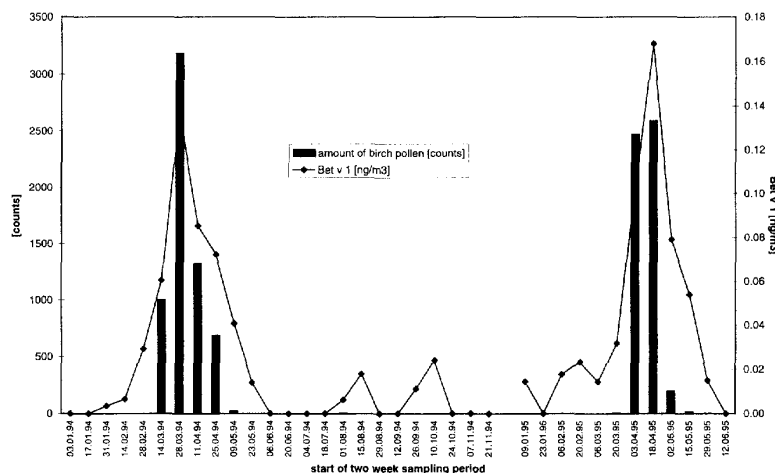


Fig. 5. Total Bet v 1 concentrations and the corresponding birch pollen counts in Zürich, 1994.

