Pollen Characterization in Size Segregated Atmospheric Aerosol

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Abstract. The first stage of a High Volumetric Cascade Impactor - HiVol (BGI-900), used for sampling of aerosol particles larger than 10 micrometres in aerodynamic diameter, was tested for bioaerosol sampling. Low air flow-rate and low pressure-drop at the jets of the first stage and high air volume are advantageous parameters, which would favour the use of the first stage for bioaerosol sampling. The sampling went in urban, rural and background localities, Prague, Brezno and Laz respectively in the Czech Republic, in summer and autumn. Pollen was separated from the impaction substrate, polyurethane foam, into homogeneous deposit on Nylon filter. The homogeneity of the deposit varied within 4%. Representative portion of the deposit was analysed by a scanning electron microscopy - SEM. There were taken 485 SEM images from 12 samples in 3 localities. Pollen grains were identified in 295 SEM images and determined into 9 genus and 4 families. Median pollen grain concentrations/deformities were 9m⁻³/24%, 3m⁻³/18%, 8m⁻³/50% for Prague, Brezno and Laz localities respectively. The pollen grains of the Poaceae family were found with the highest frequency in all localities. Number of pollen increased with total aerosol mass in Prague locality only. There were also identified brochosomes, rather unique insect secretion products, in the samples from the Laz locality.

1. Introduction
Pollen, an important part of bioaerosol, is indispensabla part of atmospheric aerosol, omnipresent colloid dispersed in the Earth atmosphere. Recently, the impact of bioaerosol particles on atmospheric processes has been studied with increasing intensity [1, 2]. Bioaerosol play a vital role in the Earth system and particularly in the interactions between atmosphere, biosphere and climate. Pollen grains are essential for the reproduction and spread of organisms across various ecosystems, and they can cause or enhance human, animal, and plant diseases. Additionally, bioaerosol serves as nuclei for cloud droplets and ice crystals and thus influences the hydrological cycle. Nevertheless, effects of pollen are, however, not yet well characterized and constitute a large gap in the scientific understanding of the interaction in the Earth system. Therefore, pollen analysis became indispensable part of comprehensive aerosol characterization studies at nowadays.

A High Volume Cascade Impactor was originally developed to sample large masses of size-segregated aerosol particles required for toxicology studies [3]. There is aerosol sampled onto rather soft polyurethane foam substrates and additionally at low flow speed in the first stage of the impactor [4].

The aim of presented study is to verify, whether the first stage of HiVol cascade impactor, constructed for ambient aerosol toxicology studies, can be also used for pollen sampling and subsequent analysis.
2. Experimental

Ambient air aerosols were sampled using a High Volume Cascade Impactor – HiVol (BGI-900, USA) at a flow rate of 900 l min\(^{-1}\) consecutively in urban, rural and background localities Prague, Brezno and Laz respectively, in late summer and early Autumn 2009 (Table 1). Aerosol from the first stage of the HiVol, which collects particles >10µm in aerodynamic diameter on polyurethane foam - PUF substrates, were used for the evaluation. The exposed PUF substrates were equilibrated at 21°C and 50% RH in a desiccator, weighed (MC-210, Sartorius), wrapped with aluminium foil, and stored in a double-wall polyethylene zipper bags at -22°C.

| Locality | GPS coordinates | Sample No | Start time | End time | Volume m\(^3\) | Aerosol mass mg |
|----------|-----------------|-----------|------------|----------|----------------|----------------|
| Prague   | 50°4'16.638"N, 14°25'15.305"E | 29A | 5.6.09, 12:16 | 7.6.09, 10:23 | 2490.3 | 11.3 |
|          | 50°24'0.545"N, 13°25'19.909"E | 31A | 9.6.09, 11:10 | 11.6.09, 10:55 | 2578.5 | 10.7 |
| Brezno   | 50°24'0.545"N, 13°25'19.909"E | 32A | 11.6.09, 11:35 | 13.6.09, 10:30 | 2533.5 | 7.4 |
|          | 49°39'35.206"N, 13°53'43.743"E | 33A | 13.6.09, 11:07 | 15.6.09, 11:30 | 2612.7 | 12.6 |
| Láz      | 49°39'35.206"N, 13°53'43.743"E | 38A | 7.8.09, 13:25 | 9.8.09, 10:58 | 2459.7 | 32.8 |
|          | 49°39'35.206"N, 13°53'43.743"E | 39A | 9.8.09, 11:45 | 11.8.09, 10:12 | 2508.3 | 37.1 |

The removal of aerosol particles from the PUF substrates was carried out in a clean hood (HB2436, LaminAir, Holten) to prevent contamination. The substrates were cut with laboratory scissors to about 1cm\(^2\) pieces, placed into 150ml beaker, wetted with several drops of ethanol, poured with 100 ml deionized water and sonicated at 60W for 3 minutes (Micro Ultrasonic Cleaner CT-400). The water extract was filtrated in a plastic apparatus (Thermo Scientific™ Nalgene™) through membrane nylon filter (47mm diameter, 0.45µm pore diameter, Cronus). The sonication and filtration was repeated once again. Then the filters were dried and homogeneity of the deposit evaluated using a reflectance measured by a Smoke Stain Reflectometer (M43D, EED). The filters were stored in Petri-slides, kept in polyethylene zipper bags and wrapped in aluminium foil.

To view aerosol deposited on the filter by Scanning Elector Microscopy – SEM, piece in 1x2 cm was cut-out from the exposed filter, affixed to aluminium target and sputtered with Au at 23°C for 5 minutes (SCD 050, Bal-Tec). Then, the samples were viewed by electron microscope (JEOL JSM-6380LV) at maximum magnification of 11000x.

3. Results and discussion

To evaluate homogeneity of the deposition on Nylon filters, reflectance measurements were conducted at 10 different spots for each filter and average reflectance and relevant relative standard deviation – rstdev were calculated. While the average reflectance decreased with aerosol mass as can be expected, relative standard deviation slightly varied around 5% for reflectance values >15 but suddenly increased to 25% when the reflectance drops below 15 (Figure 1.). Undesired increase of the inhomogeneity can be avoided by using two Nylon filters for one sample, which decrease filter load.
Figure 1. Aerosol mass and relative standard deviations of reflectance measurements versus reflectance of the deposit on Nylon filter after aerosol separation from the PUF substrate of the first stage of HiVol impactor.

Low average value of the rstded <5% of the reflectance, implying low inhomogeneity of the deposit, allows good estimation of pollen grain concentrations in the ambient air in each locality. Pollen grains were identified in 290 SEM pictures of total number 490 ones. Pollen grains concentrations in ambient air were rather low (Table 2.) in comparison with other European localities [5]. The highest pollen grain concentrations and number species were found in Prague. There were identified pollen grains of oak, elm, maple, basswood, pine and Poaceae (Figure 2). Poaceae pollen grains had the highest appearance among other grains and were also found in other two localities. Appearance of pollen grains mostly reflected flowering season. Therefore, in Brezno and Laz, there were identified pollen grains of ambrosia and amaranth, which start to flower in August and July (Figure 3.). Additionally, since the sampling in the Prague locality was conducted in the university botanic garden, though in rooftop station at height of 25m situated, there were found some rare pollen types Rosaceae (Figure 4).

Table 2. Summary of sampling localities, time of sampling and air volume, aerosol mass sampled by the first stage of HiVol cascade impactor.

| Locality    | Sample No | No. of pollen grains in SEM picture | Area of SEM picture mm² | No. of pollen grains in aerosol sample | Pollen grains concentration # m⁻³ |
|-------------|-----------|-------------------------------------|--------------------------|---------------------------------------|----------------------------------|
| Prague      | 29A       | 8                                   | 0.238                    | 36175                                 | 14.53                            |
| urban       | 31A       | 6                                   | 0.238                    | 27132                                 | 10.52                            |
|             | 32A       | 5                                   | 0.238                    | 22610                                 | 8.92                             |
|             | 33A       | 8                                   | 1.690                    | 5087                                  | 1.95                             |
| Brezno      | 38A       | 11                                  | 1.690                    | 6995                                  | 2.84                             |
| rural       | 39A       | 4                                   | 0.608                    | 7066                                  | 2.82                             |
|             | 40A       | 3                                   | 0.238                    | 13566                                 | 5.38                             |
|             | 42A       | 3                                   | 0.423                    | 7631                                  | 2.98                             |
| Láz         | 45A       | 4                                   | 0.238                    | 18088                                 | 7.13                             |
| background  | 46A       | 2                                   | 0.106                    | 20349                                 | 7.98                             |
|             | 47A       | 2                                   | 0.106                    | 20349                                 | 8.03                             |
|             | 48A       | 2                                   | 0.238                    | 9044                                  | 3.49                             |
Figure 2. SEM images of pollen grains of oak (upper left), elm (upper right), maple (middle left) and basswood (middle right), pine (lower left) and Poaceae (lower right) sampled in Prague center in June.
Figure 3. SEM images of pollen grains of amarant (left) and amborisa (right) sampled in rural locality Brezno and background Laz in September

Figure 4. SEM images of pollen grains of different species of Rosaceae sampled in Prague in June

Besides pollen grains, other types of bioaerosol were also found in aerosol samples. They were frequently fungi spores and rarely brochosomes (Figure 5.). Brochosomes are secretion products of insect, mostly leafhoppers, which were though to help them to repel water droplets and honeydew [6].

Figure 5. SEM images of Cladosporium fungi spores (left) and brochosomes sampled in Laz background locality in September
Though the brochosomes are considered to be the most abandon type of bioaerosol particles, they are found rather rarely due to two reasons. Brochosomes are airborne not as individual species, but in the form of rather large clusters containing up to 100,000 particles or stick to other giant aerosol particles (Figure 5.). Also, brochosomes are easily distorted during size-segregated aerosol sampling, applying high flow-rate in a jet, and are highly reactive with mostly nanoparticles. Low aerosol concentrations and low filter load favours brochosome detection since they are not covered with other aerosol particles [7].

Pollen deformities were also found and evaluated in the aerosol samples. The proportionality of pollen grain deformities was 24% and 18% for urban and rural localities respectively, while the highest proportionality 50% were found in background locality Laz. It is difficult to specify, whether the deformities are results of the grain manipulation or dehydration [8]. Nevertheless, deformities are not considered to occur here by aerosol sampling [7]. Pollen grain aging probably causes higher frequency of pollen grain deformities in the Laz background locality, since the sampling there occurred near the end of September, and at distinctly higher altitude of the Laz locality in comparison with other two localities.

4. Conclusions
Aerosol samples were separated by sonication and filtration from PUF substrate of the first stage of the HiVol cascade impactor and successfully evaluated by the SEM for reasonable pollen grain types reflecting flowering season. Homogeneity of the aerosol deposit on the Nylon filter allows precise estimation of pollen grain concentration in the ambient air comparable to other studies. To conclude, the first stage of the HiVol impactor was proven to be suitable for airborne pollen grain sampling and evaluation.

Acknowledgment
Study was supported by the project CENATOX under grant GAČR P503/12/G147.

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