



# Use of TEOM monitors for continuous long-term sampling of ambient particles for analysis of constituents and biological effects

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## Abstract

Many countries have implemented exposure limits for the concentration of ambient particulate matter and do therefore have to monitor their concentration. This could be performed with TEOM monitors (Tapered Element Oscillating Microbalance-monitors) that contain a filter on which particles are collected. These filters are regularly exchanged for new ones. The aim of this study was to test the feasibility of collecting used filters from monitors at different locations and establishing a method to extract particles and then study them with respect to their ability to generate oxidants, their endotoxin content, and ability to activate inflammatory cells. Filters from nine geographically spread locations in Sweden were collected during a 21-month period by local technicians who then sent them to the laboratory where they were extracted and analyzed. The procedure to let local technicians perform the filter exchange and send used TEOM filters to the laboratory worked well. A method was established in which pyrogen-free water was used to extract particles that then were aliquoted and stored for later analysis. Particulate matter (PM<sub>10</sub>) from different locations showed both a considerable seasonal and spatial-dependent difference with respect to oxidative potential (oxidize glutathione), endotoxin content, and ability to activate blood monocytes to release interleukin-1 $\beta$ . This study shows that, instead of discarding TEOM filters, they can be collected and extracted so that particles that have been sampled in a standardized way could be analyzed with respect to variables that reflect their toxicity. This could be done at a low cost. In combination with information about the ambient particle concentration, such information could be helpful in the evaluation of differences in the risk of breathing air at various locations.

**Keywords** Air pollution · TEOM monitor · Ambient particles · Surface reactivity · Endotoxin · Seasonal variation

## Introduction

Exposure to respirable ambient particles increases the risk of cardiovascular diseases, lung diseases, and worsened asthma symptoms (Maier et al. 2008; Cosselman et al. 2015; Taneepanichskul et al. 2018; Pope III et al. 2018). Such

exposure has also been shown to have an adverse effect on the development of lung function in children and teenagers between 10 and 18 years (Horak et al. 2002; Gauderman et al. 2004; He et al. 2010) and could also cause increased respiratory symptoms in children with asthma and other respiratory diseases (Sugiri et al. 2006; O'Connor et al. 2008; Schachter et al. 2016). It has also been shown that long-term exposure to fine particulate matter is associated with a reduced and faster decline in lung function and an increased risk of chronic obstructive lung disease in subjects that are 20 years and older (Guo et al. 2018). To minimize possible health risks, it is therefore important to reduce the concentration of respirable particles in the air. Many countries have now adopted regulations for how high a concentration of particulate matter with a diameter under 10  $\mu\text{m}$  (PM<sub>10</sub>) or 2.5  $\mu\text{m}$  (PM<sub>2.5</sub>) that can be tolerated (EEA 1999; Heal et al. 2012). Cities and local communities do therefore have to monitor and report the mass concentration of PM<sub>10</sub> or PM<sub>2.5</sub> and have to intervene if these limits are exceeded.

There are several methods for real-time monitoring of the concentration of respirable sized particles in ambient air. One

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way is to use special laser-based instruments in which particles under a certain diameter are registered by a laser beam in a flow cell. Another method is to use instruments based on the Tapered Element Oscillating Microbalance (TEOM) technique. In TEOM monitors, air is drawn through a filter placed on the top of an oscillating glass rod. The air flow rate through the filter is constant and the mass of the particles that attaches onto the filter will influence the oscillation frequency, which in turn makes it possible to calculate the particle mass and express this per volume of air. These monitors do depend on what sampling head that is installed and measure either PM<sub>10</sub>, PM<sub>2.5</sub>, or PM<sub>1</sub>. A traditional way to measure particle concentration is otherwise to use air pumps that draw air through filters that then are weighted. An advantage with this procedure is that particles on filters then could be extracted and analyzed regarding their chemical composition or toxic properties (Lee and Vu 2010; Kulkarni et al. 2011). However, particle concentrations in air are normally low and therefore long sampling times or high-volume pumps are necessary to collect enough particles to perform an accurate gravimetric analysis and/or perform more specific chemical or biological analysis of the collected material.

Particulate (and other) air pollution is today considered to play a considerable role for the overall mortality in the world (Landrigan et al. 2018). Particles will vary considerably with regard to size, surface reactivity, composition and their toxicity, and inflammatory-mediating capacity (Thorpe and Harrison 2008; Steenhof et al. 2011). There are different ways that such particle characteristics could be studied. One way to study surface reactivity is to analyze the oxidative potential which could be carried out by determining the particle-mediated oxidation of glutathione in a cell-free system. Glutathione is a very important airway antioxidant that loses its protective property if being oxidized (Boogaard et al. 2012; Janssen et al. 2014; Styszko et al. 2017). Particles could also be analyzed on their content of various toxic and/or pyrogenic agents. Endotoxin is a part of the cell wall of gram-negative bacteria and traces of it do therefore possess a strong inflammatory potential (Liebers et al. 2008). Others have studied endotoxin on particles and also then shown that it could vary depending on the season (Carty et al. 2003; Salonen et al. 2004). Apart from endotoxin, there are also other types of pyrogenic compounds such as fragments from gram-positive bacteria, fungi, and virus and inhalation of those will also potentiate inflammatory reactions. The total pyrogenic effect of particles (endotoxin and non-endotoxin pyrogens) could be studied with cell models. One way is then to use monocytes from whole blood that are mixed and incubated with particles and then analyze the release of pro-inflammatory cytokines. This formation is then used as an index of the particle-mediated inflammatory potential (in vitro). The assay is often called In vitro Pyrogen Test (IPT) or Monocyte Activating Test (MAT), (Daneshian et al. 2009; Stoppelkamp et al. 2017).

TEOM monitors are today used solely for measurement of PM<sub>10</sub>, PM<sub>2.5</sub>, or PM<sub>1</sub> concentrations. In the instruments, particles are however also collected on filters that are exchanged and discarded at regular intervals, normally 2–4 weeks. The principal aim in this study was to test the feasibility of using TEOM monitors as particle samplers and establish a methodology in which used TEOM filters are extracted and the obtained TEOM particles are analyzed with respect to chemical and biological characteristics. We also studied the spatial and temporal variations of these variables in particles collected from TEOM monitors located at various sites in Sweden.

## Materials and methods

### Sampling and sample preparation

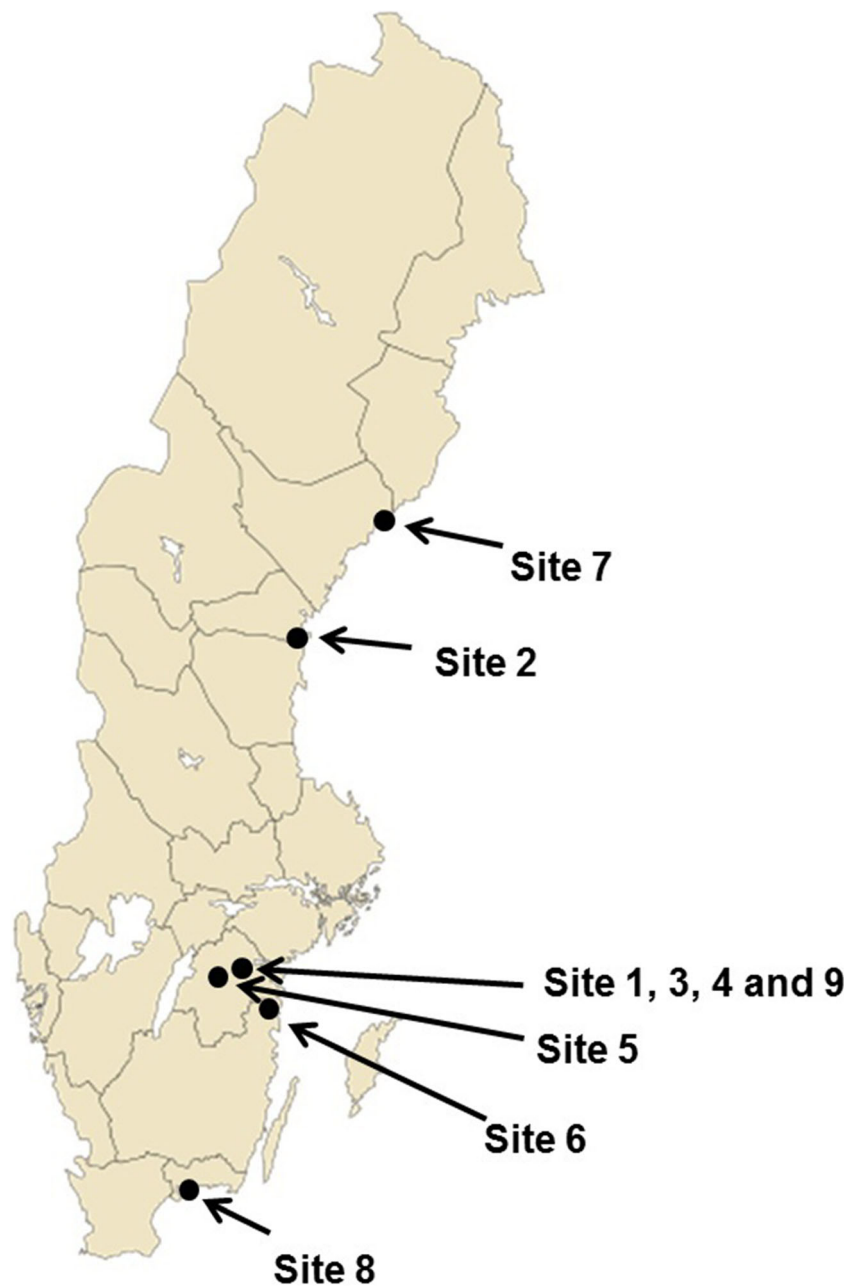
#### Sampling locations and sampling periods

TEOM filters were collected from TEOM monitors at nine sites located in six cities in both southern and northern Sweden (Fig. 1). The distance between the most southern and northern site is approximately 1000 km. These locations differed with respect to factors such as annual mean temperature, the presence of snow during the winter season, humidity, road care, traffic intensity, and type of vehicles. The precise location and the number of filters analyzed from each sampling site are shown in Table 1. All TEOM monitors at these sites sampled particles with a diameter less than 10 µm (PM<sub>10</sub>). Two main series of samples were obtained and were analyzed. First, one series collected at site 1 and site 2 during 21 consecutive months from January 2009 to September 2010. A second series was collected at sites 1–9 during the period February to April. We had no such complete series from the other seven sites. They did however supply filters regularly for most months but we chose to analyze as many samples as we could obtain for the period February to April. This is when the particle levels normally reach their annual peak and this period was therefore considered as especially interesting. The monitors were located close to a trafficked street or road in an average or small city. In no site was there any big industry close to the monitors, so the origin of the majority of particles therefore is assumed to be related to traffic (wear particles or tail-pipe particles). The information about the average monthly temperature at sites 1 and 2 was obtained from the Swedish Meteorological and Hydrological Institute.

#### TEOM monitors

TEOM monitors are used worldwide to measure the ambient concentration of particulate matter in real-time and they consist of two basic components: a sensor unit and a control unit. It is a gravimetric instrument that draws air through a filter at a constant flow rate (3 l/min), continuously weighing the filter

**Fig. 1** Map of Sweden showing the locations of the different sampling sites. For further information and exact geographical location see Table 1



and measuring particulate mass concentrations (Patashnick and Rupprecht 1991). Each TEOM filter is mounted on the top of an oscillating hollow tapered glass tube. The oscillating frequency is proportional to the mass of the particles that get trapped on the filter. So, the change in mass affects the oscillating frequency which is used to calculate the particle concentration. The basic components of the sensor unit are shown in Fig. S1 of the electronic supplementary material.

#### Filter exchange

The filter exchange procedure was performed on the first week of the month. All filters that were used and mounted in the

monitors were supplied by the laboratory. The technician who was responsible for the exchange had, some days beforehand, received a package by mail with the filter in a marked sterile 50-ml polypropylene tube, sterile nitrile gloves, and a pre-paid envelope with the return address to the laboratory. To avoid contamination, during the filter exchange, the technician used sterile gloves. A clean filter exchange tool was also used to handle the filter and to take each filter directly from the filter holder to the sterile tube so it could be sent back to the laboratory by mail. On arrival at the laboratory, each tube with filter was registered and transferred to a  $-82\text{ }^{\circ}\text{C}$  freezer. A detailed description and a film that describes the filter exchange procedure are to be found on the manufacturer's website (Thermo Scientific 2017).

**Table 1** Description of the nine different sampling sites with location, geographical coordinates, the traffic intensity, and the number of analyzed filters. The number of daily traffic of vehicles was obtained from each municipality

| Site | City*               | Street        | Coordinates            | Vehicles/day<br>(speed limit, km/h) | Number<br>of filters |
|------|---------------------|---------------|------------------------|-------------------------------------|----------------------|
| 1    | Norrköping (100000) | Ö. Promenaden | N58.591681, E16.198043 | 19,800 (50)                         | 21                   |
| 2    | Sundsvall (60000)   | Eur.route E4  | N62.401024 E17.3353623 | 30,000 (50)                         | 21                   |
| 3    | Norrköping (100000) | Kungsgatan    | N58.588870, E16.177822 | 12,580 (50)                         | 6                    |
| 4    | Norrköping (100000) | Packhusgatan  | N58.595948, E16.192336 | 31,900 (50)                         | 3                    |
| 5    | Linköping (110000)  | Hamngatan     | N58.410905, E15.630488 | 12,000 (50)                         | 3                    |
| 6    | Valdemarsvik (3000) | Storgatan     | N58.202800, E16.601717 | 4300 (30)                           | 2                    |
| 7    | Umeå (90000)        | V. Esplanaden | N63.828292, E20.257949 | 28,500 (50)                         | 6                    |
| 8    | Karlshamn (20000)   | Ågatan        | N56.171277, E14.859532 | 5900 (50)                           | 3                    |
| 9    | Norrköping (100000) | Ingelstad     | N58.610428, E16.160668 | 41,800 (50)                         | 6                    |

\*The population in the central parts of the cities in parenthesis

### Sample preparation

The initial tests used to elaborate different parts of sample preparation were performed with filters ( $n = 20$ ) that had been collected from local monitors before the start of the study. The method described below is the one that was found usable and that also was used when samples from the nine different locations were analyzed (sample series 1 and 2, see above). At each weighing/extraction procedure, a set of 15 filters were handled simultaneously. To determine the mass of particles, each filter was weighed with a precision microbalance with a reading precision of 10  $\mu\text{g}$  (Satorius Micro MC5 P). The weighings were performed on two occasions, once before extraction and once again after extraction. Before the first weighing, each filter was placed on its tube cap in a weighing room with a controlled temperature and humidity (24  $^{\circ}\text{C}$  and 55%  $\pm$  2%). In one experiment, the result of weighing with the microbalance was compared with that of an ordinary analytical balance (Precisa XR 205A). After 48 h, the filter was weighed and transferred back to its storage tube. An ultrasonic probe (Soniprep 150 Ultrasonic disintegrator, MSE, England) was used to extract the particles. Before sonication, 2 ml pyrogen-free water (Braun Malsungen AG, Germany) was added in each tube. The extraction was done in two 1-min cycles with a 10-s pause in between. During extraction, the tubes with filters were also chilled in a beaker with ice and the probe was submerged between 3 to 5 mm in the extraction media. The tip of the probe was also placed on the back side of the filter to avoid direct contact with the particle-containing side. After extraction, the samples were aliquoted into 300  $\mu\text{l}$  portions in polypropylene micro-vials (stock particle solution) which were stored in a low temperature freezer. The filter was again placed on its tube cap and allowed to acclimatize in the weighing room for 1 week before it was weighed again a second time. The mass of the extracted particles was obtained by calculating the difference between the two weighings.

Directly after extraction, the particle suspension was divided into six 300  $\mu\text{l}$  aliquots that were frozen in  $-82^{\circ}\text{C}$ . Before analysis, each sample was diluted with pyrogen-free water to a particle concentration of 100  $\mu\text{g}/\text{ml}$  and the results were expressed as mass units per milligram of particles or effect/capacity per milligram particles. For analysis of cell-activating effect, the samples were diluted further in 1.8% sterile NaCl to obtain a normal saline (0.9% NaCl).

### Analysis

#### Oxidative capacity

Particle samples (100  $\mu\text{g}/\text{ml}$  in 100  $\mu\text{l}$  water) or blanks (extracts of particle-free filters) were mixed with 100  $\mu\text{l}$ , 100  $\mu\text{mol}/\text{l}$  reduced glutathione (GSH) in 1.5 ml polypropylene micro tubes. The mixtures were incubated on a rotator at slow speed for 30 min at 37  $^{\circ}\text{C}$  and were then centrifuged for 3 min at 14000 $\times$ g. Supernatants (25  $\mu\text{l}$ ) were transferred to new micro tubes and mixed with 25  $\mu\text{l}$  tris-buffer (100 mmol/l, pH 8.5). To derivatize GSH, 130  $\mu\text{l}$   $\text{Na}_2\text{HPO}_4$  (500 mmol/l, pH 7.0) and 50  $\mu\text{l}$  OPA (orto-phthalaldehyde, 50  $\mu\text{mol}/\text{l}$ ) were added and the samples were incubated at room temperature for 5 min. The generated GSH-OPA complex was analyzed with high performance liquid chromatography (HPLC). The column was a Chromolith Performance column (100  $\times$  4.1 mm, C18, Merck Millipore) and the mobile phase consisted of 15% methanol and 85% 25 mmol/l  $\text{NaH}_2\text{PO}_4$  with a pH of 6.5 and the flow rate was 2 ml/min. Twenty microliters of each sample was injected and GSH-OPA was detected with a fluorescence detector (Merck Hitachi, F1000) with the excitation and emission wavelengths set at 340 nm and 420 nm, respectively. Samples without particles were used as negative controls and the level of GSH in those was set as 0%. Samples with 2 mmol/l hydrogen peroxide were used as positive controls

and were set as 100%. This was a modification of a method previously described by Cereser et al. (2001).

### Cell-activating effect (monocyte activation test)

This assay is often called In vitro Pyrogen Test (IPT) or Monocyte Activation Test (MAT) (Daneshian et al. 2009; Stoppelkamp et al. 2017). We studied the ability of the particles to activate monocytes in whole blood and initiate a formation of pro-inflammatory interleukine-1 $\beta$  (IL-1 $\beta$ ). We then used a method that we previously have described (Jayawardena et al. 2009). In this, the formation of IL-1 $\beta$  in samples with particles was related to that in standards with zymosan A (0–10  $\mu\text{g/ml}$ , Sigma Z-4250) and the cell-activating effect was expressed as zymosan equivalents ( $\mu\text{g/ml}$ ). In brief, blood was drawn from the forearm of healthy volunteers (personnel in the laboratory) into heparinized tubes (BD Vacutainer, Plymouth, UK). Samples with particles (140  $\mu\text{l}$ , particles in pyrogen-free water and 140  $\mu\text{l}$  1.8% sterile NaCl-solution) or blanks (extracts of particle-free filters) were mixed with 70  $\mu\text{l}$  whole blood in wells of a 500  $\mu\text{l}$  round bottom polypropylene 96-well plate (Nunc A/S, Roskilde, Denmark, no: 267245). The plate was covered with a plastic lid and incubated for 18 h in a cell culture chamber (37  $^{\circ}\text{C}$  and 5%  $\text{CO}_2$ ). The ELISA (Enzyme-Linked Immunosorbent Assay) was performed with antibodies and standards from Diaclone (Human IL-1 $\beta$  Eli-pair, Diaclone, Besancon, France, no: 851-610-010). The protocol from the manufacturer was followed with the exception that white 384-well polystyrene plates (Maxisorp, Nalge Nunc International, Rochester, USA, no: 460372) were used instead of clear 96-well plates and that chemiluminescence was used instead of absorbance to quantify the signal. For further details, see Jayawardena et al., (2009). The coefficient of variation when one sample was analyzed in different wells (mean of two wells) was between 5 and 10%.

### Endotoxin

Endotoxin was determined with a Limulus assay (LAL kit, Chromogenic Endpoint Assay, HIT302, Hycult Biotech, Holland). In brief, 25  $\mu\text{l}$  of each working particle solution (100  $\mu\text{g/ml}$ ) was mixed with 25  $\mu\text{l}$  of LAL reagent in the wells of an endotoxin-free 384-well microplate (Maxisorp, Nunc, no: 464718) and the change in absorbance (405 nm) was measured every 5 min during 1 h in a microplate reader (FLUOstar, BMG, Germany). A standard curve with known concentrations of endotoxin (0.04 to 10 EU/ml) was used for quantification. The limit of quantification was 0.04 EU/ml. Together with the samples, also two negative controls (extractions of filters without particles) and four wells with pyrogen-free water were included. All samples were analyzed in duplicate and on the same plate. The coefficient of variation when a

sample with a concentration of 0.40 EU/ml was analyzed in ten wells was 5%.

### Statistics

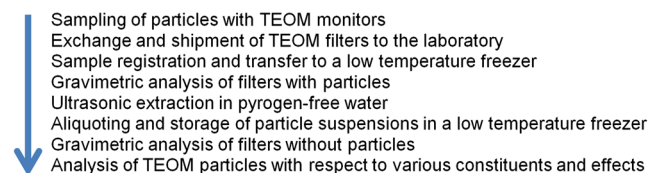
Differences between groups were calculated with the Student's *t* test and *p*-values less than 0.05 were considered as significant. The change of various variables during the period February to April was calculated by one-way ANOVA. The reason to choose this particular period was because the annual concentration of particles then peaked and that the particles then are considered as especially important. Correlations were calculated with Pearson's test and expressed as Pearson's correlation coefficients. All calculations and constructions of Graphs were performed with the software Graph Pad Prism 4.

## Results

### Particle sampling and sample preparation

The principal aim of the study was to try out a methodology in which particles on TEOM filters are extracted and used for various chemical and biological tests. The different steps of the sampling and sample preparation procedure are shown in Fig. 2. Filter exchange was performed by technicians at the participating cities. In order to avoid contamination, the technicians received written instructions about how the exchange procedure should be performed. In the instructions, it was pointed out that during the filter change, it was crucial to use sterile nitrile gloves and carefully transfer the filters directly from the filter holders in the monitors to marked sterile 50 ml polypropylene tubes. These tubes were then sent to the laboratory by mail and normally arrived 1 day after they had been taken from the TEOM monitors. On arrival at the laboratory, each tube was registered and transferred to a low temperature freezer ( $-82\text{ }^{\circ}\text{C}$ ).

The sample preparation procedure was performed in different steps. First, the particle-containing filters were placed in the weighing room where they were acclimatized for 2 days before they were weighed. In a second step, the filters were extracted in 2 ml pyrogen-free water as described below. After the extraction, they were again placed in the weighing room to acclimatize before they were weighed a second time so that the differences between the two masses could be calculated.



**Fig. 2** Different steps of the procedure, from particle sampling to analysis

The mass of the extracted particles varied between 0.20 and 6.0 mg (the mean mass of particles from 71 filters was 1.63 mg). For weighing the filters, we also tested if an ordinary analytical balance could be used instead of the type of microbalance that we used. A reference filter was then weighed ten times both with a microbalance and an analytical balance (Precisa XR 205A). The results obtained for these two series of weighings were very similar ( $108.44 \pm 0.0027$  mg vs.  $108.5 \pm 0.048$  mg).

In this study, we wanted to study surface properties, bioactive components on the particles, and how particles affected living cells and it was important to preserve the particles as close to their original constitution as possible. We therefore used pyrogen-free water as extraction medium and the extraction procedure was tried out with respect to variables such as volume of extraction medium and extraction time. The extraction was done with a probe sonicator with the filters placed in sterile 50-ml polypropylene tubes. An appropriate volume was found to be 2 ml and this was sufficient to cover the whole filter and that volume did not result in an unnecessary dilution of the particle samples. Different extraction times were also tested out and after visual examination, it was apparent that extraction for 2 min was sufficient to detach the particles from the filters. Sonication generates heat and to avoid overheating, the extraction procedure was carried out in two 1-min cycles with a 10-s pause in between. During extraction, the tubes with filters were also chilled in a beaker with ice and the probe was submerged between 3 to 5 mm in the extraction media. The tip of the probe was also placed on the back side of the filter to avoid direct contact with the particle-containing side. Before extraction of each sample, the probe was cleaned with 70% ethanol and deionized sterile water. A practical number of samples that would be handled simultaneously during one weighing and extraction procedure were tested out and found to be between 15 and 20. Directly after extraction, the particle suspension was divided into six 300  $\mu$ l aliquots that were frozen in  $-82$  °C until the different analyses could be performed. Before analysis, the samples were diluted as described in the “Materials and methods” section.

### Variation in particle mass, composition, and reactivity at two sites during 21 months

The monthly variation in the mass of particles extracted from filters at site 1 and site 2 is shown in Fig. 3. The monthly average mass of particles on filters from the whole period ( $n = 21$ ) was  $1.57 \pm 0.63$  mg (site 1) and  $1.30 \pm 0.43$  mg (site 2) and there was a fourfold difference between the months with the lowest and the highest mass. At site 2, a location in northern Sweden with a lower mean temperature, the highest particle mass was found on filters from May whereas the highest mass from the more southerly site 1 was found on filters from March.

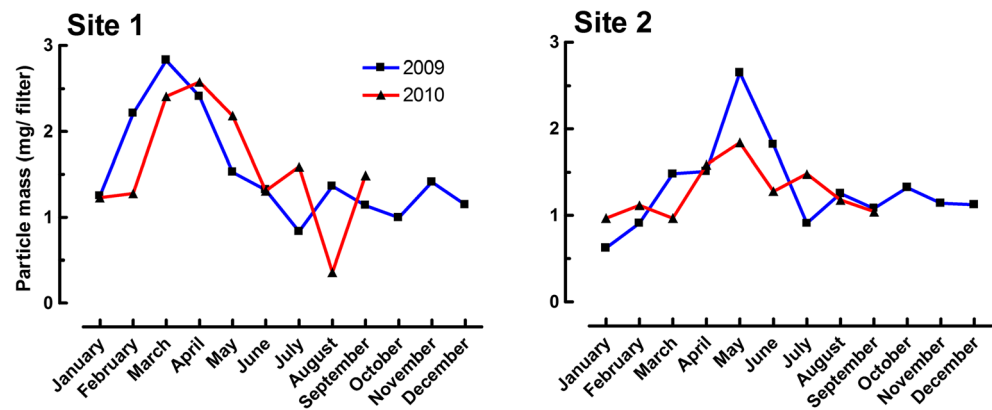
Figure 4 shows the oxidative capacity (oxidation of glutathione) of particles from sites 1 and 2. When both sites were compared, the yearly mean oxidative capacity for all samples ( $n = 21$ ) was higher at site 1 than at site 2 ( $30.5 \pm 8.3$  vs.  $18.3 \pm 6.7\%$  oxGSH (oxidized glutathione),  $p < 0.001$ ). At site 1, the highest activities were found during the winter and summer months and the lowest activity was found in particles collected in spring. A similar pattern was also seen at site 2, but here the increase during summer was less pronounced. Thus, the highest oxidative capacity was found on particles collected during winter and summer, that is, the months with lower concentrations of particles in the air (Fig. 3). There was also an inverse significant correlation between oxidative capacity and mass of particles on filters both at site 1 ( $r = -0.63$ ,  $p = 0.002$ ) and site 2 ( $r = -0.44$ ,  $p = 0.04$ ).

The cell-activating effect and endotoxin content in particles collected at sites 1 and 2 are shown in Fig. 5. The cell-activating effect of the particles was studied using a “monocyte activating test” and formation of the pro-inflammatory cytokine interleukin-1 $\beta$  (expressed as zymosan equivalents,  $\mu$ g/ml). The general trend for both years and both sites was that the activity was lowest during the winter months and that it increased during spring and summer. The average mean formation of interleukin-1 $\beta$  for all samples was significantly higher at site 1 than at site 2 ( $12.0 \pm 1.6$  and  $8.7 \pm 2.4$  zymosan equivalents,  $\mu$ g/ml,  $p < 0.0001$ ). The endotoxin content varied between less than 0.5 to over 10 ng/mg particles (Fig. 5, lower part). The average levels were also higher at site 1 than at site 2 ( $4.4 \pm 3.1$  ng/mg and  $2.5 \pm 2.0$  ng/mg,  $p < 0.02$ ). Similar to the cell-activating effect, the endotoxin levels increased during spring and peaked during summer. There was a significant correlation between the cell-activating effect and endotoxin content ( $r = 0.67$ ,  $p < 0.0001$ ,  $n = 42$ ). At both sites, the monthly mean temperature correlated significantly with both the cell-activating effect and endotoxin content (Fig. 6).

### Variation in particles collected at all sampling sites during February to April

Figure 7 shows the variation of the mass of the extracted particles, oxidizing capacity, endotoxin content, and cell-activating effect in 41 samples collected from nine different sites between February and April. This period was of special interest as it covers conditions with winter road care e.g., de-icing with road salt and spreading sand and then during spring, the road surface has dried and most of the particles that have been trapped on the road surface during winter become airborne. During this period there was a significant increase in the mass of particles extracted from the filters (from  $1.5 \pm 0.8$  to  $2.9 \pm 1.6$  mg,  $p = 0.013$ ). The opposite was true for the oxidizing capacity which decreased (from  $29.6 \pm 14.1$  to  $6.4 \pm 6.3\%$  oxGSH,  $p < 0.001$ ). The endotoxin content increased significantly during the same time period (from  $0.8 \pm 0.5$  to

**Fig. 3** The mass of particles extracted from TEOM filters collected at sites 1 and 2



$2.6 \pm 1.8$  ng/mg,  $p < 0.0001$ ). An increase, however not significant, was also found for the cell-activating effect (from  $7.5 \pm 3.7$  to  $9.7 \pm 1.0$  zymosan equivalents,  $\mu\text{g/ml}$ ,  $p = 0.06$ ).

## Discussion

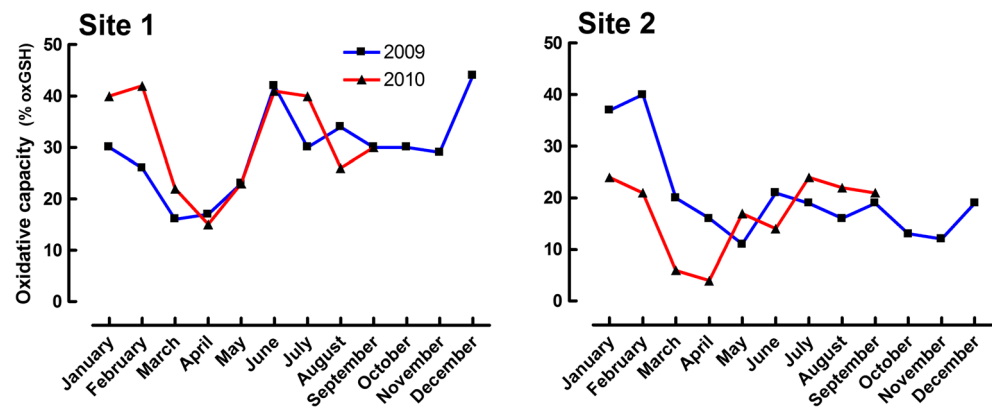
The technicians that performed the exchange of filters were employed by the local community and were trained to maintain the TEOM monitors. It was therefore easy to instruct them to make the relatively small changes in their normal routine so that the filter exchange could be performed without any risk of contamination. After being sent by mail, the filters normally arrived at the laboratory after 1 day. The overall impression was that the communication with the technicians, filter-changing procedure, and shipment of the samples worked well. In the laboratory, the tubes with filters were registered on the same day they arrived and were then transferred to a freezer. A low temperature freezer was chosen because filters were collected during a relatively long time period and we also wanted to analyze the biological activity of the particles. However, if the particles are going to be analyzed on stable constituents such as metals, the samples could just as well be stored in an ordinary freezer.

We used water as extraction medium as we wanted to preserve the surface as much as possible when our aim was to

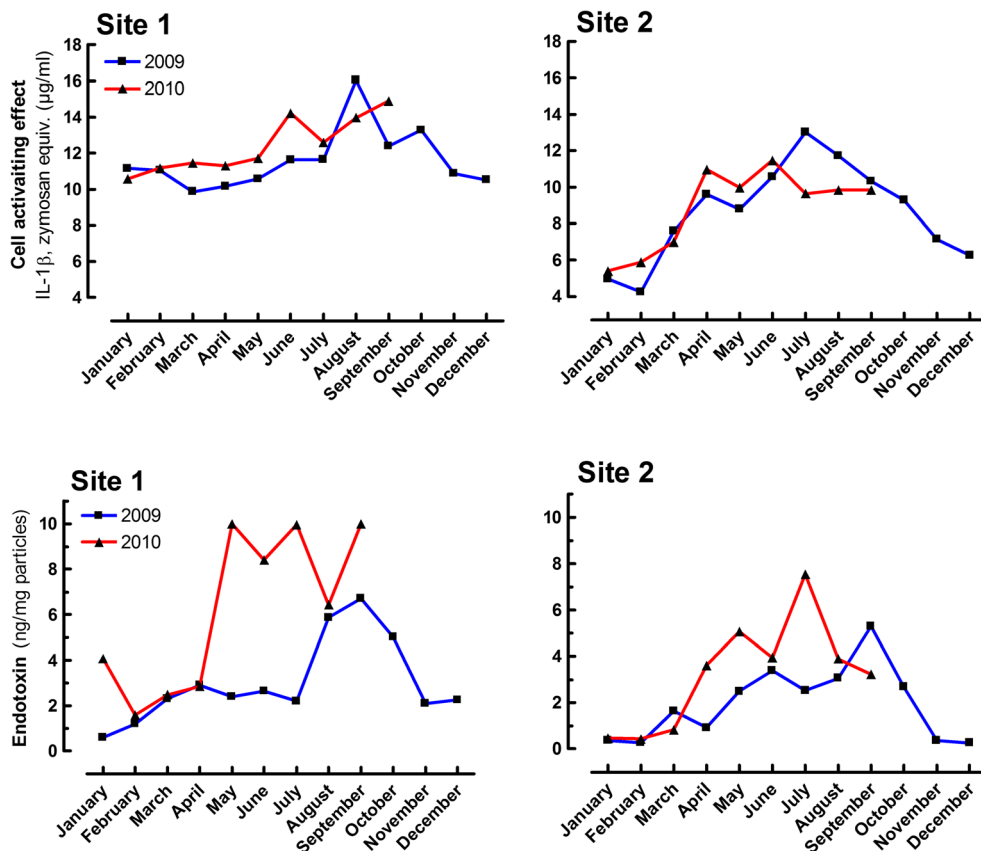
analyze the endotoxin content, the oxidative capacity of the particle surface and how the particles affected inflammatory cells. Water, which is a gentle extraction media, could be used if the intention is to also analyze polycyclic aromatic hydrocarbons or other organic constituents on the particles. The particle-water suspension must then however be extracted further with toluene or a similar organic solvent before analysis. After extraction, the particle suspensions were divided into six aliquots. Each of these could then be analyzed directly after dilution to a concentration of  $100 \mu\text{g/ml}$  or diluted further with 1.8% NaCl and used for studying the effect on cells. This way of dividing the sample gave the possibility to analyze the samples at different time points. If it is desirable, this also opens up the possibility to easily distribute samples to other laboratories.

To test the usability of the method, we used particles that had been collected from nine different sampling sites. These sites differed considerably with respect to variables such as annual mean temperature and the presence of snow. The highest particle mass was recorded during spring. The reason for this is probably due to the fact that the vast majority of airborne particles during spring originate from road wear and other materials that have accumulated on the wet and icy roads during the winter months. During spring, the road surface dries out and then it is more likely that surface-bound particles become airborne. The fact that the particle masses are higher during spring and that more particles become airborne as the

**Fig. 4** Oxidative capacity of particles collected at sites 1 and 2



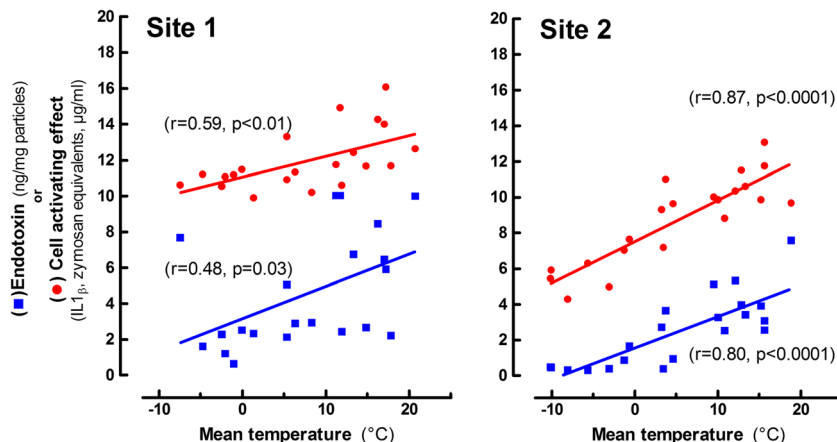
**Fig. 5** Cell-activating effect (top) and endotoxin content (bottom) in particles collected at sites 1 and 2. Mean of two measurements



roads dry after winter has also previously been shown by Johansson et al. (2007). On the contrary, the ability to oxidize glutathione was lowest on particles collected during spring (Fig. 4). The reason for the higher oxidative capacity during winter and summer could be due to the fact that the vast majority of the particles that become airborne during these seasons are more freshly produced and that they therefore have a surface that is more prone to donate electrons and generate oxidants that could oxidize glutathione. Another factor that could impact the surface reactivity could be proximity to traffic and particles collected close to roads have previously

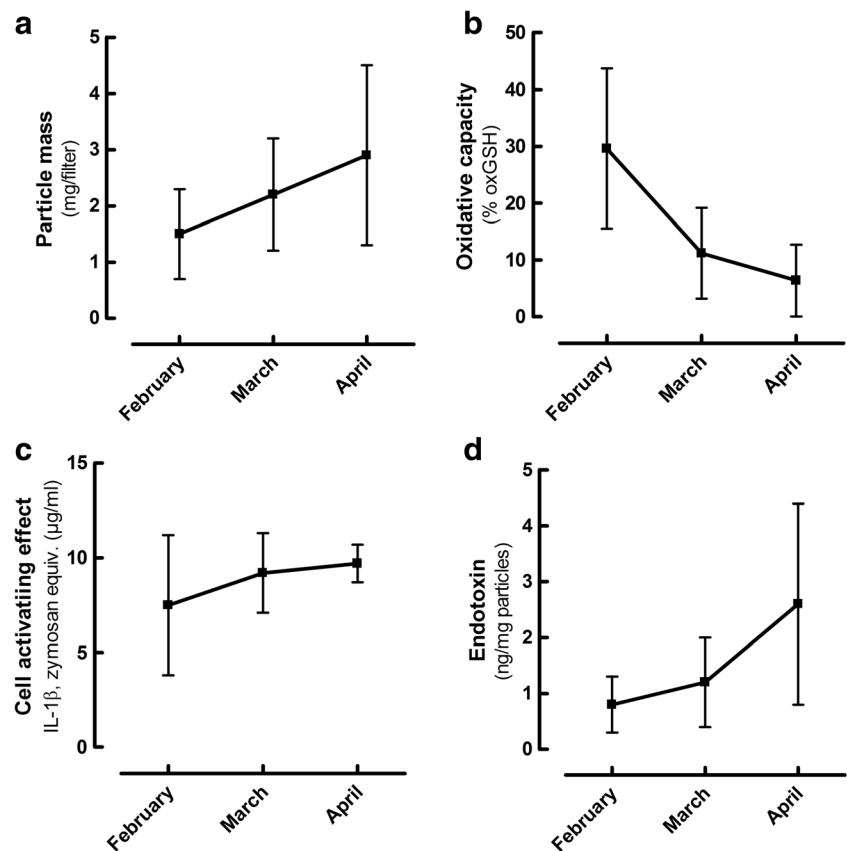
been shown to have a greater oxidative capacity than particles collected further away (Boogaard et al. 2012). Particles from site 1 had both a higher mean endotoxin level and cell-activating effect than particles from site 2. It was also apparent that both these increased significantly with the average monthly temperature (Fig. 6). The fact that the endotoxin levels are significantly higher during the warmer season has previously also been shown by others (Carty et al. 2003; Salonen et al. 2004). Higher endotoxin levels and pro-inflammatory response (interleukin-6 and nitric oxide), used as a proxy for cell-activating effects of particles collected during the warmer

**Fig. 6** Endotoxin content (blue squares) and cell-activating effect (red circles) in relation to mean temperature (monthly average) at sites 1 and 2. For further details, see the “Materials and methods” section





**Fig. 7** Mass of particles on filters, oxidative capacity, cell-activating effect, and endotoxin content in samples from nine sites collected from February to April. Mean and standard deviation



season, has also been found by Jalava et al. (2015). It was then also concluded that not only endotoxin but also other microbiological components may have a significant effect for the toxicity. The fact that there was a seasonal variation in the variables that were measured in our study was not unexpected. Seasonal variations in particle mass and levels of various other pollutants have also been reported by others and are due to variables such as weather conditions, residential biomass burning, pollution events, and other factors (Heinrich et al. 2003; He et al. 2018).

TEOM monitors require a relatively limited amount of maintenance and are also managed by trained technicians that ensure that they are operating correctly and that filters are changed using a standardized protocol and at regular intervals. These monitors are also being used in many countries and this makes it possible to compare particles from filters that have been collected at sampling sites located far apart (regions or continents) and during long periods of time. A big advantage here is also that all TEOM monitors do collect particles in exactly the same way and on the same type of filter. They could however be equipped with different sampling heads and then either collect PM<sub>10</sub>, PM<sub>2.5</sub>, or PM<sub>1</sub>. The size range is therefore fixed and it is possible to compare results from tests of TEOM particles of a certain size range with results of the same type of tests carried out on particles collected with other sampling methods (filter-pump configurations). To our

knowledge, only a few studies of particles from TEOM monitors have been performed previously. Jones and co-workers studied the presence of various salts and metals on particles collected with TEOM monitors stationed in south Wales (Jones et al. 2001) and the mineralogical content of TEOM particles has also been analyzed on the filters in situ with QEMSCAN technique (Williamson et al. 2013). We show here that it is also possible to analyze other variables. These were surface reactivity and content of and effect of pyrogens but, depending on the extraction medium (water or organic solvents), a number of other constituents on or in the particles or various biological or toxic effects could be studied.

A limitation in this study is that we analyzed a relatively small number of filters. From site 1 and site 2, we had access two filters for the whole 21-month period. The other sites did send in filters for most of this period but we chose not to analyze all of them. Instead we chose to analyze as many filters as we had from the period February to April. This is when we normally have the highest levels of particles and it was therefore considered as especially important. It had been better to have analyzed particles from more filters. Another limitation is the lack of information about the proportion of different particle sizes. We only obtained PM<sub>10</sub> particles and had no information about the ratio of PM<sub>10</sub>/PM<sub>2.5</sub> or PM<sub>10</sub>/PM<sub>1</sub>. We were thus not able to discriminate between coarse or fine respirable particles and that would have been an advantage. The monitors were all placed close to a trafficked

street or road and the particle source has therefore mostly been the traffic. Wear particles are generally bigger than tail-pipe (combustion) particles and the proportion between these two could have differed at different time points during the year. A third limitation is that we chose to analyze only three variables and those were related to surface reactivity and the content or effect of pyrogens. It is of course possible to also analyze many other variables. These could include metal content or the level of polycyclic aromatic hydrocarbon or a more specified analysis of organic and inorganic carbon that could give information about the particle sources. It is also possible to perform a more thorough toxicological testing of various effects that the particles could have on living cells. However, here instead we wanted to focus primarily on the use of TEOM monitors as samplers and the collection, extraction, and sample preparation procedure. The analysis of particles with respect to very many different characteristics was not a major goal.

## Conclusion

Our study shows that it is possible to use TEOM monitors, not only as detectors of ambient particle concentration, but also as particle samplers. TEOM filters could then be collected in a cost-efficient way and after extraction, the TEOM particles could be analyzed as regards variables that reflect their toxicity. The results could, if combined with information about the ambient particle concentration, give valuable information about possible health risks due to breathing the air at various locations.

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