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RESEARCH ARTICLE

Characterization of ambient and extracted PM_{2.5} collected on filters for toxicology applications

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Abstract

Research on the health effects of fine particulate matter (PM_{2.5}) frequently disregards the differences in particle composition between that measured on an ambient filter versus that measured in the corresponding extraction solution used for toxicological testing. This study presents a novel method for characterizing the differences, in metallic and organic species, between the ambient samples and the corresponding extracted solutions through characterization of extracted PM_{2.5} suspended on filters. Removal efficiency was found to be 98.0 ± 1.4% when measured using pre- and post-removal filter weights, however, this efficiency was significantly reduced to 80.2 ± 0.8% when measured based on particle mass in the extraction solution. Furthermore, only 47.2 ± 22.3% of metals and 24.8 ± 14.5% of organics measured on the ambient filter were found in the extraction solution. Individual metallic and organic components were extracted with varying efficiency, with many organics being lost entirely during extraction. Finally, extraction efficiencies of specific PM_{2.5} components were inversely correlated with total mass. This study details a method to assess compositional alterations resulting from extraction of PM_{2.5} from filters, emphasizing the need for standardized procedures that maintain compositional integrity of ambient samples for use in toxicology studies of PM_{2.5}.

Keywords

PM_{2.5} filter extractions, PM_{2.5} filter sampling, PM_{2.5} toxicity

History

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Introduction

Ambient fine particulate matter (PM_{2.5}) has long been associated with respiratory and cardiovascular morbidity and mortality (Dockery et al., 1993; Franklin et al., 2007; Pope et al., 1995). Recently, PM_{2.5} and related health effects have been shown to vary across the USA (Bell et al., 2009; Sampson et al., 2013), highlighting the importance of researching the impact of compositional differences in ambient PM_{2.5}. It is particularly important to understand differences in components that are relevant to human health, such as metallic and organic species (Melaku et al., 2008; Ravindra et al., 2001; Schaumann et al., 2004). As epidemiological evidence of PM_{2.5}-associated health effects continues to be strengthened, toxicology studies to understand the mechanisms behind these outcomes and impacts of compositionally differing PM_{2.5} have advanced.

Toxicology studies allow for research into PM_{2.5} health effects while avoiding confounders present in many epidemiological studies, such as lifestyle and occupational factors (Jerrett et al., 2005). In order to capture the compositional complexity of PM_{2.5}, ambient samples must

be used; however, this requires collection of samples predominantly through concentrator systems or filter-based methods (Ghio & Huang, 2004). Concentrated ambient particles (CAPs) provide PM_{2.5} samples that maintain ratios of ambient mixtures while increasing the mass to allow for both *in vitro* and *in vivo* studies (Ghio & Huang, 2004). While CAPs provide a number of benefits to research, they require an expensive concentrator system that is fixed at a single sampling location, lacking the potential to study spatial differences in ambient PM_{2.5} concentration and composition (Matte et al., 2013).

Filter sampling allows for collection of ambient PM_{2.5} that may vary by location and source while utilizing a relatively low-cost method conducive to a variety of air sampling equipment systems (Kundu & Stone, 2014). An important consideration is the translation of PM_{2.5} on the filter, to a liquid suspension of PM_{2.5} that can be used in toxicology experiments. This process is integral to maximizing extraction efficiency while maintaining compositional integrity, so that the final extraction solution yields sufficient PM_{2.5} mass that remains representative of ambient PM_{2.5}.

Preparation of PM_{2.5} is typically a multistep process involving removal from the filter into solution, recovery of dry PM_{2.5}, and re-suspension into media appropriate for the toxicology application. A variety of extraction techniques have been implemented in toxicology research, which differ

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Table 1. Methods previously implemented for extraction of ambient PM_{2.5} for use in toxicology studies.

Removal method	Solvent	Concentration	Authors
Sonication	Water	N/A	Huang et al. (2003), Schins et al. (2004), Rivero et al. (2005), Riva et al. (2011), and Deng et al. (2013)
		Lyophilization	Vincent et al. (1997), Monn and Becker (1999), Baulig et al. (2004), and Geng et al. (2006)
		Desiccator	Watterson et al. (2007), Gualtieri et al. (2012), and Longhin et al. (2013)
	Methanol	Vacuum centrifuge	Huang et al. (2014)
		Dilution	Schaumann et al. (2004)
		Vacuum and desiccator	Valavanidis et al. (2005)
Probe sonication	Toxicology media PBS	Rotary evaporator	Jalava et al. (2006, 2009), Gerlofs-Nijland et al. (2007), Happo et al. (2010, 2013), Verma et al. (2012), and Janssen et al. (2014)
		N/A	Long et al. (2001), Akhtar et al. (2010, 2014), and Kumar et al. (2015)
		Lyophilization	Choi et al. (2004)
Soxhlet extraction	Methanol Toxicology media	N ₂ blow down	Mudway (2004) and Godri et al. (2011)
		N/A	Imrich et al. (2000) and Ning et al. (2000)
Agitation	DCM And methanol And water	Evaporation	de Kok et al. (2005)
		Evaporation	Skarek et al. (2007)
		Rotary evaporator	Cavanagh et al. (2009)
	Water	Lyophilization	Dye et al. (2001)

Removal method, solvent type, concentration, and authors are listed for toxicological assessments of ambient PM_{2.5} using filter extraction.

by the type of filter used for ambient collection, removal procedure and solvent, concentration method, and the media used for re-suspension. Table 1 summarizes a literature review of preparation procedures and emphasizes the variability of extraction methods that have been used.

Variation in extraction techniques between research groups creates a potential for bias, where findings may be dependent on the extraction procedures used rather than on the characteristics of ambient material (Bein & Wexler, 2014). Well-characterized extraction solutions would avoid these biases as the exact concentration and composition of PM_{2.5} used would be known, enabling a more accurate interpretation of exposure studies. Thus far, a limited number of toxicology studies using ambient PM_{2.5} have reported chemical characterization of both metals and organics in extraction solutions (Huang et al., 2014; Lauer et al., 2009; Verma et al., 2012). Here a novel method was developed to measure compositional differences in PM_{2.5} between collected ambient material and the corresponding extraction solutions.

Materials and methods

PM_{2.5} collection

Sampling locations

In winter 2014, PM_{2.5} samples were collected in Pittsburgh, PA at five sampling locations throughout the downtown area including a regional background location in a park 14 km upwind of the downtown area.

Sampling methods

Portable ambient air samplers were deployed approximately 3 m above ground level on metal utility poles and ran for

7 consecutive days at each sampling location. Samplers were enclosed in waterproof cases and equipped with 2.5 μm size-selective Harvard impactors (HIs) with 37 mm Teflon™ (polytetrafluoroethylene [PTFE]) filters (Pall Corporation, Ann Arbor, MI) or cyclone adapted HIs (Air Diagnostics and Engineering Inc., Harrison, ME) with 37 mm quartz filters (Pall Corporation, Ann Arbor, MI). Vacuum pumps (model PCXR4, SKC Inc., Eighty Four, PA) were calibrated to 4 liters per minute air flow rate (Matte et al., 2013). Quartz filters were pre-baked at 900 °C for 4 h to remove trace organic material.

Four samplers were co-located at each sampling location to provide equivalent samples for ambient characterization as well as for extraction. For the quantification and characterization of ambient material, two samplers per location collected PM_{2.5} on either a PTFE or a quartz filter. For extraction of ambient material into solution, two samplers per location collected PM_{2.5} on PTFE filters.

Ambient PM_{2.5} characterization

PTFE filters were used to determine PM_{2.5} concentrations through gravimetric analysis of filters pre- and post-sampling. Total PM_{2.5} mass was measured on an ultra-microbalance (model XP2U, Mettler Toledo, Columbus, OH) following a 48 h equilibration in a temperature and humidity controlled chamber (20.0 °C and 35% humidity).

Ambient compositional analysis by X-ray fluorescence (XRF) of metals and by thermal desorption gas chromatography mass spectrometry (TD-GC-MS) of organics was performed on PTFE and quartz filters, respectively, at Desert Research Institutes, DRI (Reno, NV). Metals ($n = 51$) and organics ($n = 34$) analyzed are shown in Table 2. Compounds analyzed included 14 of the 16 EPA Priority polycyclic aromatic hydrocarbons (PAHs).

Table 2. Metals and organics analyzed.

Metals				Organics	
Ag	Cu	Na	Sn	1-Methyl phenanthrene	Dibenzo[a,h]anthracene
Al	Eu	Nb	Sr	2-Methyl phenanthrene	Dibenzothiophene
As	Fe	Ni	Ta	9-Fluorenone	Fluoranthene
Au	Ga	P	Tb	Acenaphthene	Fluorene
Ba	Hf	Pb	Ti	Acenaphthylene	Hopanes (<i>n</i> = 10)
Br	Hg	Pd	Tl	Benzo[a]anthracene	Indeno[1,2,3-cd]pyrene
Ca	In	Rb	U	Benzo[a]pyrene	Phenanthrene
Cd	Ir	S	V	Benzo[b]fluoranthene	Pyrene
Ce	K	Sb	W	Benzo[e]pyrene	Steranes (<i>n</i> = 4)
Cl	La	Sc	Y	Benzo[ghi]fluoranthene	
Co	Mg	Se	Zn	Benzo[ghi]perylene	
Cr	Mn	Si	Zr	Benzo[jk]fluoranthene	
Cs	Mo	Sm		Chrysene	

Metals (*n* = 51) analyzed by X-ray fluorescence and organics (*n* = 34) analyzed by thermal desorption gas chromatography mass spectrometry. All compounds were measured in both ambient and extraction solution samples.

PM_{2.5} extraction

Removal

Following sampling, PTFE filters collected for extraction (*n* = 2/sampling location) underwent gravimetric analysis, described above, to determine the total PM_{2.5} mass collected. Filters were then placed particle side down in 100 mL glass beakers containing a 9:1 solvent (methanol: sterile Milli-Q water) and sonicated for 2 min at 40 kHz in a waterbath sonicator (Branson Ultrasonics, Danbury, CT). Beakers were sufficiently wide to allow filters to lie flat, avoiding the need to cut filters into pieces. Cutting can intensify release of filter material during sonication, which creates difficulties in post-weighting of filters to determine removal mass. The extracts of the two filters collected from each location were pooled together (Baulig et al., 2004).

After sonication, filters and the beaker were rinsed with methanol to remove any residual particles and all rinses containing PM_{2.5} were stored in a closed 50 mL conical tube at -20 °C until concentration. PTFE filters were left to dry and equilibrate prior to gravimetric analysis for determination of the PM_{2.5} mass removed from each filter. Blank PTFE filters were prepared in the same manner as exposed filters to control for any loss of material throughout the removal process.

Concentration

PM_{2.5} suspended in the methanol solution were centrifuged (8000g, 15 min) prior to being frozen in liquid nitrogen and concentrated through lyophilization in a 4.5 L bench top freeze dryer (Labconco, Kansas City, MO). Dry concentrated PM_{2.5} samples were stored away from any light sources at -20 °C until further analysis.

Re-suspension

Concentrated dry PM_{2.5} samples were re-suspended in a set volume of serum-free Dulbecco's modified Eagle's medium (DMEM) for future *in vitro* research. Samples were vigorously pipetted and vortexed to distribute PM_{2.5} throughout the media, then immediately prepared for PM_{2.5} characterization. Samples of PM_{2.5} that were removed from the filter, concentrated, and re-suspended in media (hereafter referred

to as *extracted samples*) are the most accurate form of PM_{2.5} for characterization of samples used in toxicology research.

Extracted PM_{2.5} characterization

Aliquots of extracted PM_{2.5} samples in DMEM were suspended onto pre-weighed PTFE and quartz filters to allow for gravimetric and chemical analyses comparable to those performed for ambient samples. Due to the hydrophobic nature of PTFE filters, samples were mixed with methanol and then applied to the filters. PM_{2.5} in solution was left to dry on the filters and then filters were equilibrated for gravimetric analysis. PM_{2.5} mass was determined for extracted samples prior to characterization through XRF and TD-GC-MS analysis (Table 2). Expected masses of all constituents in extracted samples were calculated using the PM_{2.5} mass applied to the filter and ambient composition data. Filters suspended with DMEM-only were weighed and analyzed to allow for blank adjustment of samples due to mass and compositional components present in DMEM.

Statistical analysis

Statistical analysis for all data was performed with StataSE 13 (StataCorp, LP, College Station, TX) and Prism 6.0 (GraphPad Software, Inc., San Diego, CA). All data were reported as a mean ± standard deviation (SD). Pearson correlation coefficients were determined between PM_{2.5} mass and specific constituents. Data were analyzed using a one-way analysis of variance (ANOVA) with Bonferroni's test for multiple post-hoc comparisons where appropriate. Where ANOVA indicated significant differences and in all two-group comparisons, differences were investigated using Student's *t*-test. Differences with *p* values < 0.05 were considered significant.

Results

Sampling location differences

Masses for PM_{2.5}, metals, and organics were determined for each of the five locations. PM_{2.5} mass was determined at three stages: (1) ambient material collected over the sampling period ("PM_{amb}"); (2) recovered material measured based on pre- and post-removal filter weights ("PM_{rem}"); and (3)

recovered material concentrated and re-suspended into DMEM (“PM_{ext}”). Metals and organics masses were determined at stage 1 (“metals_{amb}, organics_{amb}”) and 3 (“metals_{ext}, organics_{ext}”). Ambient mass collected varied between sampling sites, but trends between PM_{amb}, PM_{rem}, and PM_{ext} were similar across locations (Figure 1).

Metals_{amb} and metals_{ext} (Figure 2A) were calculated by summing the masses of all species analyzed. Metals_{amb} did not correspond to PM_{amb}, yet the highest PM_{amb} sampling location also had the highest metals_{amb}. Metals_{amb} and metals_{ext} also did not trend together indicating that extraction differences between sampling sites impacted metals_{ext}. Interestingly, the lowest metals_{ext} was observed at the location with the highest PM_{amb} and metals_{amb}.

Organics_{amb} and organics_{ext} were quantified by summing all species analyzed and variability was observed between all locations (Figure 2B). Similarly to ambient metals, organics_{amb} did not trend with PM_{amb}. However, unlike metals, the location with the highest organics_{amb} was not from the sampling location with the highest PM_{amb}. All organics_{ext} were less than organics_{amb} and varied between sampling locations independent of PM_{amb}. As with metals_{ext}, the effect of extraction differences between sampling locations was observed in organics_{ext}.

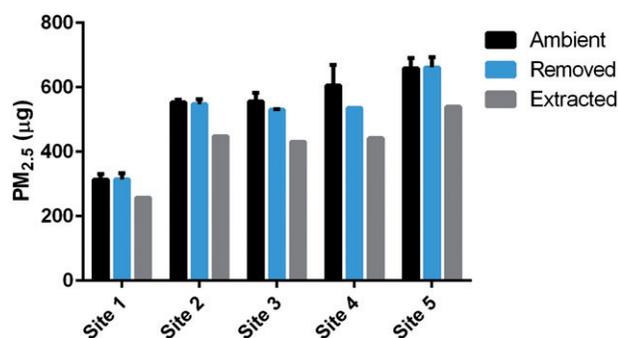
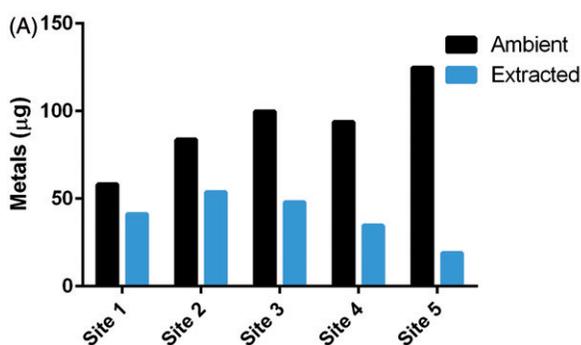


Figure 1. PM_{2.5} mass across sampling sites. Ambient mass, mass following removal from filter via sonication (“removed”), and mass following re-suspension in cell culture media (“extracted”) are displayed for each sampling location in micrograms ($n=2$ /site for ambient and removed samples – except for site 4 ($n=1$) due to equipment failure during collection and $n=1$ /site for extracted samples). Sampling sites are ordered from lowest to highest (1–5) ambient PM_{2.5} mass. Data are expressed as means \pm SD.



Extraction efficiency

Ambient masses of PM_{2.5}, metals, and organics were compared to extracted masses to determine the percent extracted. Removal of total PM_{2.5} was $98.0 \pm 1.4\%$ following sonication; however, extraction efficiencies following concentration and re-suspension in DMEM were found to be substantially lower at $80.2 \pm 0.8\%$ of PM_{amb} (Figure 3).

Overall extraction efficiency for metals (Table 3) was $47.2 \pm 22.3\%$, with extraction efficiencies for specific metals ranging from 0.7 (for Ce) to 73.4% (for Na). High variability in extraction efficiency was also observed for specific metals between sampling locations (i.e., $SD \pm 35.8\%$ for Ni). All averaged extraction efficiencies were less than 100% removal except for three trace metals that were present in DMEM (Ca, Mg, and P); these components were excluded from calculations of total metals. Contributions to total ambient metals for Ca, Mg, and P were 2.2, 1.1, and 0.0%, respectively.

Extraction efficiencies for organics (Table 3) are displayed for the five compounds detected in both ambient and extracted samples: 1-methyl phenanthrene (1MP), acenaphthylene (Acy), benzo[b]fluoranthene (BbFl),

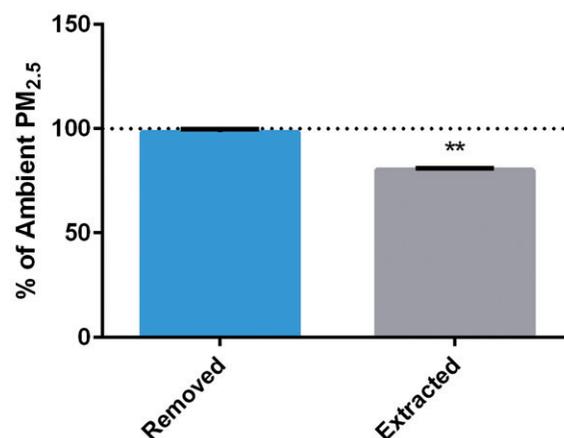


Figure 3. PM_{2.5} mass following removal and extraction. Mass following removal of PM_{2.5} from filter via sonication and following complete extraction (concentration and re-suspension in cell media) are displayed relative to total ambient PM_{2.5} mass for all sampling locations ($n=5$). Data are expressed as means \pm SD; ** p value <0.001 indicating a statistically significant difference between groups.

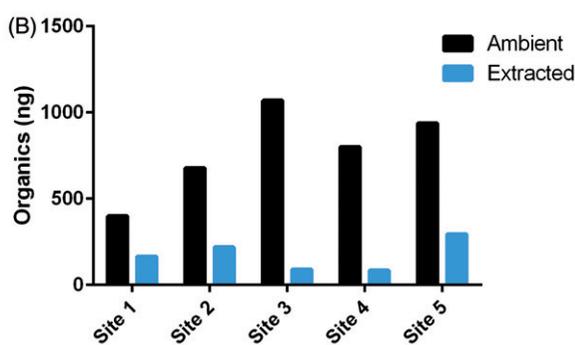


Figure 2. Ambient and extracted masses of PM_{2.5} components. (A) Total mass of metals (μg) in ambient samples and corresponding extraction solutions at each sampling site. (B) Total mass of organics (ng) in ambient samples and corresponding extraction solutions at each sampling site. Site numbering is representative of total ambient PM_{2.5} mass ordering of low to high (1–5). Constituents comprising total metals and organics are shown in Table 2.

benzo[ghi]perylene (BghiPer), and indeno[1,2,3-cd]pyrene (Ipyr). Variability of efficiency was observed between species (Ipyr to IMP: 17.0–101.5%) as well as between sampling locations for individual species (i.e., BghiPer SD \pm 43.9%). Extraction efficiency for total organics measured was 24.8 \pm 14.5%. All hopanes ($n=10$), steranes ($n=4$), and other organic compounds ($n=15$) measured were found at varying concentrations in ambient samples but were below the limit of detection in all extraction solutions, suggesting near complete loss during the extraction process.

Expected masses for constituents were calculated for extraction samples based upon PM_{2.5} mass and ambient composition. The expected values were compared to actual masses recorded through analysis of extraction solution (Tables 4 and 5).

Relationship of constituents to total PM_{2.5}

The relative contribution of measured metals and organics, as a percent of PM_{2.5} mass, was determined for both ambient and extracted samples (Figure 4). In ambient samples, the contribution of metals to PM_{amb} was 120 times higher than the contribution of organics. However, in extracted samples, the contribution of metals increased to over 705 times higher than organics.

Table 3. Extraction efficiencies.

Component	Percent extracted	SD
PM _{2.5}		
Removed	98.0	1.4
Extracted	80.2	0.8
Metals		
Al	40.0	54.8
Ca	570.4	583.3
Cd	9.0	20.0
Ce	27.0	60.3
Cl	7.9	17.8
Cr	4.8	10.7
Cs	10.4	23.3
Cu	21.4	7.7
Fe	28.8	9.5
Mg	227.8	338.5
Mn	20.7	8.7
Mo	15.1	12.1
Na	73.4	49.3
Ni	26.9	35.8
P	17 128.8	8604.4
Pb	16.8	11.5
S	0.7	1.6
Sn	20.3	45.3
Sr	3.6	5.1
Zn	6.2	11.7
Total	47.2	22.3
Organics		
IMP	101.5	23.1
Acy	31.7	20.9
BbFl	20.7	19.3
BghiPer	98.9	43.9
Ipyr	17.0	38.0
Total	24.8	14.5

Percent extracted with SDs for total PM_{2.5} mass following removal and extraction as well as extracted PM_{2.5} components (metals and organics).

Correlations

Ambient to extracted

Correlations of PM_{2.5} to metal and organic components were calculated to compare how PM_{2.5} mass and constituents were related for both ambient and extracted samples (Table 6). Statistically significant positive correlations were observed between PM_{amb} and Fe and Zn, while none of the extracted constituents exhibited a statistically significant correlation with PM_{ext}. Marked differences were observed between ambient and extracted correlations of constituents to PM_{2.5} mass (reported as ambient: extracted) for metals (0.911: -0.219), organics (0.805: -0.083), and a number of specific constituents including Al (0.967: -0.299), Cr (0.725: -0.350), Zn (0.975: -0.166), and Acy (0.734: -0.243).

To determine how specific components of PM_{2.5} related between ambient and extracted samples, correlation coefficients were determined for each component (Table 6). Several extracted constituent values were negatively correlated with ambient measurements of the same constituent, including: total metals, total organics, and specific components such as Al, S, and Sr. Positive correlations were observed for a number of components including: Cs, Fe, Mn, Sn, IMP, and BbFl. Statistically significant positive correlations between ambient and extracted values were present for PM_{2.5} mass and Tb.

Extraction percent to ambient characteristics

Correlations between calculated extraction percentages of specific components to total PM_{2.5} were made to investigate potential trends in extraction efficiency based upon PM_{amb} (Table 6). The percent of total mass extracted was

Table 4. Expected versus actual metals of extraction solution.

	Expected (μg)	Actual (μg)
Al	1.3965	0.3545
Ca	1.5980	8.7968
Cd	0.0074	0.0005
Ce	0.1141	0.0379
Cl	15.5670	1.3850
Cr	0.0499	0.0034
Cs	0.0054	0.0028
Cu	0.1621	0.0410
Fe	5.7970	2.0322
Mg	0.7899	1.1810
Mn	0.4285	0.1080
Mo	0.0766	0.0145
Na	21.7918	19.2882
Ni	0.0172	0.0059
P	0.0000	1.7684
Pb	0.1644	0.0340
S	20.3513	0.1847
Sn	0.0065	0.0048
Sr	0.0223	0.0008
Y	0.0066	0.0001
Zn	1.0115	0.0930
Total	69.3641	35.3377

Average expected and actual metals (μg) on filters. Expected metals were calculated using total PM_{2.5} mass applied to the filter and ambient composition data. Actual values were determined from XRF of extracted solutions.

Table 5. Expected versus actual organics of extraction solution.

	Expected (ng)	Actual (ng)
Acy	132.9485	36.5572
Ace	0.1473	0.0000
F	0.1520	0.0000
P	2.7877	0.0000
Flu	2.9187	0.0000
Pyr	1.9161	0.0000
9Flo	0.5447	0.0000
DBT	0.0292	0.0000
1MP	0.3019	0.2998
2MP	0.3923	0.0000
Chr	4.6438	0.0000
BbFl	4.0059	0.9423
BjkFl	6.7336	0.0000
BaAnt	2.3504	0.0000
BePyr	2.3839	0.0000
BaPyr	1.6650	0.0000
Ipyr	1.7039	0.2270
DBahAnt	0.2353	0.0000
BghiPer	7.1982	6.8868
BghiFl	0.9941	0.0000
Hopanes	1.9904	0.0000
Steranes	0.1141	0.0000
Total	176.1569	44.9131

Average expected and actual organics (ng) on filters.

Expected organics were calculated using total PM_{2.5} mass applied to the filter and ambient composition data. Actual values were determined with TD-GC-MS of extracted samples. Organics analyzed were hopanes ($n=10$), steranes ($n=4$), and PAHs ($n=20$) – abbreviated: acenaphthylene (Acy), acenaphthene (Ace), fluorene (F), phenanthrene (P), fluoranthene (Flu), pyrene (Pyr), 9-fluorenone (9Flo), dibenzothiophene (DBT), 1-methyl phenanthrene (1MP), 2-methyl phenanthrene (2MP), chrysene (Chr), benzo[b]fluoranthene (BbFl), benzo[jk]fluoranthene (BjkFl), benzo[a]anthracene (BaAnt), benzo[e]pyrene (BePyr), benzo[a]pyrene (BaPyr), indeno[1,2,3-cd]pyrene (Ipyr), dibenzo[a,h]anthracene (DBahAnt), benzo[ghi]perylene (BghiPer), and benzo(ghi)fluoranthene (BghiFl).

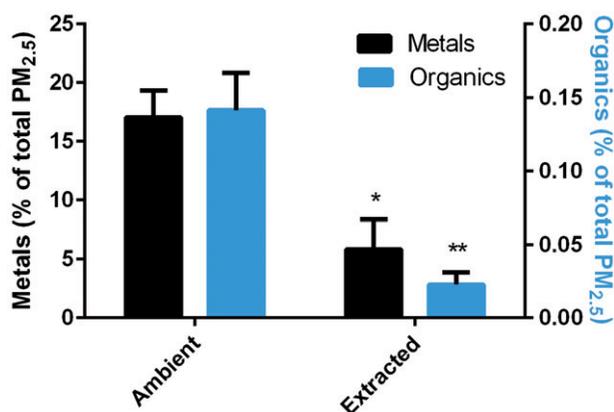


Figure 4. Contribution to total PM_{2.5} mass. Percent contribution to PM_{2.5} mass for total metals and organics (sum of constituents listed in Table 2) in ambient samples and extraction solutions. Left y-axis represents percent contribution of metals and the right y-axis indicates organics contribution. Data are expressed as means \pm SD; * p value < 0.05 and ** p value < 0.001 , indicating a statistically significant difference between ambient and extracted samples.

Table 6. Correlations of PM_{2.5}.

Component	Amb to ext	PM _{2.5} mass to constituents		
		Amb	Ext	% Ext
PM _{2.5}	0.975*			-0.273
Metals				
Al	-0.203	0.967	-0.299	-0.717
Ca	0.005	0.925	-0.530	-0.836
Cd	0.459	0.426	0.350	0.513
Ce	0.218	-0.322	-0.215	0.068
Cl	0.079	0.931	-0.215	0.068
Cr	0.189	0.725	-0.350	0.513
Cs	0.696	0.068	-0.003	0.335
Cu	0.554	0.947	0.263	-0.744
Fe	0.708	0.993*	0.502	-0.571
Mg	0.115	-0.005	-0.808	-0.125
Mn	0.571	0.940	0.474	-0.175
Mo	-0.003	0.507	-0.744	-0.590
Na	-0.639	0.707	0.051	0.033
Ni	0.045	0.951	0.048	0.184
P	N/A	N/A	-0.098	-0.928
Pb	0.504	0.736	0.213	-0.643
S	-0.189	0.858	-0.215	0.068
Sn	0.809	-0.081	0.350	0.513
Sr	-0.427	0.809	-0.555	-0.528
Tb	1.000*	0.079	0.635	0.079
Zn	0.312	0.975*	-0.166	0.108
Total	-0.610	0.911	-0.219	-0.815
Organics				
1MP	0.752	0.365	0.812	-0.123
Acy	-0.231	0.734	-0.243	-0.616
BbFl	0.570	0.953	0.670	0.467
BghiPer	0.139	0.630	0.652	0.279
Ipyr	0.078	0.579	0.635	0.079
Total	-0.083	0.805	-0.083	-0.522

Pearson's correlation coefficients are presented for total PM_{2.5} and constituents (metals and organics) between ambient ("amb") and extraction solution ("ext") samples as well as PM_{2.5} mass to: ambient values, extraction solution values, and percent extracted.

* p Value < 0.05 , indicating a statistically significant correlation.

significantly positively correlated with PM_{amb}. While both the percent of extracted metals and organics were negatively correlated with PM_{amb}. These correlations suggest that as ambient PM_{2.5} mass increases, the efficiency of extraction for total metals and organics decreases. Similar trends were seen in individual constituents including: Al, Cu, Fe, Pb, and Acy. However, not all components measured had negative correlations between extraction efficiency and total PM_{2.5}, these included: Cd, Cr, Sn, and BbFl.

Discussion

PM_{2.5} mass

The percent of mass removed via sonication was consistent across sampling locations, which is a characteristic necessary to avoid a methods bias. Less inter-filter variability was seen in removal efficiency than has previously been reported, where efficiency ranged from 59% to 95% (Imrich et al., 2000). Increased consistency in removal efficiency in this research is likely due to more deliberate selection of solvents based on anticipated chemical characteristics of PM_{2.5} components, as well as refinement of sonication methods. Extraction protocols using water as the sole solvent are common, and while effective for removal of water-soluble components and approximately 75% of PM_{2.5} mass, water is

not effective for extraction of nonpolar species, including many organics compounds (Hawthorne et al., 2000; Longhin et al., 2013; Watterson et al., 2007). It should be noted that a portion of studies neglect to report removal percentages, limiting inter-method comparisons. Based solely on mass removal from the filter, the methods outlined here maintained a high PM_{2.5} yield and were consistent between filters and sampling locations.

The significant positive correlation between ambient and extracted PM_{2.5} mass suggests that ambient mass loadings do not impact the extraction of total PM_{2.5}. Consistent extraction independent of mass makes the outlined methods translatable to many regions and sampling timescales. The methods are also effective in reducing release of filter material into the extracted solutions, as no significant loss of mass was observed with blank sonicated filters. The lack of observed fiber loss is likely due to a combination of the filter type used and decreased time and intensity of sonication. Previous methods have utilized potentially destructive probe sonication or extended sonication times, which can necessitate filtering of samples to remove fibers but also introduces a potential loss of PM_{2.5} (Godri et al., 2011; Huang et al., 2014; Riva et al., 2011; Van Winkle et al., 2015).

Components of PM_{2.5}

Differences in extraction efficiency between total PM_{2.5} and constituents of PM_{2.5} demonstrate a key limitation of filter extraction methods, discussed below. Importantly, a vast majority of the PAHs (15 of the 20 analyzed), hopanes, and steranes were not extracted at any of the locations. While loss in organics was not unexpected due to the volatility of the compounds (EPA, 2014), quantifying the shift from ambient contributions is useful to establish differences from filter samples.

A number of individual components, total organics, and total metals were inversely related to corresponding ambient masses, a result of decreased extraction efficiencies as mass increased. Ideally, ambient and extracted components would be equally correlated to total mass, indicating that composition of extracted PM_{2.5} was similar to that of ambient PM_{2.5}. However, positive correlations in ambient samples alone suggest that the relative composition of the ambient material is changed during the extraction process. Additionally, as ambient PM_{2.5} mass increases, the percentage of metals and organics extracted decreases.

Loss between removal and concentration

Translating the removal solution into dry particulate material is an imperative step before re-suspension into cell culture media to create an extraction solution for toxicology experiments. In this study, this process created a significant loss of mass and presumably loss of compounds that were volatile or soluble in the removal solution (9:1 methanol in water). However, characterization was only performed following re-suspension in cell culture media; additional characterizations of the removal and extraction solutions could identify at what point in the process the losses occurred. Better characterization of these losses is essential to accurate research, as extraction percentages are frequently reported as total mass

removed from filter, without consideration of losses that occur during the subsequent preparation steps.

Impacts on toxicology applications

Studies using ambient PM_{2.5} extracted from filters are an integral component for assessing biological impacts of PM_{2.5} both *in vitro* and *in vivo*. In some cases, responses are correlated to ambient concentrations, without regard for changes that occur during the extraction process (de Kok et al., 2005). The shift in relative contributions of specific components to total PM_{2.5} demonstrates that the resultant extraction solution in this work is not representative of the ambient mixtures. Recently, different extraction methods were found to result in distinct biological impacts (Van Winkle et al., 2015). Identifying the specific components of PM_{2.5} that are not representatively extracted by protocols can suggest mechanisms responsible for the varying biological responses. In this work we identified the loss of numerous health-relevant compounds including: Cr, Fe, Ni, Pb, Zn and 10 of the 16 EPA Priority PAHs (Chen & Lippmann, 2009). There is a need to further understand what effects these losses have on subsequent toxicology analyses.

Extraction efficiencies of components of PM_{2.5} were shown in this research to vary between sampling locations. Similar investigations found that extraction efficiencies were influenced by the source mixture and therefore composition of PM_{2.5} (Bein & Wexler, 2014). These findings are particularly important for studies examining multiple sampling locations or the impacts of mixed sources. With inconsistent extraction efficiency, the variation of ambient samples will be obscured or lost, and toxicology results will not be representative of the actual exposure of interest. An additional concern is the finding that extraction efficiencies of metals and organics are inversely related to total PM_{2.5} loadings. High mass loadings are necessary in toxicology studies to provide adequate material for exposures, but it is ineffective to collect such loadings when they decrease the yield of metal and organic species.

In this study, extraction was performed on samples with spatially varying ambient PM_{2.5} concentration and composition. The impacts of temporal and seasonal variation in PM_{2.5} composition on extraction efficiencies were not investigated here, but future research in these areas would strengthen the correspondence to studies using temporally variant ambient samples. Furthermore, results from this study are only generalizable to the methods utilized, and efficiencies will differ based upon the extraction procedures employed (Bein & Wexler, 2015). In this study, PM_{2.5} was re-suspended into cell culture media; to accurately determine how composition may be impacted by re-suspension in different toxicology medium (i.e. saline, PBS, or water), further studies should be conducted. However, based on this and previous works, it is clear that complete extraction has not been achieved using any current methodology; therefore, extraction solutions will differ compositionally from ambient source material, and the issues highlighted by this extraction protocol are likewise of concern with other methods (Akhtar et al., 2014; Happo et al., 2010).

Characterization of PM_{2.5} was performed only on ambient material and final extraction solutions; thus, changes in

composition during the intermediate stages are unknown. Identifying specific steps in extraction procedure that is most impactful on the recovery of PM_{2.5} components would help to establish refined procedures that maintain ambient compositions. While this work has begun to uncover compositional differences, it examined only a subset of the key components of PM_{2.5} other substantial contributors to mass including inorganic ions and total elemental and organic carbon would further elucidate the compositional changes following extraction.

Conclusions

This research has outlined a method for the extraction of PM_{2.5} from filter samples, which was effective in high mass recovery while maintaining filter integrity. Comparison of ambient and extracted samples suggests that the method was more effective in recovering metals in the extraction solution compared to organics. To the authors' knowledge, only one study has performed a well-characterized extraction solution analysis, and this research highlighted the variance in extraction of components of PM_{2.5} based on the extraction procedures implemented (Bein & Wexler, 2015). However, this current work is the first to compare components measured in PM_{2.5} filter extract with those measured on collocated ambient filters from multiple sampling locations. The narrow understanding of alteration to PM_{2.5} composition as a result of extraction is a limitation that persists throughout a vast majority of toxicology research using PM_{2.5} collected on filters. Further awareness of the underlying mechanisms for the observed compositional shifts, in addition to the adoption of standardized extraction techniques that more efficiently extract all components of PM_{2.5}, would allow for biological impact studies that are more readily translatable to ambient exposures, and would facilitate comparisons between studies.

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Declaration of interest

The authors report no declarations of interest.

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