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Monthly variation in masses, metals and endotoxin content as well as pro-inflammatory response of airborne particles collected by TEOM monitors

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Abstract

Particle exposure has been linked to an increased incidence of cardiovascular disease. Furthermore, particle exposure has been shown to have a chronic inhibitory effect on lung development in young people and may result in increased respiratory problems in adults or children with respiratory-related diseases. In today's urban environments, particle levels are mainly monitored gravimetrically; however, other factors such as particle size, shape and surface reactivity have recently been noted as highly important in relation to possible health outcomes. Here, particles from TEOM monitor filters placed in three different cities were studied. The purpose of the study was to investigate whether there are variations in particle masses, cadmium and lead contents, as well as endotoxin levels between locations and time points over the year and if this can be correlated to the particles ability to induce a pro-inflammatory response in vitro. Results showed that it is possible to detect variations at different locations and at different time points over the year and that cadmium, lead and endotoxin levels did not coincide with the increased total particle masses while endotoxin levels coincided with pro-inflammatory responses in vitro. The present study shows that filter analysis is a useful complement to gravimetric or particle-counting measurements in studies of particle-related health effects and will give useful information regarding future air quality measurements.

Keywords Particles · PM10 · Exposure · TEOM · Metals · Endotoxins · Pro-inflammatory response

Introduction

A number of studies have shown that exposure to air pollution increases the risk of mortality in lung as well as cardiovascular diseases (Pope et al. 2018; Stockfelt et al. 2017; Fiordelisi et al. 2017). The term air pollution, however, includes a variety of substances. In Sweden's air quality regulation (SFS 2010:477), air pollution includes the presence and air content of nitrogen dioxide, sulphur dioxide, particles (PM10 and PM 2.5), benzene, carbon monoxide, ozone, polyaromatic hydrocarbons (with benzo (a) pyrene as indicator) and the metals arsenic, lead, nickel and cadmium. In connection with the introduction of environmental quality standards, particles have been recognised as an increasing air pollution problem (IVL Swedish Environmental Research Institute 2016). Particle exposure, in addition to the increased incidence of cardiovascular disease, has been shown to have a chronic inhibitory effect on lung development in young people between 10 and 18 years of age and may result in increased respiratory problems in children with respiratory-related diseases (Gauderman et al. 2004; O'Connor et al. 2008).

In today's urban environments, particle levels are mainly monitored gravimetrically, which means that the particle contents are based on mass. However, other factors such as particle size, shape and surface reactivity have recently been noted as highly important in relation to possible health outcomes (Golokhvast et al. 2015; Steenhof et al. 2011; Ristovski et al. 2012). These factors are likely to be of interest since small particles may end up deeper in the lungs and have a larger combined surface area compared with large particles. Variation in particle properties have also been shown to be of importance for the degree of toxicity and inflammation-inducing potential which has been demonstrated for wear

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particles from tires and road surfaces (Araujo 2010; Lindbom et al. 2006, 2007; Karlsson et al. 2011; Gustafsson et al. 2008; Boogaard et al. 2012).

This study is based on a previous study, where the possibility of using filters from existing TEOM stations for further characterisation of particle properties was explored (Nosratabadi et al. 2019). The earlier study showed that the particle masses peaked in March/April while the oxidative capacity of the particles was at its highest early in the spring and the content of endotoxins and the pro-inflammatory response were highest in late summer. This indicates that the source and composition of the particles are different over the year and may pose different health risks (Nosratabadi et al. 2019). In the present study, a more in-depth analysis of TEOM filters collected monthly in three cities in the southeast part of Sweden are presented. The main purpose of the study was to investigate whether there are variations in particle masses, metal contents (Cd, Pb) and endotoxins between locations and time points over the year and if this can be correlated to the particles ability to induce pro-inflammatory response in vitro.

Methods

Particulate matter (PM10) in air

Particulate matter with a size less than 10 μm (PM10) were collected on filters by TEOM monitors. Filters were collected at four sites located in three different cities in the southern part of Sweden during 1 year (September 2014–August 2015). In detail, particles have been collected from two different TEOM stations placed in two different streets (street 1 "Kungsgatan" and street 2 "Promenaden") in a city connected to the Baltic Sea via a bay, (city 1, Norrköping, 96,500 inhabitants), from one TEOM station placed in an inland city, (city 2, Linköping, 100,000 inhabitants) and from one TEOM station placed in a smaller city located in direct connection to the Baltic Sea (city 3, Kalmar, 36,500 inhabitants). From street 2 in city 1, a similar filter collection has previously been done for one year (September 2009–August 2010).

Mean monthly values for the ambient particle concentrations were calculated based on data from the TEOM monitors that had been sent to a national database handled by the Swedish Meteorological and Hydrological Institute (SMHI). Information regarding temperatures in the three cities was collected from open data provided by SMHI.

Extraction of particles from TEOM filters

The TEOM monitors and the collection of particles from the TEOM filter are described in detail in a previous study (Nosratabadi et al. 2019). In short, TEOM monitors, which are used worldwide, measure the ambient concentration of particles (PM10 or PM2.5) in real time. TEOM monitors are gravimetric instruments that draws ambient air through a filter at a constant flow rate (3 L/min), while continuously weighing the filter, measuring ambient particulate mass concentrations (Patashnick and Rupprecht 1991). Replacement of the TEOM filters was carried out according to instructions every month for 1 year (September 2014–August 2015) by personnel from the respective municipality. A total of 48 filters were collected from the four TEOM stations. After each replacement, the TEOM filters were sent to the Occupational and Environmental Medicine laboratory at the Linköping University Hospital. Upon arrival at the laboratory, each sample was recorded and the tube with filters was then placed in a low temperature freeze (− 70 °C) while awaiting weighing, particle extraction and analysis.

To determine the mass of the particles, each filter was weighed with a precision microbalance with a reading precision of 10 μg (Sartorius Micro MC5 P). Before the weighing, each filter was stored for 48 h in a room with a controlled temperature and humidity (24 °C and 55% ± 2%). At the same time, two unexposed control filters were also weighed. The weighing was performed twice, before and after the extraction to calculate the mass of particles released during the extraction.

After the first weighing, each filter was placed in a sterile 50-mL Falcon tube and 2 mL of endotoxin-free water was added (Braun Malsungen AG, Germany). To extract the particles from the filter, an ultrasonic probe was used for 5 × 10 s per filter (MSE Soniprep 150 Ultrasonic Disintegrator, Heathfield, UK). The suspension with extracted particle was allocated in fractions of 500 μL and frozen at − 30 °C pending analysis.

Metal analysis

For determination of relative amounts of lead and cadmium in the samples, an atomic absorption spectrophotometry (ContrAA 700 with graphite furnace, Analytik Jena AG, Jena, Germany) was used. The frozen samples were thawed at room temperature and transferred to a Teflon tube. The Teflon tubes were placed in a heating cabinet (50 °C) for 15 h until dried, then 300 μL of nitric acid and 100 μL of water were added to each tube. The Teflon tubes were then incubated in a heating cabinet (80 °C) overnight for 16 h. After incubation, 600 μL of deionised water was added to each tube and mixed for 5 s using a vortex before the analysis.

Endotoxins

The endotoxin content on each filter was analysed using a specific Limulus assay (Pierce™ LAL Chromogenic endotoxin quantitation kit, Thermo Fisher Scientific, Waltham, MA, USA) according to the manufacturer's instructions. Briefly, 10 µg of particles in suspension and standard was added to wells of a 96-well plate and incubated for 5 min at 37 °C. To this was added 50 µL of limulus amebocyte lysate (LAL) and the plate was incubated for 10 min before a chromogenic substrate was added followed by 6 min incubation at 37 °C. The endotoxin levels were determined using a spectrophotometer measuring at 405 nm (FLUOstar, BMG Labtech, Ortenburg, Germany).

Pro-inflammatory response

The potential for the collected particles to induce a pro-inflammatory response was measured by incubating plasma from a healthy donor with particles and then measuring the formed content of IL-1β with a commercial ELISA kit (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. Briefly, 100 µL of whole blood was mixed with 20 µg of extracted particles and incubated for 18 h at 37 °C. The following day, the sample was centrifuged at 14,000*g* for 5 min. Residual supernatants (blood plasma) were analysed with ELISA kits in which antibodies bound in a 96-well plate bind to IL-1β and, via an enzymatic method, dye is read at 450 nm (FLUOstar, BMG, Germany).

Statistics

Differences between the cities were investigated using Friedman test with Dunns multiple comparison post-test. Differences between the two measured periods at the same location were analysed by Wilcoxon matched pairs test. A p value below 0.05 was considered significant.

To investigate the different measures variability over the year, a multivariate model (OPLS-DA) was created using SIMCA 15.

Results

Comparison of the three cities

Particle levels (PM10) in air were calculated gravimetrically using TEOM stations and are reported as monthly mean values in Fig. 1a. These values were calculated from data sent from the TEOM monitors and collected in a national database at the Swedish Meteorological and Hydrological Institute (SMHI). There were significantly higher particle levels in city 1 location 1 and city 2 compared with city 3 in pair-wise monthly comparisons ($p < 0.05$).

The average monthly temperature differed between the cities where city 2,which is located inland, showed significantly lower temperature than both locations in city 1 ($p < 0{,}01$) and city 3 ($p < 0.001$, Fig. 1b). These differences were however very small in absolute numbers as can be seen in Fig. 1b.

Extracted particles from the TEOM filters were analysed for their relative metal content. Cadmium levels were significantly higher in city 3 compared with city 1 location 1 ($p < 0.001$) as well as city 2 ($p < 0.01$, Fig. 1c). For lead, city 1 location 2 showed significantly higher levels compared with both city 1 location 1 ($p < 0.05$) and city 2 ($p < 0.01$, Fig. 1d). Lead levels in city 3 showed a clear increase compared with city 2 and city 1 location 1 but this was not statistical significant.

Endotoxin tests and pro-inflammatory responses via IL-1β analysis showed a similar trend comparing all locations over the year but no significant differences between the cities was found (Fig. 1e, f). However, a positive correlation could be seen between the levels of endotoxin and IL-1β when comparing all measures (Spearman correlation, $R = 0.5$, $p < 0.001$).

Co-variation over the year

The co-variation of the investigated variables over the year was investigated using a multivariate model with OPLS-DA. This model aims at separating a set of y-variables (in this case, the different months) using a set of x-variables (in this case, the measures of particle mass, temperature, metal levels, endotoxin content and IL-1β) that allows for detecting underlying co-variation. This co-variation can be seen as a clustering of investigated y- and x-variables in a two-dimensional plane in a so-called loading plot of the investigated factors. As can be seen in Fig. 2, the endotoxin content and pro-inflammatory response (IL-1β) showed the highest levels during the summer months (May to August), illustrated as a clustering to the right in the image. The PM10 particle concentrations were highest in March, while both the lead and cadmium concentrations on the TEOM filters were higher during the winter months (especially November, seen as clustering to the bottom left).

Time trend of particles and metal content in city 1

At location 2 in city 1, data was available from 5 years prior to the current study, which allowed a comparison of time trends at this location. Particle concentrations were in general lower 2014–2015 compared with 2009–2010, and the highest peaks occurred in April 2010 and in March 2015 (Fig. 3a). The delay in 2010 may be explained by that the temperature was in general higher during 2014–2015 (Fig. 3b). When looking at the metal content of the collected particles, cadmium showed a significant and lead a close-to-significant decrease years 2014–2015 compared with 2009–2010 (Fig. 3c, d). Interestingly, the highest peaks of the metals occurred in November to February in 2009–2010, a period when the total particle masses collected by the TEOM station were relatively low.

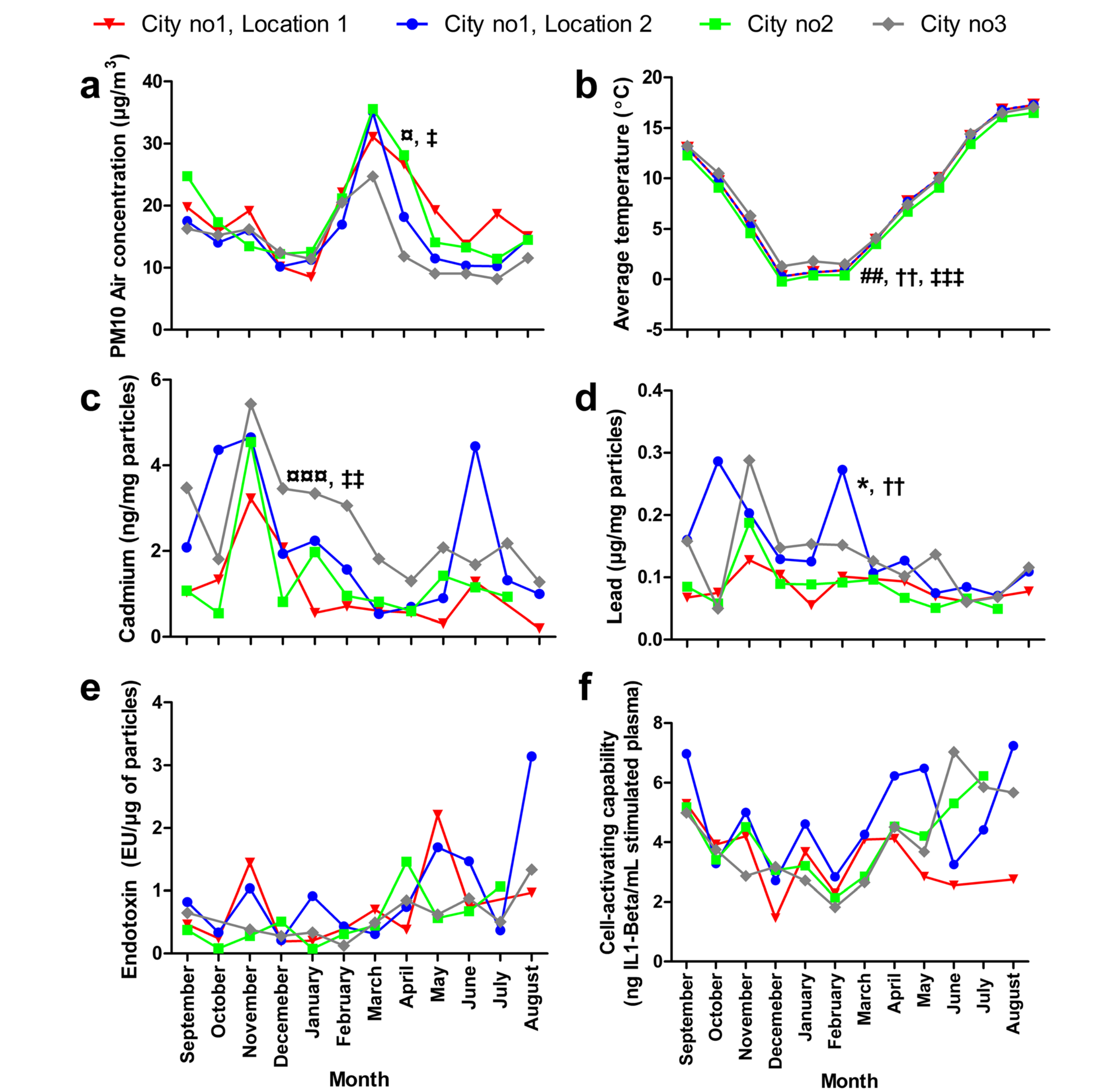

Fig. 1 Comparison of monthly values at four different sites in three different cities. **a** PM10 particle concentrations (μg/m^3) in air as reported by the TEOM stations. **b** Average temperatures. **c** Concentration of cadmium in the collected dust from the TEOM stations. **d** Concentration of lead in the collected dust from the TEOM stations. **e** Endotoxin levels in the collected dust from the TEOM stations. **f** Pro-inflammatory response measured by the amount of IL-1β produced after incubation of human plasma over night with collected particles. $^{*}p < 0.05$ city 1 location 1 vs location 2, $^{\#\#}p < 0.01$ city 1 location 1 vs city 2, $^{¤/¤¤¤}p < 0.05/0.001$ city 1 location 1 vs city 3, $^{††}p < 0.01$ city 1 location 2 vs city 2, $^{‡/‡‡/‡‡‡}p < 0.05/0.01/0.001$ city 2 vs city 3 as determined by Friedman's test with Dunn's multiple comparison post-test

Discussion

Particles in air

Particulate matter (PM10) masses in air were calculated during the period September 2014–August 2015, based on information from TEOM stations located in three different cities in Sweden (Fig. 1a). The highest monthly averages for PM10 in the four locations coincided in March 2015. The two measuring locations in city 1 (Norrköping) showed the highest monthly averages that approached 40 μg/m^3, while the monthly average in city 2 (Linköping) reached just over 30 μg/m^3 followed by city 3 (Kalmar) with about 25 μg/m^3. High levels of airborne particles in the spring have for a long time been considered a problem in Nordic countries (Nosratabadi et al. 2019). However, the timing of when the highest levels occur during the spring may vary slightly from year to year depending on the weather as can be seen in Fig. 3a, b. The

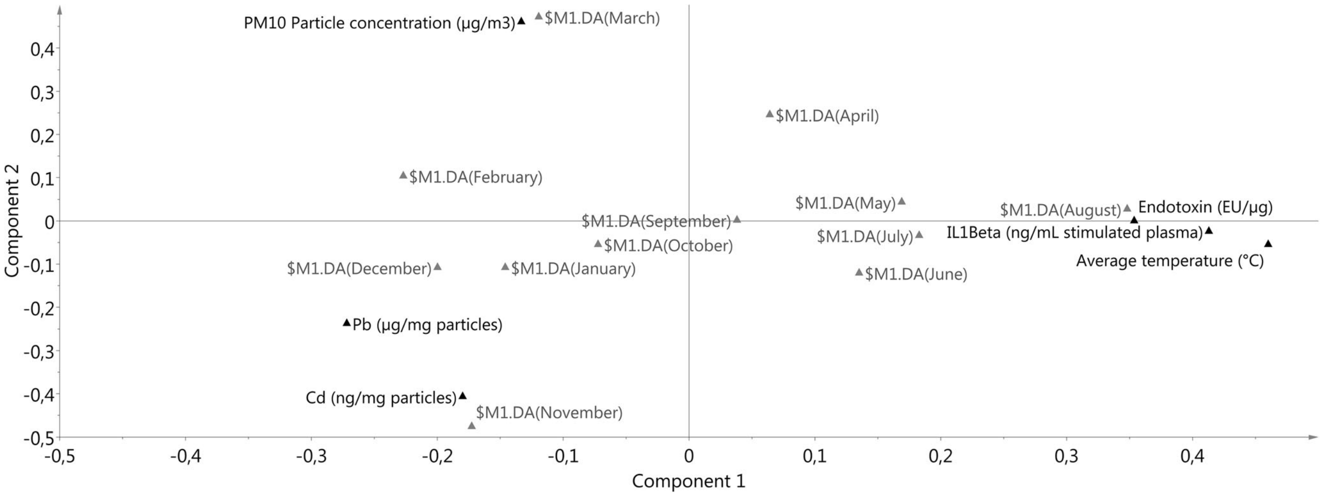

Fig. 2 Loading plot of the multivariate modelling of the investigated variables (black triangles) at the four TEOM stations and their variability during the year (months in grey triangles). Variables and months clustering indicate an underlying pattern of increased level of the variable during that month. The *x*-axis of the plot is the so-called predictive component that explains most of the variability between months while the *y*-axis is the orthogonal component indicating further variability.

high particle levels in the spring are to a high extent resulting from wear particles that are formed during friction between studded tires and roadway during the winter. When spring arrives and the road becomes dry, generated particles are free to become airborne and thereby inhaled (Gustafsson et al. 2008). PM10 in air is a rough measure of possible particle-

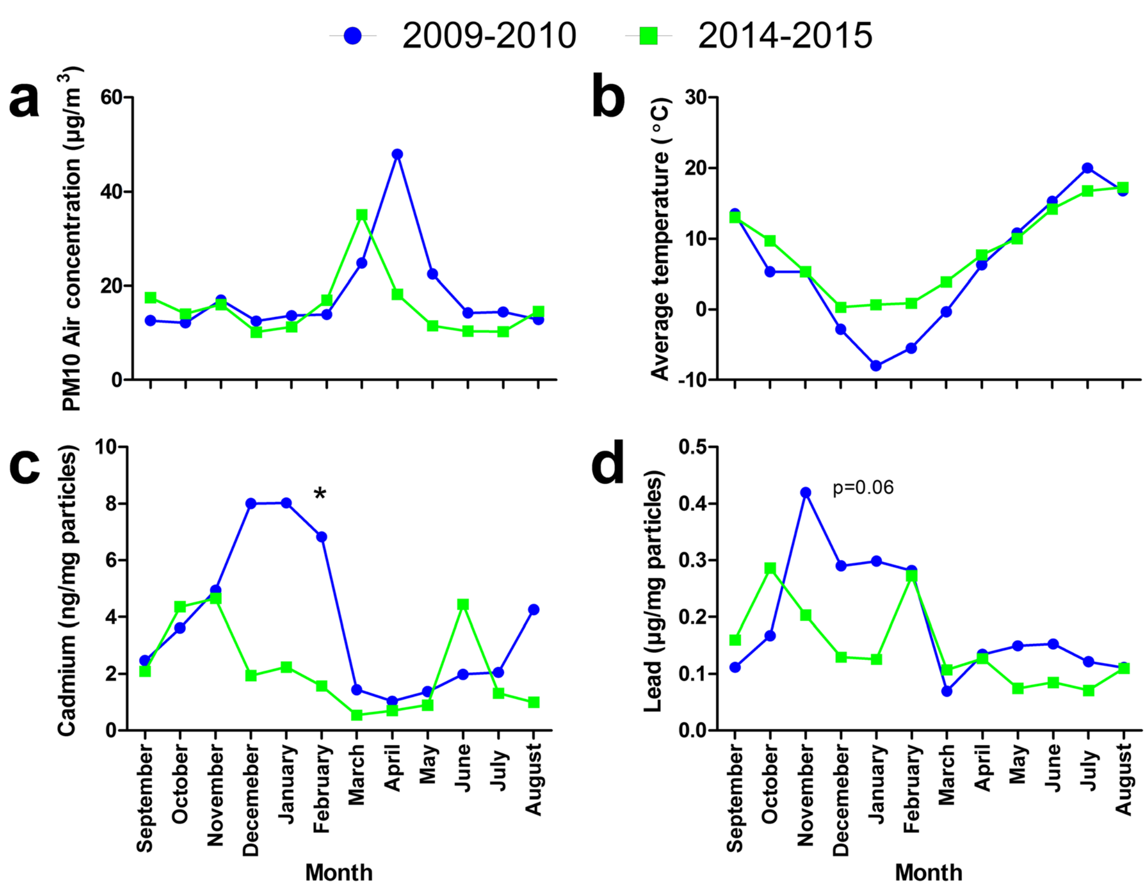

Fig. 3 Comparison of PM10 particle concentrations, temperature, cadmium and lead content years 2009–2010 and 2014–2015 in city 1 at location 2. **a** Monthly mean values of particle concentration (µg/m^3) in air as reported by the TEOM stations. **b** Average temperature as collected from open data hosted by the Swedish Meteorological and Hydrological Institute (SMHI). **c** Concentration of cadmium in the collected dust from the TEOM stations. **d** Concentration of lead in the collected dust from the TEOM stations. $^{*}p < 0.05$

related health effects since recent research has shown that smaller combustion particles may be more relevant to study (Xing et al. 2016) and that it is also of great importance what element the particles consist of and what they carry on their surface (Ali et al. 2018). The reason why monthly averages are used in the present study is that these values can be related to the monthly filter changes and thus the particle masses. Unfortunately, monthly average values cannot be directly related to the environmental quality standard (EQS), but they can reflect measures taken by authorities responsible for air quality in the street environments. None of the cities participating in this study exceeded EQS during the study period 2014–2015 but Fig. 3 a reflects the lowering of airborne particles in city 1 location 2, comparing the two periods 2009–2010 with 2014–2015. This lowering is probably due to performed action plans to reduce particles after previous overruns of EQS in city 1 in years 2005 and 2009.

Metal content of collected particles

Filters from all locations showed a similar trend in cadmium and lead levels over the year (Fig. 1c, d). Interestingly, elevated cadmium level did not coincide with monthly mean values for PM10 levels in air and was surprisingly higher in the smaller city 3 than in the larger city 1 and city 2. The origin of this cadmium has to be further studied but according to the Swedish Environmental Health Report 2017 (administered by the Public Health Agency of Sweden), participants in this particular region report that they are disturbed by smoke from private wood burning. Furthermore, the city also has metallurgical industry as well as shipping activities that may contribute to cadmium levels. Lead on the other hand showed a significant increase in city 1 location 2 compared with city 1 location 1 as well as to city 2. The differences found within city 1 may depend on that location 2 has more traffic (58% more as analysed by the municipality) and that it is located closer to a larger industrial area as well as shipping activities. The latter is interesting in the light of the fact that city 3 that had a clear but non-significant increase of lead compared with city 1 location 1 and city 2 also has shipping activities that may contribute to the detected levels on the TEOM filters.

However, in city 1 location 2, the levels of cadmium years 2014–2015 were significantly reduced compared with years 2009–2010 (Fig. 3c, $p < 0.05$). Lead showed a similar trend although not significant (Fig. 3d). Lead and cadmium are included in the Swedish air quality regulation (SFS 2010: 477). The regulation states, in order to protect human health, the annual mean levels of cadmium should not exceed 5 ng/m^3. In addition, lead should not exceed an annual mean value of 0.5 μg/m^3. These units are not directly comparable with the units in the present study, but it can be clearly seen in Fig. 3 that both lead and cadmium content per particle dust mass have decreased comparing years 2014–2015 with years 2009–2010. Metal measurements in moss in this area during the period 1985–2015 support our findings regarding lead and cadmium, showing a decreasing trend (IVL Swedish Environmental Research Institute 2016).

Endotoxins

Endotoxins are bacterial components often found in our immediate environment and when we are exposed, the immune system reacts strongly to be prepared for a possible infection (Levels et al. 2011). The internalisation of endotoxins in macrophages and endothelial cells results in local production of inflammatory cytokines with subsequent migration of inflammatory cells into the lung and the penetration of cytokines into the blood (Rylander 2002).

Endotoxin exposure is a well-known health risk for, among other things, personnel handling organic waste or cutting fluids as well as agricultural workers. Chronic inhalation exposure has been linked to health effects including cough, shortness of breath, fever, headache and inflammation in various occupational settings (Liebers et al. 2008). Endotoxins can adhere to the surface of particles and accompany them into the lungs and the endotoxins may result in more pronounced immunological reactions than the particles themselves. It is thus of interest to know if there are endotoxins extracted with the particles from the TEOM filters and if the levels vary with time points or locations.

The highest peaks of endotoxins at the different locations were obtained late spring/summer, similar to what has been described before (Carty et al. 2003). During the summer, the particle levels are relatively low, indicating the importance of analysing also what the particles carry on their surface. These results confirm the previous study (Nosratabadi et al. 2019) and are realistic since at this time of the year, when it is warm and humid, conditions are particularly beneficial for bacterial growth (Fig. 1e).

Pro-inflammatory response

As a marker for pro-inflammatory response, the cytokine interleukin-1β (IL-1β) was analysed in the present study. To mimic an in vitro fever reaction, the abundance of IL-1β was measured in blood samples exposed to particle solutions from the different sites and time points. An increase in pro-inflammatory response could be seen during summer and early autumn in all cities, which largely coincided with the time points when the endotoxin levels were elevated in the collected particles (Fig. 1f).

These results indicate that it is important to know what the particles may carry on their surface and that it might be of interest to evaluate endotoxin occurrence in connection with particle content analysis in the future.

Limitations

Each TEOM monitor contains one filter that for this study was collected monthly for subsequent analysis. Thereby, only one filter per month has been available from each location. Exact instructions on how the filter change should be performed were distributed, but it cannot be ruled out that filters may have been contaminated in connection with handling.

No morphological characterisation was performed on the collected particles; thus, nothing can be concluded regarding location or seasonal variations in particle size or shape. The mass-based measurement methods did not allow analysis of nanoparticles and therefore, nothing can be said about their presence. In addition, the methods were not suitable for analysing hydrophobic substances such as polycyclic aromatic hydrocarbons (PAH), which would have been highly relevant.

The method used for digestion in this paper will not result in a total digestion of the silicates which might be present in the ambient air. The metal content might therefore be underestimated. However, for the metals presented in the present paper, previous studies have shown that a milder digestion is sufficient (Tursic et al. 2008).

Future perspectives

Measuring PM10 levels, which already show a decreasing trend in Sweden, is probably not an optimal tool for the future when assessing possible health outcomes. There are also indications on that the development in the automobile industry produces an increased amount of small combustion particles (Liu et al. 2013). Furthermore, there are reports showing increased addition of nanoparticles (e.g. Ce nanoparticles) in certain propellants (Erdakos et al. 2014) that may contribute to an increased risk of high nanoparticle levels in street spaces in the future. Studies of the smaller airborne particles that can move deeper into the lungs and that are able to carry a larger amount of substances on their surface probably give a better picture of possible health risks. In the future, increased measurements of PM2.5 should be considered or the use of particle-counting instruments equipped with a filter for particle surface component analyses.

Conclusion

The present study of PM10 particles collected on filters, as complement to particle mass calculation, confirms that improved knowledge regarding possible health effects from particle exposure is needed. By analysing filters from TEOM stations, we show that it is possible to detect variations at different locations and at different time points over the year. Interestingly, cadmium, lead and endotoxin levels did not coincide with the total particle masses while endotoxin levels coincided with inflammatory responses in vitro.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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