

UC Davis

UC Davis Previously Published Works

Title

Compositional variance in extracted particulate matter using different filter extraction techniques

Permalink

<https://escholarship.org/uc/item/6bw1w5v1>

Authors

Bein, KJ
Wexler, AS

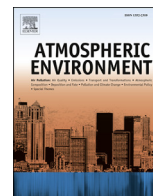
Publication Date

2015-04-01

DOI

10.1016/j.atmosenv.2015.02.026

Peer reviewed



Compositional variance in extracted particulate matter using different filter extraction techniques



K.J. Bein ^{a, b, c, *}, A.S. Wexler ^{a, c, d, e, f}

^a Air Quality Research Center, University of California, Davis, USA

^b Center for Health and the Environment, University of California, Davis, USA

^c Crocker Nuclear Laboratory, University of California, Davis, USA

^d Mechanical and Aeronautical Engineering, University of California, Davis, USA

^e Civil and Environmental Engineering, University of California, Davis, USA

^f Land, Air and Water Resources, University of California, Davis, USA

HIGHLIGHTS

- Ability to provide exhaustive chemical characterization of single PM extract.
- Significance of directly characterizing extracted PM for toxicological testing.
- Existence of substantial compositional biases between different extraction methods.
- Importance of standardizing PM extraction objectives and procedures.

ARTICLE INFO

Article history:

Received 7 October 2014

Received in revised form

5 February 2015

Accepted 9 February 2015

Available online 11 February 2015

Keywords:

Filter extraction

Filter sampling

Particulate matter extract

Particulate matter composition

Compositional bias

ABSTRACT

Collection and subsequent extraction of particulate matter (PM) from filter substrates is a common requirement for in vivo and in vitro toxicological studies, as well as chemical analyses such as ion chromatography and inductively coupled plasma mass spectrometry. Several filter extraction protocols exist and different laboratories employ different methods, potentially biasing inter-study comparisons. Previous studies have shown significant differences in extraction efficiency between techniques and identified the relevant extraction artifacts. However, a comprehensive inter-comparison of different methods based on the chemical composition of the extracted PM has never been conducted. In the current study, an exhaustive suite of chemical analyses is performed on PM extracted from glass micro-fiber filters using techniques commonly employed in different laboratories: Multi-solvent extraction (MSE) and spin-down extraction (SDE). PM samples were collected simultaneously during field studies conducted in an urban and rural setting using a high-volume PM_{2.5} sampler. Results show remarkable compositional variance between the PM extracts for all chemical components analyzed, including metals, water soluble ions, polycyclic aromatic hydrocarbons, non-aromatic organics, elemental carbon and organic carbon. Mass closure was greater than 90% for MSE but deviated substantially for SDE. Detailed retrospective gravimetric analysis of archived SDE samples revealed that a process-based loss of PM mass is the root cause of the differences. These losses are shown to be compositionally biased, both externally between different PM mixtures and internally within a given PM mixture. In combination, the results of this study are the first to demonstrate (i) an exhaustive chemical characterization of a single PM extract, (ii) the significance of directly characterizing the extracted PM used in toxicological studies, (iii) the existence of substantial compositional biases between different filter extraction techniques and (iv) the importance of standardizing filter extraction objectives and procedures to avoid introducing study bias into toxicological studies.

© 2015 Elsevier Ltd. All rights reserved.

1. Introduction

In air quality science, particulate matter (PM) is commonly sampled from an environment – e.g. the ambient atmosphere,

* Corresponding author. Air Quality Research Center, University of California, Davis, One Shields Avenue, Davis, CA 95616, USA.

E-mail address: kjbein@ucdavis.edu (K.J. Bein).

smokestacks, building interiors and laboratory generated exhaust streams – by drawing air across a filter, or some other type of substrate, to separate the PM from the gases. PM collected on filters can then be analyzed for composition using various analytical techniques and/or toxicity using *in vivo* and *in vitro* systems. In almost all cases, the PM must be removed, or extracted, from the filter prior to analysis. Depending on the objective, the filter extraction process can be exhaustive – i.e., maximizing the amount of total PM removed from the filter – or selective, i.e. extracting only certain PM components or compound classes.

For toxicological studies, the primary objective of filter extraction is to conserve, as much as possible, the physical and chemical properties of the PM as it originally existed in the atmosphere or exhaust stream – including particle size, number concentration, morphology and individual particle compositional and structural integrity – so that the results of these studies are representative of true population exposure. Currently, the most widely applied filter extraction technique involves sonication in ultra-pure water followed by lyophilization to remove the water and recover dry PM (Devlin, 2009; Bowser, 2009). The PM is then suspended in the delivery vehicle and sonicated and/or vortexed immediately prior to instillation or aspiration. Extraction efficiencies – i.e. the mass of PM removed by extraction relative to the mass of PM collected on the filter – on the order of 60–70% are commonly reported for this technique and this efficiency may be compositionally biased. Recently, an exhaustive, multi-solvent extraction (MSE) technique including sonication, liquid–liquid extraction, selective filtration and solvent removal was introduced, resulting in extraction efficiencies consistently exceeding 90% (Bein and Wexler, 2014).

Chemical composition studies, however, require a suite of analytical techniques given the chemical complexity of PM and each technique measures a certain class of compounds; e.g. metals via Inductively Coupled Plasma Mass Spectrometry (ICP-MS), inorganic ions via Ion chromatography (IC) or polycyclic aromatic hydrocarbons (PAHs) via Gas Chromatography Mass Spectrometry (GC-MS). In this case, the goal of filter extraction is to selectively extract certain compounds while minimizing co-extraction of potential interfering species to eliminate matrix effects – i.e. in complex multi-component systems, the presence of certain components can interfere with the detection of others, either synergistically or antagonistically – and thus each analytical technique typically requires its own filter extraction protocol. For example, trace element analysis via ICP-MS requires an initial organic solvent extraction followed by acid digestion using a strong acid (Herner et al., 2006). The initial organic solvent extraction is necessary for most combustion generated aerosol and/or secondary organic aerosol (SOA) since (i) the trace metals are typically encapsulated by layers of organic compounds and (ii) most organic compounds are hydrophobic and thus are not likely removed from the filter to any significant degree by water alone. Once the organic layers are removed, acid digestion dissociates the metal oxides and salts, bringing the metal ions into solution for analysis. Similarly, there are several different sample preparation protocols for molecular speciation of particulate organic carbon via GC-MS that are based on organic solvent extraction followed by post-extraction cleanup steps to dissolve the organics into solution and separate them from the particle matrix (Schauer et al., 1996, 1999; Sheesley et al., 2004; Fine et al., 2001, 2004; Ham and Kleeman, 2011).

The possible existence of toxicological matrix effects – an extrapolation of the idea of chemical matrix effects – is a new concept that is largely unstudied. For particle toxicity, the basic idea is that the sum of endpoint-specific toxicological responses to individual PM components may be different than the response to the composite of those components, i.e. the presence of endpoint-specific toxicologically inert PM components may interfere with

the response to the toxicologically active PM components. This may further depend on the physical form in which the components are present; e.g., dissolved in solution, individual particles or particle aggregates. In this context, toxicological response may vary significantly depending on the filter extraction technique employed. A filter extraction technique designed according to one set of objectives may inadvertently alter the composition of the particle mixture in such a manner as to enhance or inhibit toxicological response relative to another technique designed with a different set of objectives. The current study was designed to test this hypothesis.

Separate filter extraction techniques commonly used in different laboratories and designed with different sets of objectives were used to extract ambient PM collected simultaneously from an urban and rural sampling site using high-volume PM_{2.5} sampler systems. The extracted PM was exhaustively characterized both chemically and toxicologically using a suite of analytical techniques and toxicological endpoints. A comprehensive inter-comparison of the filter extraction techniques based on the chemical composition of extracted PM is presented in what follows. Results from the toxicological studies are published separately (Van Winkle et al., *in press*). Overall, and to the authors' knowledge, this is the first study to (i) provide an exhaustive chemical characterization of a single PM extract, (ii) analyze the same PM extracts as used in subsequent exposure studies and (iii) inter-compare different filter extraction techniques in terms of the chemical composition of extracted PM.

2. Methodology

2.1. PM sampling

Field studies were conducted simultaneously during winter 2011 at two separate sampling sites representing an urban and rural environment using PM_{2.5} high-volume sampler systems (Tisch Environmental Inc., TE-6070V-2.5-HVS) equipped with PM₁₀ size selective heads (Tisch Environmental Inc., TE-6001), operating at a flow rate of 40 cfm and loaded with aluminum foil substrates for collecting the coarse PM fraction ($PM_{10-2.5} = 2.5 < D_{p50} < 10 \mu\text{m}$) and Teflon coated borosilicate glass microfiber filters (Pall Corporation, TX40H120WW-8X10) for collecting the fine PM fraction ($PM_{2.5} = D_{p50} < 2.5 \mu\text{m}$). Aluminum foil substrates were pre-baked at 500 °C for 24 h and glass microfiber filters were pre-cleaned via successive sonication in milli-Q H₂O, dichloromethane (DCM) and hexane (Hx). Field blanks were included for all studies. The **urban** sampling site was located on the rooftop of a two story building at the northeast corner of T St. and 13th St. in downtown Sacramento, CA, surrounded by a mixture of residential, commercial and industrial sources and within a quarter mile of a major freeway interchange. The **rural** site was situated on top of a single story laboratory in the southeast corner of the Center for Health and the Environment complex on the south campus of U.C. Davis and surrounded by agricultural lands. PM_{2.5} filter samples and field blanks from both sites were extracted using two different filter extraction techniques detailed below and the extracts subjected to an exhaustive chemical characterization using a range of analytical techniques, as discussed later.

2.2. Filter extraction techniques

In total, five different filter extraction techniques commonly employed by different groups, including the US Environmental Protection Agency, were prescreened via qPCR analysis of THP-1 monocyte cell line response to the extracted PM samples using a six panel assay, including inflammatory mediators and PAH

response elements: IL-1b, IL-4, IL-8, GM-CSF, CYP1A1 and COX-2. Based on the results of this analysis, the two extraction techniques eliciting the most robust responses relative to filter blank extract controls were chosen for detailed compositional and toxicological characterization using a suite of analytical techniques and pulmonary and cardiovascular endpoints. Both filter extraction techniques chosen for this study have been described in detail elsewhere (Bein and Wexler, 2014; Van Winkle et al., in press; Chanet et al., 2013; Carosino et al., in press) so only a summary is provided here.

Multi-Solvent Extraction (MSE) is an exhaustive method comprising a combination of sonication in multiple solvents, liquid–liquid extraction, microporous membrane filtration and detailed gravimetric analysis designed specifically to (i) maximize extraction efficiency, (ii) minimize compositional biases, (iii) minimize extraction artifacts and (iv) provide precise and accurate direct measurements of extracted PM mass. A comprehensive gravimetric characterization of this method showing that extraction efficiencies consistently exceed 90% for a wide range of PM samples was presented in a previous study (Bein and Wexler, 2014). An outline of the various steps follows:

- Sonication in milli-Q H₂O (**H₂O Ex**)
- Liquid–liquid extraction of **H₂O Ex** in dichloromethane (**DCM soluble**) and hexane (**Hx soluble**)
- Microporous membrane filtration of **H₂O Ex**, **DCM soluble** and **Hx soluble** fractions
- Lyophilization of **H₂O Ex** followed by gravimetric analysis
- N₂ blowdown of **DCM soluble** and **Hx soluble** fractions followed by gravimetric analysis
- Sonication in dichloromethane (**DCM Ex**)
- Microporous membrane filtration, N₂ blowdown and gravimetric analysis of **DCM Ex**
- Sonication in hexane (**Hx Ex**)
- Microporous membrane filtration, N₂ blowdown and gravimetric analysis of **Hx Ex**
- Reconstitution of all fractions into single composite sample and final gravimetric analysis

The fractional distribution of total extracted PM mass among the various steps outlined above for the urban, rural and field blank filter samples is shown in Fig. 1. The **Hx soluble** and **Hx Ex** fractions were below the minimum detection limit of the gravimetric analysis in all cases and thus are excluded from the figure. Extraction efficiencies of $95.4 \pm 0.7\%$ and $96.9 \pm 0.5\%$ were obtained for the urban and rural PM_{2.5} samples, respectively, and residual filter material equaling less than 2% of total extracted PM mass was recovered during extraction of the field blank.

Spin-Down Extraction (SDE) was designed primarily to minimize the amount of contaminant filter material co-extracted with the PM. For both methods, filter glass microfibers (FGMs) are unavoidably shed from the filter during the sonication process and retained in the extracted PM either as freely suspended microfibers or agglomerated with PM. Although borosilicate glass is chemically inert, there is concern about potential interference effects induced by the size and morphology of FGMs on the animals and cell cultures used in in vivo and in vitro studies (Bein and Wexler, 2014). The microporous membrane filtration steps of the MSE method described above have been shown to selectively remove roughly 60–70% of FGMs by mass from the extraction of filter blanks but removal efficiencies have not been measured for the extraction of PM samples (Bein and Wexler, 2014). The objective of the SDE method is to maximize FGM removal efficiencies in PM extracts.

As with MSE, sonication is the basic extraction method in SDE. However, SDE differs substantially by employing a

microcentrifuge-based cellular homogenization technique serving as the selective filtration step separating FGM from the PM and soluble PM components. In cellular and molecular biology, homogenization refers to the mechanical shearing of higher-molecular weight cellular components to form a reduced viscosity, homogenous lysate after the initial cellular disruption step during RNA and DNA purification from cell and tissue samples. Centrifugal force provides the necessary pressure differential across the filter membrane to separate lower-molecular weight components from other cellular material. Applied to the extraction of PM, the concept is that the mechanical shear will break apart particle–particle and particle-FGM agglomerates to form a homogenous extract that can be selectively filtered to remove the FGM. The following is an outline of the SDE method:

- Top layer of filter membrane with PM deposit is removed, leaving filter backing behind
- Filter membranes added to top of QIAshredder[®] column (Qia-gen, cat. # 79654)
- QIAshredder[®] column weighed to obtain extraction pre-weight
- 500 μ L Dulbecco's PBS without CaCl₂ or MgCl₂ added to column
- Filter membranes probe sonicated for 5 s
- Collection tubes attached to column and centrifuged at $7600 \times g$ for 4 min
- Supernatant collected from tubes and transferred back to column
- Membranes sonicated in supernatant and then centrifuged; process repeated twice
- Final centrifuged PM sample resuspended in supernatant and filtered through clean column
- Supernatant lost during process replaced with fresh PBS to obtain 500 μ L final volume
- Extracted membranes in original column washed with 500 μ L distilled H₂O and centrifuged
- Extracted membranes and column dried in SpeedVac concentrator for 6 h
- Extracted membranes and column weighed to obtain extraction post-weight
- Extraction pre- and post-weights subtracted to obtain extracted PM mass

The fundamental differences between the MSE and SDE methods are:

- Extraction solvents: H₂O, DCM and Hx followed by solvent removal in MSE versus sonication directly into the PBS delivery vehicle for SDE
- Post-extraction cleanup steps: microporous membrane filtration for MSE versus centrifugal homogenization and filtration in SDE
- Gravimetric analysis: direct measurement of extracted PM mass for MSE versus difference between pre- and post-extracted filter mass for SDE

The decision to extract directly into PBS rather than water was motivated by the scaling limitations of the microcentrifuge apparatus used in the SDE technique relative to the PM mass surface density on the filter and desired dosing ratios for the exposure studies. Since PBS was used as the delivery vehicle, extracting directly into PBS maximizes the PM concentration in the stock solution. Extracting into water first would require further dilution with PBS, lowering PM concentration. It was unclear prior to the extractions whether the desired dosing ratios could be obtained in this manner so PBS was used.

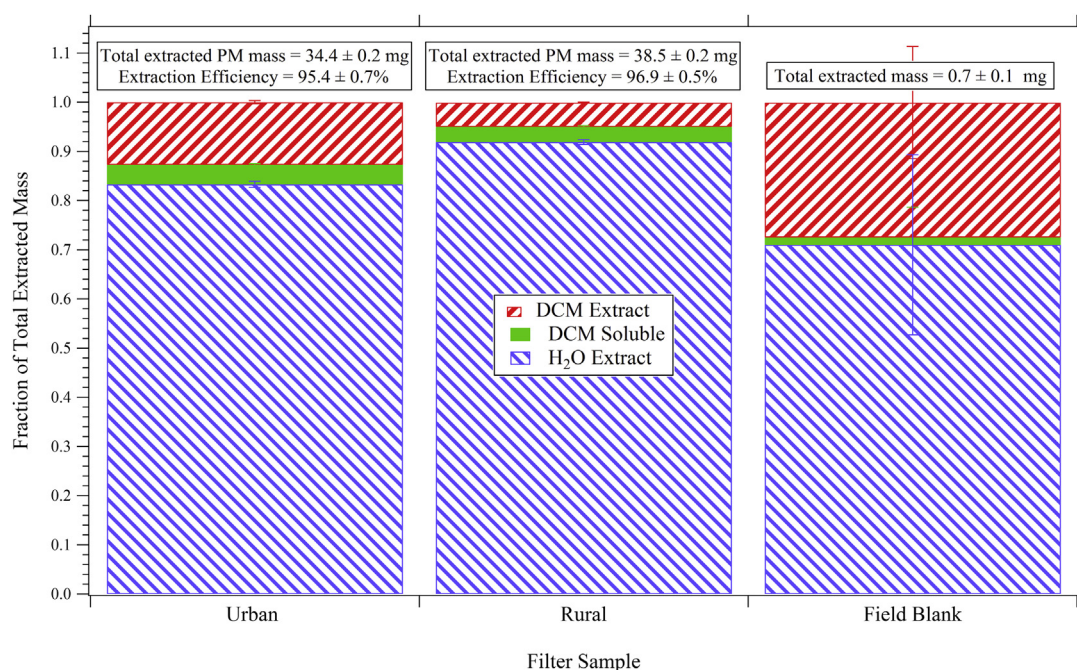


Fig. 1. The fractional distribution of total extracted mass among the various steps of the multi-solvent extraction (MSE) for the urban, rural and field blank filter samples; see text for discussion.

2.3. Sample preparation and chemical characterization

The main objective of the current study is a compositional comparison between different filter extraction techniques. As a result, all sample preparation and subsequent chemical analyses were performed on the material extracted from PM and field blank filters rather than on the filters themselves. As will be shown later, this is a key distinction from the traditional approach to chemically characterizing exposure studies. Almost exclusively, collocated instrumentation running in parallel with the PM samplers are used to characterize the physical and chemical properties of the collected PM. Even in those cases where this instrumentation is also filter based, multiple filter types and sample preparation methods are required to cover the full spectrum of relevant PM components since different protocols have been developed independently for analyzing different PM components. Furthermore, these protocols vary significantly from those commonly used in toxicological laboratories to extract PM for *in vitro* and *in vivo* studies. Therefore, it is not clear that the chemical characterization is a true representation of the exposure. This is especially true since the analytical protocols have been optimized to be highly quantitative while the extraction efficiency standards of toxicological studies have as yet been defined. Instead, the focus of extraction techniques for toxicological studies is normalizing mass dose while assuming that the original PM composition distribution has been conserved, regardless of extraction technique or extraction efficiency. To the authors' knowledge, this is the first study to (i) provide an exhaustive chemical characterization of a single PM extract, (ii) analyze the same PM extracts as used in subsequent exposure studies and (iii) inter-compare different filter extraction techniques in terms of the chemical composition of extracted PM. As such, design and development of novel sample preparation methods was necessary. These methods are outlined according to chemical component and associated analytical technique in what follows.

All PM extracts were divided into multiple aliquots of known PM mass concentration in solutions specific to the various analytical

techniques employed. Field blank extracts were divided into the same number of aliquots using the same solvent volumes as the PM extracts to ensure proper field blank correction. A process blank was also included for each method to quantify any potential sources of contamination during sample preparation. All PM composition data were field blank and process blank corrected. Measurement errors were propagated through all calculations to obtain 99% confidence intervals for the final reported compositional mass fraction data.

Trace Metals were measured via Inductively Coupled Plasma–Mass Spectrometry (ICP-MS) according to the SOPs, calibration standards, QA/QC and MDL/error analyses of the Interdisciplinary Center for Plasma Mass Spectrometry at U.C. Davis (<http://icpms.ucdavis.edu/>). The elements analyzed include Li, Be, Na, Mg, Al, K, Ca, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Rb, Sr, Ag, Cd, Cs, Ba, Tl, Pb and U. Sample preparation was accomplished by successive liquid–liquid extractions of the PM, field blank and process blank aliquots suspended in 3 mL milli-Q H₂O using DCM and Hx to remove any organics. Concentrated nitric acid and milli-Q H₂O were then added to the solvent washed suspensions to obtain a 6 mL volume of 1 M solution. These solutions were bath sonicated for ~1 h and refrigerated until analysis.

Water Soluble Inorganic and Organic Ions were measured via the combination of Ion Chromatography (IC), Automated Colorimetry (AC) and Atomic Absorption Spectrophotometry (AAS) according to the SOPs, calibration standards, QA/QC and MDL/error analyses of the Desert Research Institute (DRI) in Reno, NV (Chow et al., 2012). The ions analyzed include NH₄⁺, Cl[−], NO₂[−], NO₃[−], SO₄^{2−}, PO₄^{3−}, Na⁺, Mg⁺², K⁺, Ca⁺², 17 different organic sugars and 9 organic acids. Sample preparation was accomplished by diluting the PM, field blank and process blank aliquots to a 10 mL volume with milli-Q H₂O, bath sonicated for 30 min and then filtering the solutions using a 0.2 μm pore size. Samples were refrigerated until analysis.

Molecular Organic Compounds, including 38 different polycyclic aromatic hydrocarbons (PAHs), 27 high molecular weight alkanes/alkenes, 10 iso/anteiso-alkanes, 2 methyl-alkanes, 3 branched alkanes, 5 cycloalkanes, 18 hopanes and 12 steranes, were

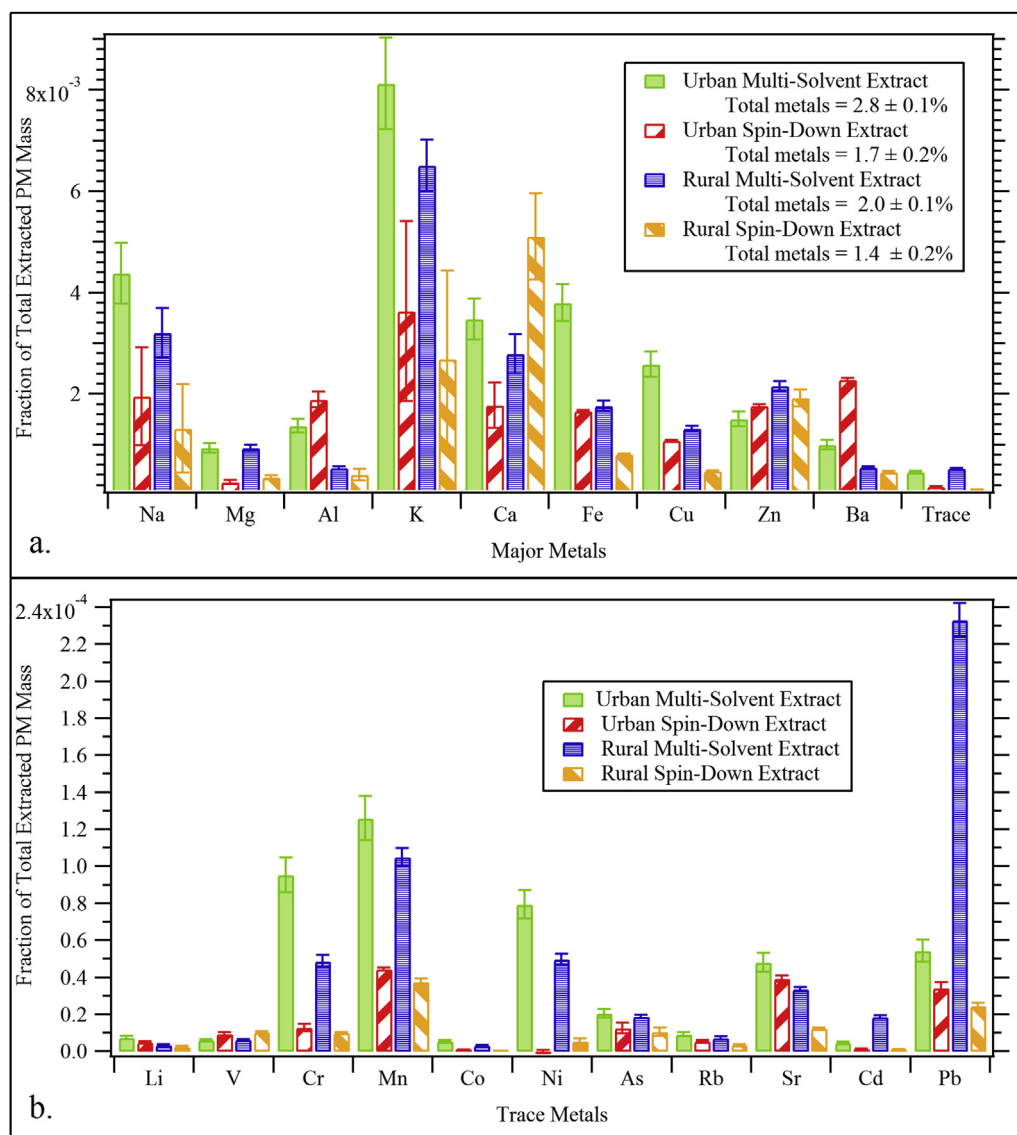


Fig. 2. Fraction of total extracted PM mass accounted for by (a) major and (b) trace metals detected during ICP-MS analysis of the urban and rural PM samples extracted via MSE and SDE; error bars represent 99% confidence intervals.

measured via Thermal Desorption-Gas Chromatography Mass Spectrometry (TD-GCMS) according to the SOPs, calibration standards, QA/QC and MDL/error analyses of DRI (Chow et al., 2012).

The thermal desorption technique of DRI is designed for sample deposits on pure quartz filters so a novel method was developed during this study to deposit the PM and field blank extracts onto 25 mm Tissuquartz™ filters. The PM, field blank and process blank aliquots were diluted to 5 mL volume using MeOH and then sonicated for ~30 min. Immediately following sonication, the MeOH suspensions were slowly dripped (~100 $\mu\text{L}/\text{min}$) onto a 25 mm Tissuquartz™ filter housed in a custom filter holder attached at the bottom to a filtration flask. A N_2 tank was attached to the vacuum port of the filtration flask and a 5 lpm reverse N_2 flow applied through the flask to (i) prevent solvent bleed-through and sample loss by providing back pressure at the bottom of the filter and (ii) accelerate MeOH evaporation from the filter.

Since the mass of the individual PM and field blank aliquots from the MSE method were directly measured, it was possible to quantify the mass transfer efficiency of the deposition process by

taking the difference between the pre- and post-deposition masses of the Tissuquartz™ filters for these samples. The average mass transfer efficiency over all samples was $97 \pm 8\%$, demonstrating quantitative transfer well within the detection limits of the analysis and thus validating the deposition technique.

Elemental Carbon and Organic Carbon (EC/OC) were measured via Thermal Optical Reflectance according to the SOPs, calibration standards, QA/QC and MDL/error analyses of DRI (Chow et al., 2012). Similar to TD-GCMS, the TOR measurements require sample deposits on pure quartz filters so the exact same procedures described above were also applied here. The mass transfer efficiencies for the EC/OC analysis were included in the TD-GCMS calculations.

3. Results and discussion

3.1. Compositional mass fraction data

Fig. 2 through 6 show the fractional distribution of total extracted PM mass among the individual chemical components

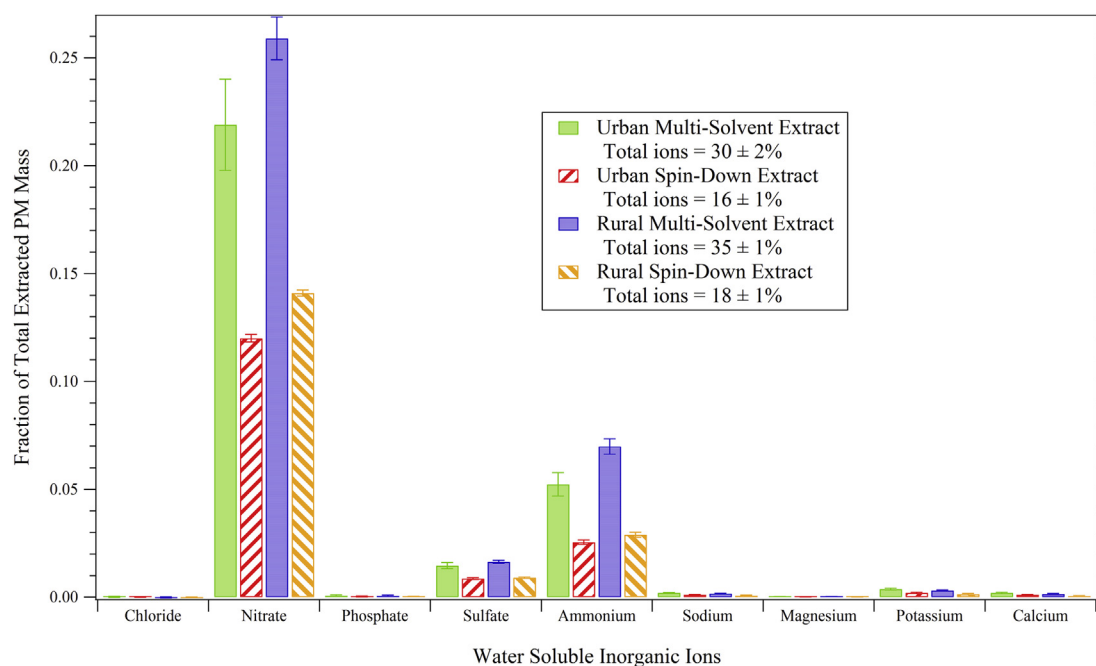


Fig. 3. Fraction of total extracted PM mass accounted for by the water soluble inorganic ions detected during IC, AC and AAS analysis of the urban and rural PM samples extracted via MSE and SDE; error bars represent 99% confidence intervals.

measured for the MSE and SDE extractions of the urban and rural PM samples. Fig. 2 shows (a) major and (b) trace metals, Fig. 3 shows water soluble inorganic ions, Fig. 4 (a) major and (b) minor PAHs, Fig. 5 (a) major and (b) minor nonaromatic molecular organic compounds and Fig. 6 organic carbon (OC), elemental carbon (EC) and total organic matter (OM), where OM was calculated using an OM-to-OC ratio of 1.6 ± 0.2 (Turpin and Lim, 2010). These data have been filter blank and process blank corrected and the error bars represent 99% confidence intervals.

The water soluble organics (i.e. sugars and acids) are not shown since their detection was inhibited by the high detection limits of the analytical technique relative to the PM mass measured. For example, the average detection limit of the individual species was 50 ppb while the average PM concentration measured was 100 ppm. This means that the specie would have needed to be present in the PM at concentrations greater than 500 ppm to be detected, which is at least an order of magnitude larger than the measured PAH and nonaromatic organic concentrations. In future studies, this can be alleviated by significantly increasing PM concentrations above 100 ppm. This was not possible in the current study due to limited PM availability. The two major exceptions to this general trend were levoglucosan, which was present at substantial concentrations in the MSE extracts (urban MSE = 1400 ± 100 ppm and rural MSE = 760 ± 50 ppm) but undetected in the SDE extracts, and methanesulfonic acid, which was present in all extracts (urban MSE = 130 ± 60 ppm, urban SDE = 90 ± 70 ppm, rural MSE = 140 ± 60 ppm and rural SDE = 90 ± 50 ppm).

Since the SDE samples were extracted directly into PBS, which contains potassium chloride, potassium phosphate, sodium chloride and sodium phosphate, the sodium and potassium values in the metals data (Fig. 2a) and sodium, potassium, chloride and phosphate values in the ions data (Fig. 3) have been estimated from the MSE data using the average ratio of MSE to SDE data for the other metals and ions, respectively. This was done independently for the urban and rural samples and the calculated ratios showed surprisingly small spread.

In combination, there is a strikingly nonrandom difference in the compositional mass fraction data between the MSE and SDE techniques for all measured components in the urban and rural PM extracts. The MSE data are consistently higher than the SDE data. For example, the ratio of SDE to MSE mass fraction data for the urban and rural extracts are: 0.60 ± 0.08 and 0.67 ± 0.09 for total metals, 0.54 ± 0.04 and 0.52 ± 0.02 for total inorganic ions, 0.35 ± 0.05 and 0.8 ± 0.1 for total PAHs, 0.41 ± 0.04 and 0.32 ± 0.04 for total molecular organic compounds, and 0.8 ± 0.1 and 0.49 ± 0.06 for OM + EC. These trends indicate a significant amount of uncharacterized mass in the SDE extracts, as discussed next.

3.2. Mass closure

A mass closure analysis was performed to further investigate the trends in the compositional mass fraction data discussed above. Fig. 7 shows the results of this analysis for the MSE and SDE methods as the component sum of the compositional mass fraction data for the major PM components in the urban and rural PM extracts. Although the MSE data demonstrate good mass closure – $92 \pm 8\%$ and $95 \pm 9\%$ for the urban and rural extracts, respectively – it is immediately clear that a significant fraction of the PM mass extracted via SDE is unaccounted for, or missing, in both the urban ($36 \pm 7\%$) and rural ($52 \pm 4\%$) samples. Given that (i) the MSE extracts are well characterized by the measured chemical components and (ii) the total extracted PM mass was never directly measured in the SDE method but rather estimated by the difference in pre- and post-extraction filter weights, it is most likely that the unaccounted PM mass was actually lost somewhere in the SDE process. A retrospective mass reconciliation effort was made to test this hypothesis using archived SDE samples, as discussed next.

3.3. Retrospective mass reconciliation

The primary issue with directly measuring the mass of the SDE extracts is that the samples were extracted into PBS which contains

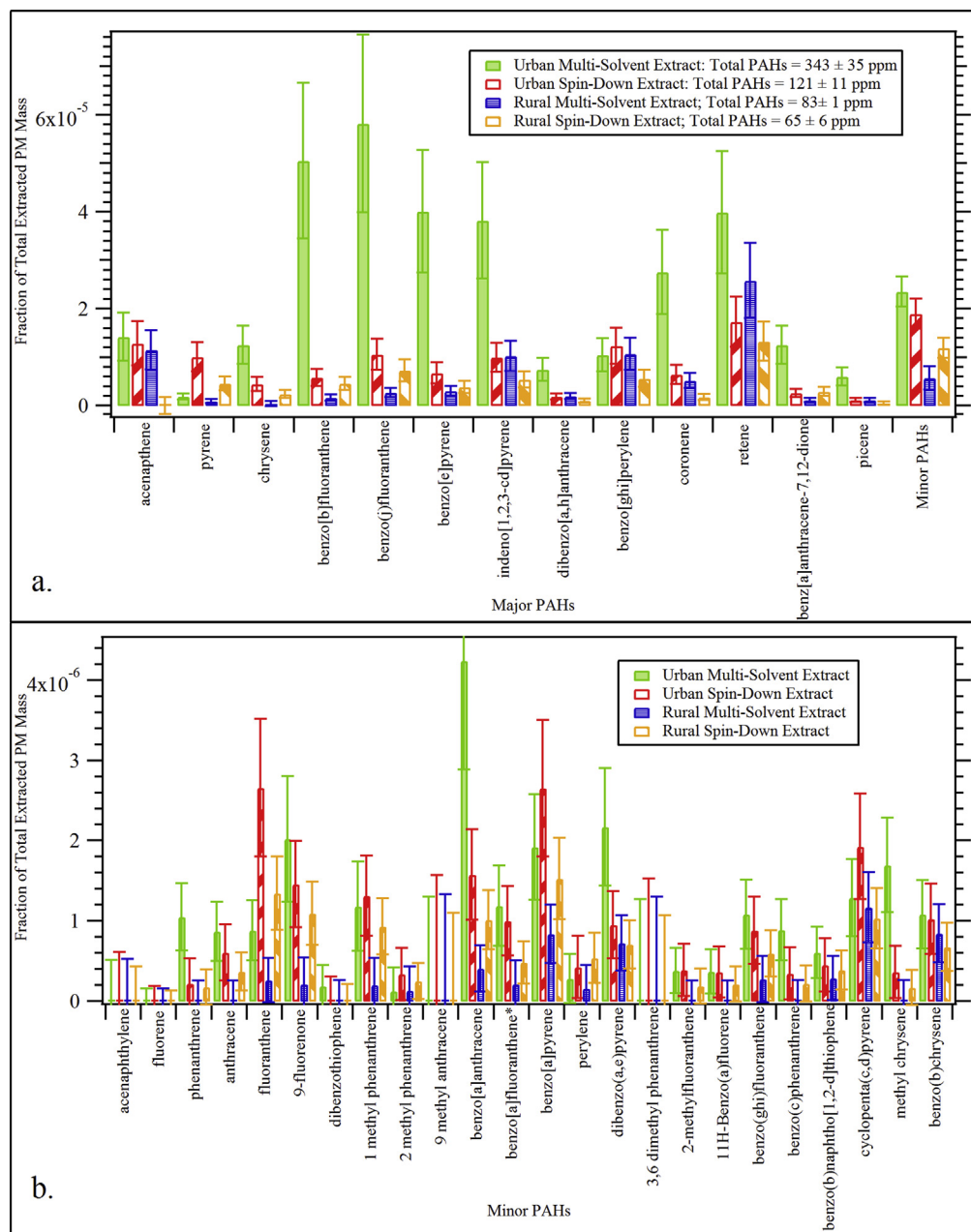


Fig. 4. Fraction of total extracted PM mass accounted for by (a) major and (b) minor PAHs detected during TD-GCMS analysis of the urban and rural PM samples extracted via MSE and SDE; error bars represent 99% confidence intervals.

high salt concentrations relative to the concentration of extracted PM. For example in the current study, a 500 μL volume of Dulbecco's PBS without CaCl_2 or MgCl_2 was used. It contains 5.28 mg of buffering salts compared to the $\sim 4\text{--}5$ mg of PM mass that can be extracted into this volume via the SDE method. Therefore, the accuracy of subtracting the volume-calculated mass of PBS salts from the total measured mass to obtain the mass of extracted PM not only depends on the accuracy of the gravimetric analysis but will be highly sensitive to the accuracy of the PBS volume measurements as well. To estimate the magnitude of this error, an archived aliquot of the SDE field blank extract was diluted with minimal MeOH, bath sonicated and evenly partitioned among six new aliquots. The MeOH and residual PBS H_2O content were evaporated under a N_2 atmosphere and the dry extract weighed using an analytical microbalance. The average percent difference between the measured

mass and volume-calculated PBS mass for all six aliquots was $0 \pm 6\%$, showing (i) excellent agreement between measured and calculated mass and (ii) excellent FGM removal efficiency for the SDE technique.

The exact same procedures described above were applied to archived aliquots of the SDE urban and rural extracts. The PBS-adjusted measured masses were then compared to the original pre- and post-extraction filter mass differences to show that, in fact, $44 \pm 9\%$ and $52 \pm 8\%$ of the extracted urban and rural PM mass, respectively, was lost during the SDE process. Using the adjusted masses in the mass closure analysis described previously increases the percent mass closure from $64 \pm 4\%$ to $110 \pm 13\%$ for the urban SDE extract and from $48 \pm 3\%$ to $100 \pm 10\%$ for the SDE rural extract, further substantiating the analysis and hypothesis. However, the relative distribution of PM mass among the measured components

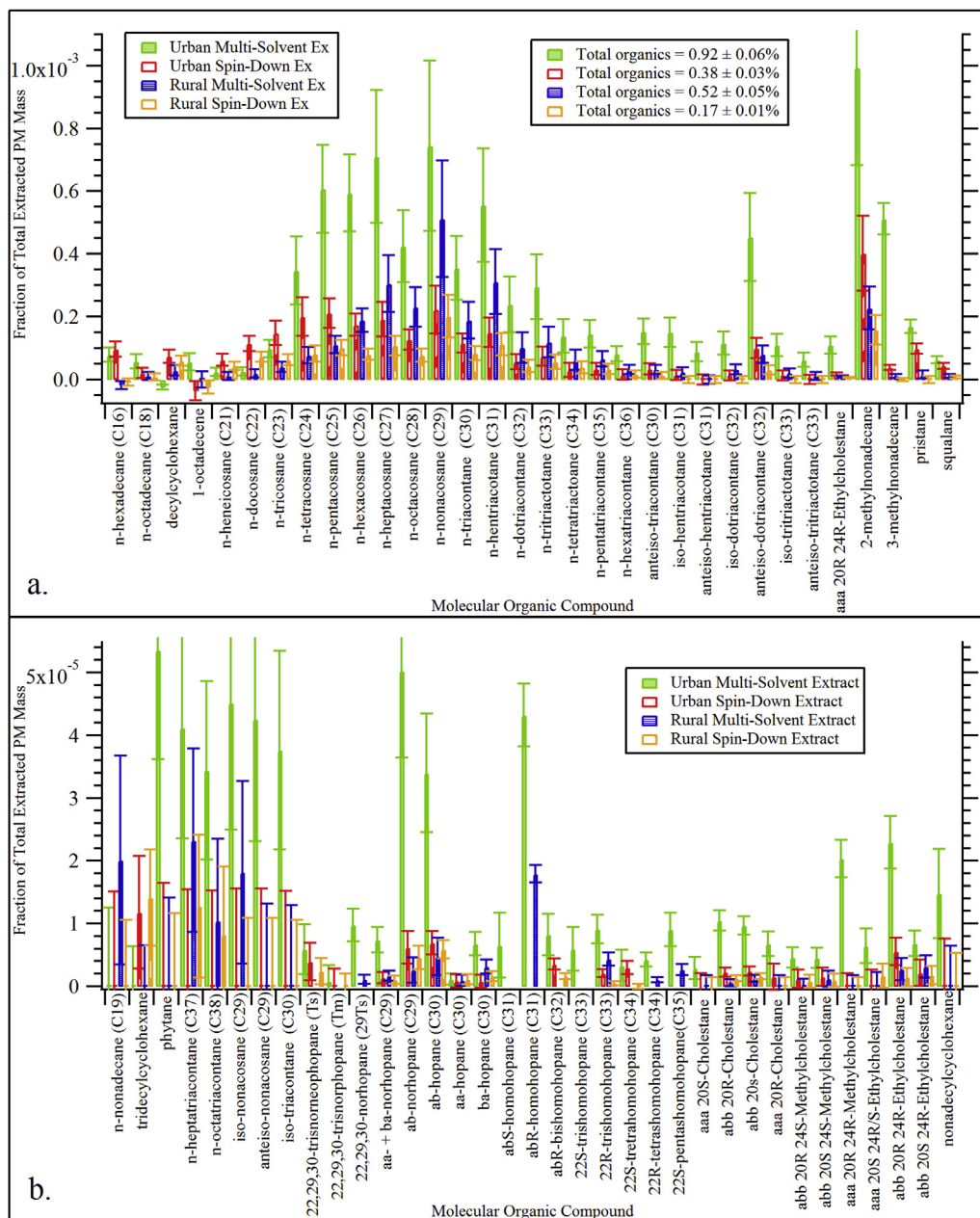


Fig. 5. Fraction of total extracted PM mass accounted for by (a) major and (b) minor molecular organic compounds detected during TD-GCMS analysis of the urban and rural PM samples extracted via MSE and SDE; error bars represent 99% confidence intervals.

does not change since the data are simply scaled by a constant factor.

It is not immediately clear at this point what step(s) of the SDE method is responsible for the missing PM mass but the most likely sources include (i) any supernatant lost during the process, which was approximated to be roughly 10% based on the amount of makeup volume required at the end of the extractions, (ii) any PM lost during the final filtration through the clean QIAshredder® column, (iii) any PM retained in the original QIAshredder® column but removed upon final rinsing prior to post-extraction weighing and (iv) any organics and ammonium nitrate retained on the shredded filter membranes but lost to evaporation during SpeedVac drying.

As a final analysis, the enrichment factors of the measured chemical components in the SDE extracts relative to the MSE

extracts were calculated using the adjusted PM masses. These results are shown in Fig. 8 for both the urban and rural PM samples. Values close to one indicate that the component was lost in constant proportion to the total loss of PM mass and thus was not enriched or depleted in the SDE extract while values above/below one indicate enrichment/depletion relative to the MSE extract. It is clear from the mass closure analysis that inorganic ions and organic matter constitute the largest sources of missing mass for both samples simply because they represent the largest fraction of total PM mass. However, the enrichment factors vary widely from component to component and between samples. For example, the urban sample is highly depleted in PAHs and inorganic ions and only slightly depleted in metals while the rural sample is highly enriched in PAHs and metals and neither enriched nor depleted in ions. Similarly, both samples are highly depleted in molecular

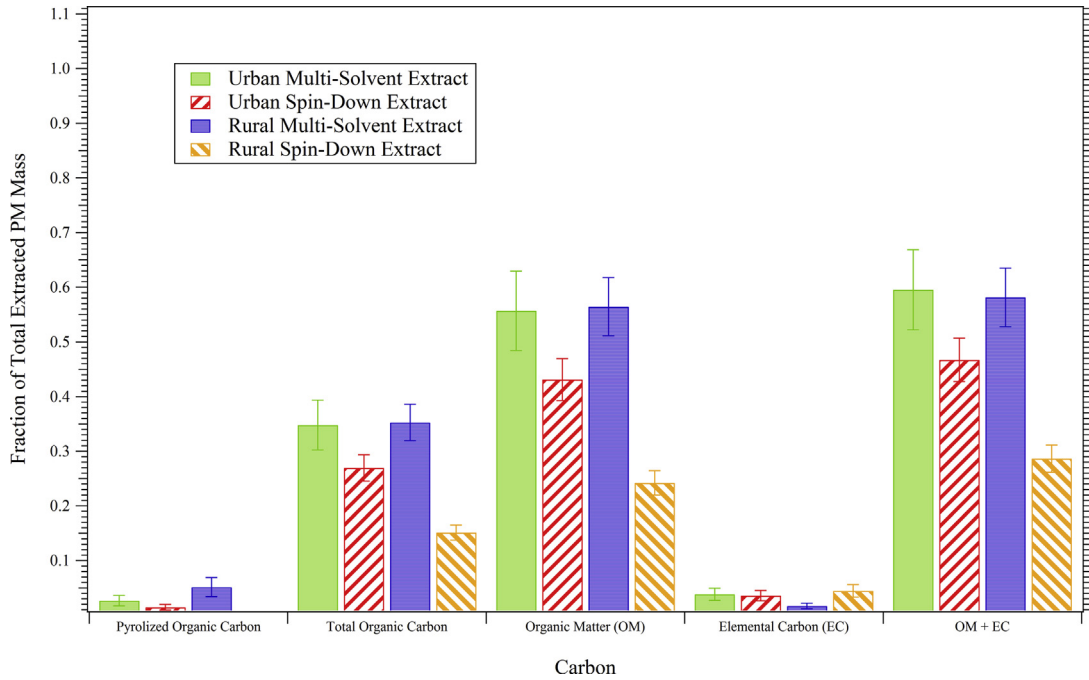


Fig. 6. Fraction of total extracted PM mass accounted for by various carbon fractions determined from TOR measurements of the urban and rural PM samples extracted via MSE and SDE; error bars represent 99% confidence intervals.

organic compounds and highly enriched in EC but show opposite trends in OC. This represents a severe compositional bias between the two extraction techniques that could potentially manifest as differences in toxicological response and underscores the importance of characterizing and standardizing the filter extraction process.

4. Conclusions

Separate filter extraction techniques commonly employed by different laboratories were used in the current study to extract

PM_{2.5} samples collected simultaneously at an urban and rural sampling site. A comprehensive inter-comparison of the extracted PM showed significant compositional variance between the two techniques attributed to a process-based loss of PM mass from the SDE method. This was confirmed retrospectively using detailed gravimetric analyses. To the authors' knowledge, this is the first study to demonstrate (i) an exhaustive chemical characterization of a single PM extract, (ii) the importance of chemically characterizing the extracted PM used in toxicological studies rather than relying on parallel measurements, (iii) the relevance of directly measuring extracted PM mass rather than using pre- and post-extraction

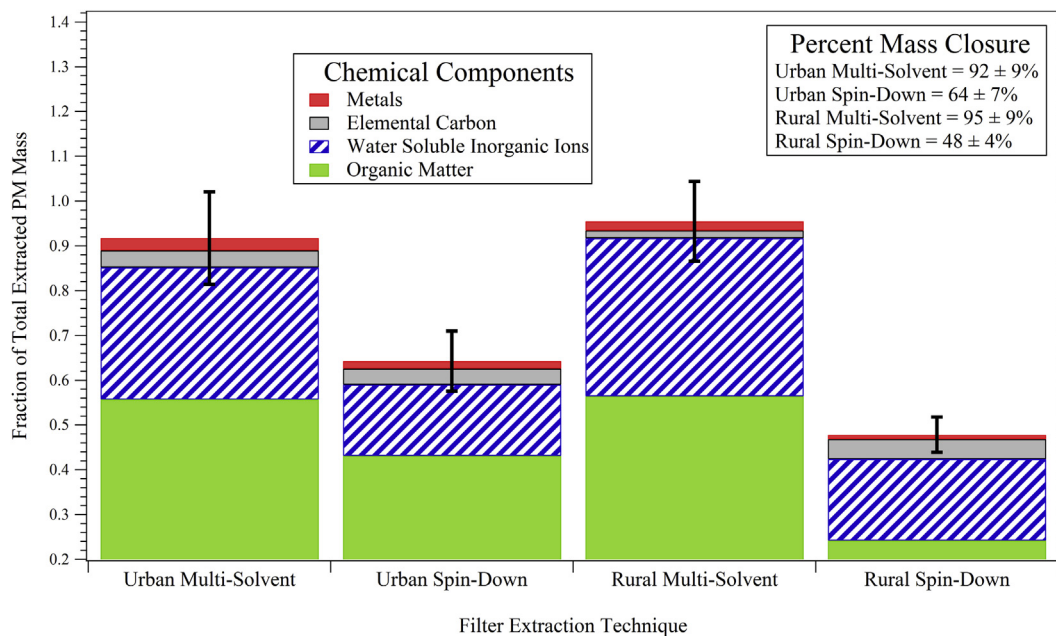


Fig. 7. Fractional distribution of total extracted PM mass among the chemical components measured for the rural and urban PM samples extracted via MSE and SDE; error bars represent 99% confidence intervals for the component sum.

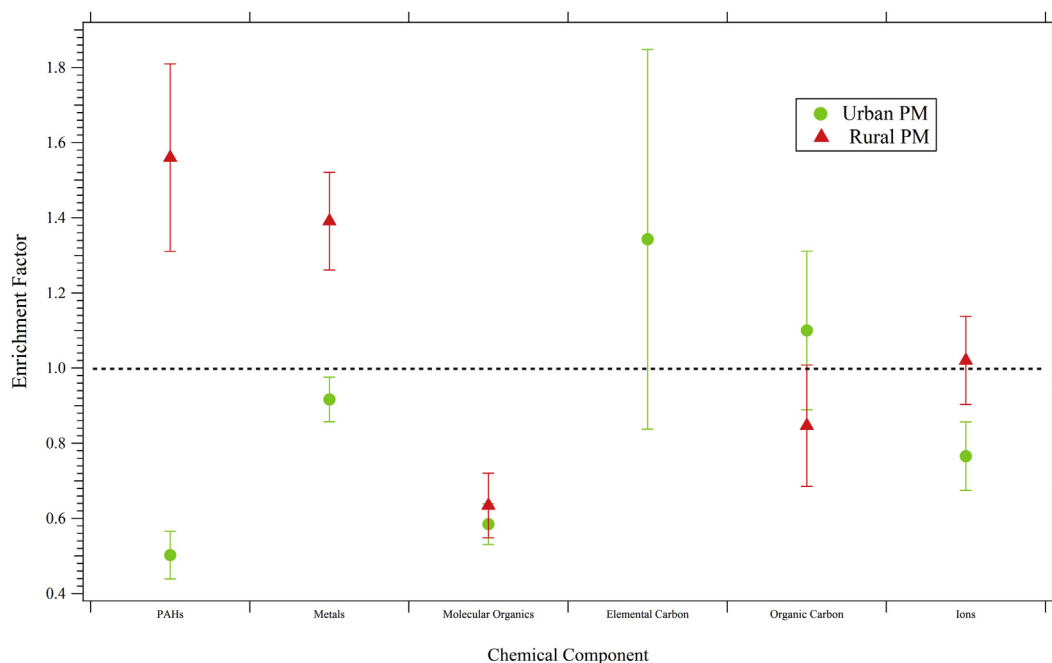


Fig. 8. Enrichment factors for the various chemical components in the SDE extracted urban and rural PM samples relative to the MSE samples after adjusting the SDE data for the true mass of extracted PM; see text for discussion.

differences in filter weights, (iv) the existence of substantial compositional biases between different filter extraction techniques and (v) the importance of standardizing filter extraction objectives and procedures to avoid introducing study bias into toxicological studies.

A detailed toxicological inter-comparison of the MSE and SDE extracted urban sample, published separately (Van Winkle et al., in press), revealed an unintuitive trend opposite that of the chemical characterization described here. With the exception of PAH response, the SDE-extracted PM sample consistently elicited the largest and most robust toxicological response for almost all endpoints tested. This is especially intriguing considering that the mass reconciliation of the SDE method occurred long after the toxicology studies were conducted such that incorrect PM masses were used to determine dose. This means that the elicited responses were observed at roughly half the intended SDE dose. In combination, these two studies present a paradox. The data presented here clearly demonstrate that the MSE method best conserves the original chemical composition of the sampled PM while the largest and most robust toxicological responses were generally elicited from the SDE method. The obvious question becomes which method is more appropriate. It may be the case that toxicological matrix effects are being observed; i.e. SDE removed a large fraction of toxicological inert components, thus amplifying the response to the active components, while MSE maximized extraction of all components and inert ones diluted the response to active ones. Conversely, it may be the case that MSE altered the physical composition of the PM – e.g., particle size distribution, internal distribution of chemical components or inclusion of FGM – which inhibited the bioavailability of the extracted PM. Although the current study sets the stage for this discussion, further research is required to fully address these issues and answer this question.

Lastly, glass microfiber filters were chosen for the current study given their ubiquitous use throughout the aerosol sampling community – due to their ultrahigh performance, especially in the retention of organic species – thus making the results relevant to the largest audience. However, great effort was made in both

extraction protocols to separate contaminant glass microfibers from the PM samples due to potential toxicological interference effects. As was shown here, this effort led to large compositional biases in the PM extracts, essentially introducing new artifacts for the sake of another and underscoring the importance of evaluating alternative filter media for PM toxicity testing. Studies aimed at quantifying differential toxicity as a function of filter media and PM extraction technique are essential to resolving these issues.

In summary, the MSE method presented here extracts over 90% of the PM from filter substrates enabling pulmonary instillations that better mimic atmospheric PM exposure compositions. This work still leaves open the question of which components and sources are toxic and which endpoints they effect.

Acknowledgments

This work was supported by the California Air Resources Board through contract number 09-330. The statements and conclusions in this publication are those of the contractor and not necessarily those of the California Air Resources Board. The mention of commercial products, their source, or their use in connection with material reported herein is not to be construed as actual or implied endorsement of such products. The authors gratefully acknowledge the assistance of Michael Lame in performing the spin-down extractions.

References

- Bein, K.J., Wexler, A.S., 2014. A high-efficiency, low-bias method for extracting particulate matter from filter and impactor substrates. *Atmos. Environ.* 90, 87–95.
- Bowser, D., 2009. Standard Operating Procedure for PUF and Filter CAP Extract. New York University School of Medicine, NY. Personal communication.
- C. M. Carosino et al., Allergic airway inflammation is differentially exacerbated by daytime and nighttime ultrafine and submicron fine ambient particles: heme oxygenase-1 as an indicator of PM-mediated allergic inflammation. *J. Toxicol. Environ. Health. Part A*, (in press).
- Chan, J.K.W., et al., 2013. Combustion derived ultrafine particles induce cytochrome P-450 expression in specific lung compartments in the developing neonatal and

- adult rat. *Lung Cell. Mol. Physiology* 304, L665–L667.
- Chow, J.C., Watson, J.G., 2012. In: Ruzer, L., Harley, N.H. (Eds.), *Aerosols Handbook: Measurement, Dosimetry, and Health Effects*, vol. 2. CRC Press/Taylor & Francis, New York, NY, pp. 179–204.
- Devlin, R., 2009. Standard Operating Procedure for Extraction of Concentrated Particulate Matter from PUF and G5300 Media. National Health and Environmental Effects Research Laboratory, U.S. Environmental Protection Agency, Research Triangle Park, NC. Personal communication.
- Fine, P., Cass, G., Simoneit, B., 2001. Chemical characterization of fine particle emissions from fireplace combustion of woods grown in the northeastern United States. *Environ. Sci. Technol.* 35, 2665–2675.
- Fine, P., Cass, G., Simoneit, B., 2004. Chemical characterization of fine particle emissions from the wood stove combustion of prevalent United States tree species. *Environ. Sci. Technol.* 21, 705–721.
- Ham, A., Kleeman, M., 2011. Size-resolved source apportionment of carbonaceous particulate matter in urban and rural sites in central California. *Atmos. Environ.* 45, 3988–3995.
- Herner, J., Green, P., Kleeman, M., 2006. Measuring the trace elemental composition of size-resolved airborne particles. *Environ. Sci. Technol.* 40, 1925–1933.
- Schauer, J., Rogge, W., Hildemann, L., Mazurek, M., Cass, G., 1996. Source apportionment of airborne particulate matter using organic compounds as tracers. *Atmos. Environ.* 30, 3837–3855.
- Schauer, J., Kleeman, M., Cass, G., Simoneit, B., 1999. Measurement of emissions from air pollution sources 1. C1 through C29 organic compounds from meat charbroiling. *Environ. Sci. Technol.* 33, 1566–1577.
- Sheesley, R., Schauer, J., Bean, E., Kenski, D., 2004. Trends in secondary organic aerosol at a remote site in Michigan's upper peninsula. *Environ. Sci. Technol.* 28, 6491–6500.
- Turpin, B.J., Lim, H.-J., 2010. Species contributions to PM_{2.5} mass concentrations: revisiting common assumptions for estimating organic mass. *Aerosol Sci. Technol.* 35, 602–610.
- L. S. Van Winkle, et al., Biological dose response to PM_{2.5}: effect of particle extraction method on platelet and lung responses. *Toxicol. Sci.*, (in press).