# MINERALOGICAL, CHEMICAL AND TOXICOLOGICAL CHARACTERIZATION OF THE URBAN AIR PARTICLES

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## Introduction

Persistent organic pollutants (POPs) such as polyaromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs) or organochlorine pesticides (OCPs) represent a risk for human health[1] as well as for the ecosystems of remote areas as they are bioaccumulative, resist degradation and cycle in the environment for a long time. Despite of a significant improvement of the air quality in recent years, the atmospheric levels of POPs remain a subject of great public health concern[2] especially in the large cities.

The health hazard of ambient air can be determined by conventional gaseous pollutants (ozone, nitrogen oxides) and particulate matter (PM). Particle size and morphology, disregarding its chemical properties, represent one of the hazards. Respirable particles are usually divided into the coarse (diameter more than 2.5 μm), fine (0.1-2.5 μm) and ultrafine (less than 0.1 μm) size fractions. Currently, governments regulate 10 μm-diameter ($PM_{10}$) and 2.5 μm-diameter ($PM_{2.5}$) particles but recent studies suggest that the unregulated ultrafine particles are potentially the most dangerous. Growing attention is given to the potential effects of the ultrafine dust particles in the human health because they can penetrate deeper into the respiration tract than fine or coarse particles do[3], and cause the respiratory problems. Pulmonary effects of PM include the triggering of inflammation in the smaller airways, which can lead to the exacerbation of asthma and chronic bronchitis, airway obstruction, and decreased gas exchange. PM can also interfere with the clearance and inactivation of bacteria in lung tissue.

The chemical composition of the particles themselves, as well as the variety and quantity of compounds sorbed on their surfaces are other factors possibly responsible for the health effects. Since fine and ultrafine particles predominantly originate from combustion of fossil fuels and vehicle emissions, PAHs are among the substances of a great interest and they appear to be significant contributors to the genotoxicity and carcinogenity of air pollution in the urban environments. Oxidative stress has emerged as a mechanism that underlies the toxic pulmonary effects of atmospheric particles. Experimental evidence[4] showed that redox-active transition metals, redox-cycling quinoids and PAHs contained in aerosols act synergistically, producing reactive oxygen species leading to oxidative DNA damage. Current evidence also indicates that PAHs are transformed enzymatically to active metabolites that react with DNA to form adducts that result in mutations [5].

A partitioning of chemicals between the phases and particle size fractions as well as processes on the aerosol particles are important as they control the atmospheric fate of these compounds (degradation and physical removal processes). They are, however, insufficiently known[6] and are to be better understood in order to describe POPs cycling and assess related impacts to the ecosystems and human health. Filling this gap is vital for future evaluation of the health effects of various fractions of the atmospheric PM.

## Methods and materials

Samples of airborne particulate matter (< PM10) for the purpose of the pilot study were collected in Brno, Czech Republic, in August, 2006. A sampling location was in the industrially influenced area affected also by road

traffic and domestic emissions. A high volume ambient air sampler PM-10 (Graseby-Andersen, USA, flow 1.13 $m^3$ $min^{-1}$, volume 1620 $m^3$ per 24 h) equipped with a multi-stage cascade impactor for particle-size fractionation was used for particle sampling. This impactor fractionates suspended particulates into six size fractions (bellow 10, 7.2, 3.0, 1.5, 0.95, and 0.45 micrometers). Particles were sampled at Slotted Collection Substrates and Whatmann quartz filters. Six sampling sites with different sources of atmospheric particles were selected for a follow-up study: the quarry, cement factory, airport, road traffic, coal combustion, glass and metal industry. Sampling duration was 7 days and sampling was repeated 4 times at each site. Four replicates were analyzed separately to assess a variability of data.

Scanning electron microscope (SEM) CamScan CS 3200 equipped with microanalytical system Link ISIS 300 (Oxford Instruments) with energy-dispersive SiLi spectrometer (EDS) was applied to characterize morphology and semiquantitative chemical composition. The compositional spectra were compared with the EDS library of clay and rock forming minerals. X-ray diffraction analysis (XRD) was performed using powder diffractometer Philips X'pert MPD system with Bragg-Brentano reflecting geometry and vertical goniometer PW 3020.

Each particulate fraction was a subject of the chemical analysis for qualitative and quantitative determination of bound organic compounds. All samples were extracted with dichloromethane in a Büchi System B-811 automatic extractor. Surrogate recovery standards (D8-naphthalene, D10-fenantrene, D12-perylene for PAH analysis; PCB 30 and PCB 185 for PCB analysis) were spiked on each filter prior to extraction. Terphenyl and PCB 121 were used as internal standards. Volume was reduced after extraction under a gentle nitrogen stream at ambient temperature, and fractionation achieved on silica gel column (30 cm length, 1 cm i.d.); a sulfuric acid modified silica gel column was used for PCB/OCP samples. Samples were analyzed using a GC-MS instrument (HP 6890 - HP 5973 and 5975) supplied with a J&W Scientific fused silica column DB-5MS) for 16 US EPA PAHs, 7 indicator PCBs, *o,p'*- and *p,p'*- DDT, DDE and DDD, α-, β-, γ-, δ-hexachlorocyclohexane (HCH), hexachlorobenzene and pentachlorobenzene. Recoveries were higher than 74 % and 72 % for all air samples for PCBs and PAHs, respectively. Recovery factors were not applied to any of the data. Recovery of native analytes measured for the reference material varied from 88 to 100 % for PCBs, from 75 to 98 % for OCPs, from 72 to 102 % for PAHs. The levels in field blanks never exceeded 1 % of the quantities detected in samples for PCBs, 1% for OCPs, and 2% for PAHs. Laboratory blanks were always lower than 1% of the amount found in the samples.

Genetically modified bacteria cells (tester strain Escherichia coli PQ 65 harboring a sulA::lacZ fusion) were employed in the study[7]. DNA is a molecular target and the reporter responds directly to the DNA damage. Cytotoxicity as a result of more general macro-molecular damage can be detected in this test as well. Solution of 4-nitroquinoline-N-oxide (4-NQO) was used as positive control in the test. The SOS induction factor (IF) was calculated for every tested concentration and positive control based on negative controls (IF (4-NQO, 0,469 ug $ml^{-1}$) = 32.06, S.D. = 2.07, IF (negative control) = 1). The samples with the induction factor higher than 1.5 for any concentration were considered to be significant genotoxins.

**Results and Discussion**

Properties of each size fraction of the airborne particles were assessed. Finest fraction (<0.45 μm) showed to have the highest mass and highest active surface (Fig. 1a), highest amount of associated PAHs (Fig. 1b) and also highest direct (Fig. 1c) and indirect genotoxic (Fig. 1d) potentials. While 43% of PAHs were associated with the fine fraction (< 0.45 μm), 20 % was found in the fraction between 0.45 and 0.95 μm and around 10 % in each of the remaining fractions. This distribution is in a very good agreement with estimated specific areas coming out from mineralogical analysis. The coarse fraction included abundant spores and plant fragments along with rock-forming minerals, such as quartz, feldspars, mica, illite-smectite, chlorite and kaolinite. The intermediate fractions consisted mainly of clay minerals, e.g. kaolinite, illite-smectite, dolomite, calcite and gypsum. The finest fractions (<0.95 μm) were dominated by carbonaceous PM, most probably soot flakes, droplets and films, poorly crystallized quartz, gypsum, lead and copper sulfates. SOS chromotest was used to study direct and indirect (without and with metabolic activation) toxicological potential of each particular fraction. All samples reached statistical significance of direct genotoxicity (IF >1.5), and genotoxicity increased with decreasing particle size.

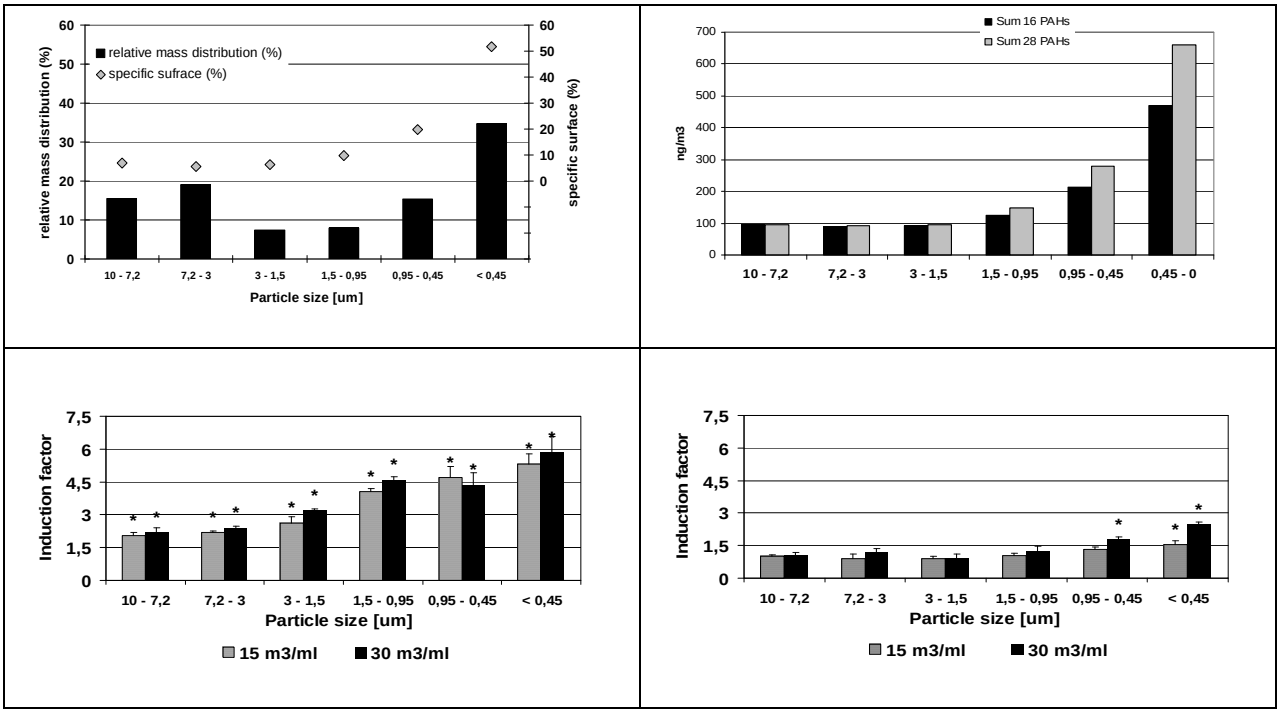


*Figure 1. (a) Relative mass distribution of airborne particles between the size fractions compared to relative distribution of their specific surface. (b) Amounts of PAHs sorbed to the individual size fractions. Genotoxicity without (c) and with (d) metabolic activation.*

To assess a variability of all these parameters, particulate matter from six sampling sites with strong and well defined sources (quarry, cement factory, airport and agricluture, road traffic, coal combustion, glass and metal industry) was collected in the next step. Gas phase associated chemicals were sampled in addition to particulate fractions, organochlorines and heavy metals were analyzed as well. A greater selection of toxicological tests (focused on the specific mode of action: dioxin-like toxicity, estrogenicity, androgenicity) was applied.

As can be seen from Fig. 2, size distribution of the atmospheric particles varied from site to site with the coarse particles dominating in the quarry and cement factory, and fine particles dominating at the sites affected by combustion sources. On the contrary, majority of organic pollutants (PAHs, PCBs, OCPs) was associated with the finest fractions at all sites and so was the strongest toxic effect. For a dioxin-like toxicity, for instance, there was much stronger activity found at the sites with combustion sources even though a total amount of particles was an order of magnitude higher in the cement factory. Gas phase associated chemicals collected in the polyurethane foam filters showed no dioxin-like activity. Four sampling campaigns from each site were analyzed separately to reveal variability less than 20% between the sampling campaigns.

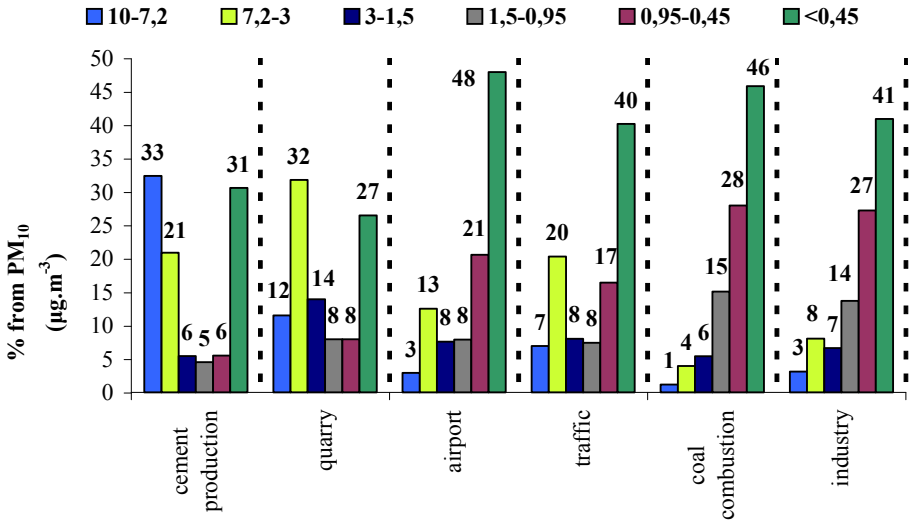


*Figure 2. Mass distribution of atmospheric particles between the individual size fractions at six sampling sites.*

## Conclusions

In spite of growing attention devoted to the effects of PM on human health, there are no studies linking the toxicological effects of particles to the PM size dependent chemical composition and the chemical compounds sorbed on their surfaces. Filling this gap is vital for future evaluation of the health effects of various fractions of the atmospheric PM. We have to determine whether in addition to monitoring the mass or the number of particles in the air we also need to consider the chemical composition of the particles as well as a number of toxic chemicals associated with their surfaces when evaluating the effects of ultrafine particles. In our study, a combination of methods was used to describe the morphology and sorption potential of various size fractions of the PM, to determine the chemicals associated with their surfaces and to link it to toxicological effects and related risks. It was confirmed that fine and ultrafine particles pose the highest environmental risk. This set of information has to be considered when assessing the fate of organic compounds in the atmosphere including long-range transport and sink processes.

## Acknowledgements

The project was supported by the Czech Ministry of Environment (SP/1a3/29/07) and Ministry of Education (MSM 0021622412).

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