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Association of IL-6 with $PM_{2.5}$ Components: Importance of Characterizing Filter-Based $PM_{2.5}$ Following Extraction

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Abstract

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Compliance with Ethical Standards

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Filter-based toxicology studies are conducted to establish the biological plausibility of the well-established health impacts associated with fine particulate matter ($PM_{2.5}$) exposure. Ambient $PM_{2.5}$ collected on filters is extracted into solution for toxicology applications, but frequently, characterization is nonexistent or only performed on filter-based $PM_{2.5}$, without consideration of compositional differences that occur during the extraction processes. To date, the impact of making associations to measured components in ambient instead of extracted $PM_{2.5}$ has not been investigated. Filter-based $PM_{2.5}$ was collected at locations ($n = 5$) and detailed characterization of both ambient and extracted $PM_{2.5}$ was performed. Alveolar macrophages (AMJ2-C11) were exposed (3, 24, and 48 h) to $PM_{2.5}$ and the pro-inflammatory cytokine interleukin (IL)-6 was measured. IL-6 release differed significantly between $PM_{2.5}$ collected from different locations; surprisingly, IL-6 release was highest following treatment with $PM_{2.5}$ from the lowest ambient concentration location. IL-6 was negatively correlated with the sum of ambient metals analyzed, as well as with concentrations of specific constituents which have been previously associated with respiratory health effects. However, positive correlations of IL-6 with extracted concentrations indicated that the negative associations between IL-6 and ambient concentrations do not accurately represent the relationship between inflammation and $PM_{2.5}$ exposure. Additionally, seven organic compounds had significant associations with IL-6 release when considering ambient concentrations, but they were not detected in the extracted solution. Basing inflammatory associations on ambient concentrations that are not necessarily representative of in vitro exposures creates misleading results; this study highlights the importance of characterizing extraction solutions to conduct accurate health impact research.

Keywords

Filter-based particulate matter; Particulate matter toxicity; Filter extraction; Toxicity bias

1 Introduction

Exposure to ambient fine particulate matter ($PM_{2.5}$) has been associated with morbidity and mortality related to respiratory inflammation (Abbey et al. 1995; Dominici et al. 2006; Tecer et al. 2008; Ostro et al. 2009). Associations have also been found between adverse respiratory health outcomes and specific constituents of $PM_{2.5}$, such as metallic and organic species, independent of total $PM_{2.5}$ concentration (Mar et al. 2000; Ostro et al. 2007; Ostro et al. 2009). To identify the mechanisms of ambient $PM_{2.5}$-induced respiratory illnesses and establish the biological plausibility of epidemiological findings, toxicology studies have employed filter-based collection of ambient $PM_{2.5}$ (Choi et al. 2004; Sawyer et al. 2010; Kumar et al. 2015).

Through the collection of ambient $PM_{2.5}$ on filters, toxicology studies can be conducted to identify associations between $PM_{2.5}$ components and biological outcomes. This practice can identify specific components that are most impactful through investigation of the genotoxicity (Dellinger et al. 2001; Topinka et al. 2011; Wang et al. 2011), mutagenicity (de Kok et al. 2005; Skarek et al. 2007; de Brito et al. 2013), oxidative potential (Godri et al. 2011; Gualtieri et al. 2012; Janssen et al. 2014), and inflammatory impacts (Happo et al. 2010; Riva et al. 2011; Akhtar et al. 2014; Kumar et al. 2015). In these previous studies, the

complex mixture of $PM_{2.5}$ was characterized prior to complete preparation for use in the toxicology studies, and this characterization data was used to make associations to biological responses. The practice of basing association of components present on the ambient filter and not on the extraction solution disregards compositional changes that may occur during the extraction process to prepare filter-based $PM_{2.5}$ for toxicology research.

Recently, complications of extraction have been demonstrated, where total $PM_{2.5}$ mass extracted can remain high (80%), while less efficient extraction and even complete loss of health-relevant components of $PM_{2.5}$ (i.e., benzo[*a*]pyrene, Ni, pyrene) can occur (Roper et al. 2015). Methods for extraction vary between research groups as detailed in Roper et al. (2015). These differences in methods implemented along with the characteristics of collected $PM_{2.5}$ (i.e., source contributions, mass loadings) can impact extraction efficiency (Bein and Wexler 2014; Roper et al. 2015). The lack of a standardized extraction protocol is of further concern, as it results in methods that vary between research groups and further inhibit cross-study comparisons.

While growing research has begun to characterize extracted $PM_{2.5}$ components for associations to oxidative potential (Verma et al. 2012) and genotoxic (Danielsen et al. 2011), mutagenic (Cavanagh et al. 2009), and inflammatory (Van Winkle et al. 2015) outcomes, associations are still routinely made to ambient values. Additionally, the resultant discrepancies in associations between using ambient or extracted values has yet to be evaluated. Enhanced understanding of how ambient samples translate to extracted solutions —and how this can impact interpretation of biological outcomes—will ultimately enable the identification of the causal components of $PM_{2.5}$-associated health effects.

In this study, the release of the pro-inflammatory cytokine, interleukin (IL)-6, was assessed in alveolar macrophages (AM) following exposure to $PM_{2.5}$ samples that were extracted from ambient filters. Importantly, this study was designed to establish the need for well-characterized extraction solutions when identifying causal components. Associations between IL-6 release and $PM_{2.5}$ were made using both ambient and corresponding extracted $PM_{2.5}$ measurements to determine if findings were dependent on the stage at which $PM_{2.5}$ was characterized.

2 Methods

2.1 $PM_{2.5}$ Collection

Sampling and extraction methods have been described in detail previously (Roper et al. 2015). Briefly, portable ambient air samplers were deployed approximately 3 m above ground level on metal utility poles for seven consecutive days. Four sampling locations were distributed throughout downtown Pittsburgh, PA, and one additional site was located in a park 14.5 km upwind of the urban area. Harvard Impactors (HI) and cyclone adapted HIs (Air Diagnostics and Engineering Inc., Harrison, ME) served as size segregators, collecting $PM_{2.5}$ on 37-mm-diameter Teflon™ (PTFE) filters and 37-mm quartz filters, respectively (Pall Corporation, Ann Arbor, MI). Four samplers were co-located at each location: two filters (one PTFE, one quartz) were collected for the characterization of ambient $PM_{2.5}$, and

two filters (both PTFE) were collected for extraction and subsequent toxicology research with in vitro exposures.

2.2 $PM_{2.5}$ Extraction

Following sampling, PTFE filters collected for in vitro exposures underwent gravimetric analysis 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). Filters were weighed pre- and post-sampling to determine ambient $PM_{2.5}$ mass collected. Using two PTFE filters per sampling location to ensure adequate mass for exposures, samples were sonicated in a 9:1 solution of methanol in sterile Milli-Q water. The resultant $PM_{2.5}$ suspensions were concentrated through lyophilization to dryness without filtration and stored at −20 °C. PTFE filters underwent gravimetric analysis following sonication to determine the mass removed from each filter. In preparation for in vitro analysis, $PM_{2.5}$ from each sampling location was re-suspended in serum-free Dulbecco's Modified Eagle Medium (DMEM). In order to maintain both concentration and compositional differences between locations, $PM_{2.5}$ from each sampling location was re-suspended in an equal volume of DMEM, which was the volume required in order to translate $PM_{2.5}$ mass from the lowest ambient concentration to an extraction concentration of 70 μg/mL. This concentration has been previously established to induce the release of cytokines (Becker et al. 2003; Sawyer et al. 2010); all extracted sample concentrations remained lower than thresholds previously observed to induce cell death (Schins et al. 2002). Aliquots of extracted samples were re-deposited onto PTFE and quartz filters (Roper et al. 2015) and were characterized according to the same procedures as ambient $PM_{2.5}$, described below.

2.3 $PM_{2.5}$ Characterization

$PM_{2.5}$ concentrations were calculated via gravimetric analysis of PTFE filters pre- and post-deployment. Ambient and extracted $PM_{2.5}$ composition was determined via x-ray fluorescence (XRF) analysis of inorganic species (from PTFE filter) and thermal desorption gas chromatography mass spectrometry (TD-GC-MS) analysis of organics (from quartz filter), at Desert Research Institutes, DRI (Reno, NV). Inorganic species, referred to hereafter as "metals" ($n = 51$), and organics ($n = 34$) analyzed are listed in Table 1. A schematic of characterization of ambient and extracted samples is provided in Supplementary Figure 1.

2.4 In Vitro $PM_{2.5}$ Exposure

Mouse alveolar macrophage cells, AMJ2-C11 (American Type Culture Collection, ATCC, Rockville, MD) were cultured in DMEM supplemented with 5% fetal bovine serum (FBS), 4 mM L-glutamine, 1.5 g/L sodium bicarbonate, 4.5 g/L glucose, 5 mM HEPES, 50 U/mL penicillin, and 50 μg/mL streptomycin, following the ATCC protocol.

Cells were seeded at 2.5×10^5 onto six-well plates and underwent a 2-h starvation at 37 °C in a 5% CO_2/95% air atmosphere. Immediately following starvation, all wells were exposed to $PM_{2.5}$ suspended in serum-free DMEM or controls for 3, 24, or 48 h. Control cells received media alone, media containing lipopolysaccharide (LPS) to control for potential

endotoxin contamination (Tager et al. 2010), or media containing extracts of unexposed filters to control for filter material released during the extraction procedures. LPS contamination of $PM_{2.5}$ samples was quantified using a chromogenic endotoxin quantitation kit (Pierce LAL Chromogenic Endotoxin Quantitation Kit; Thermo Scientific, Pittsburgh, PA) and LPS-control cells were treated with the highest LPS concentration detected from previous samples collected in Pittsburgh, PA (0.174 EU/mL). Duplicate wells for each sample/control were exposed at each time point.

2.5 Post-Exposure Analysis

Following exposure (3, 24, or 48 h), plates were vigorously shaken and cell media was collected. Total cell counts (Z1 Coulter Particle Counter; Beckman Coulter Inc., Brea, CA) were recorded; media was then centrifuged at 14,000×*g* for 5 min. Supernatants were collected and stored at −80 °C until analysis.

IL-6 concentrations were measured in duplicate for all cell supernatants following the manufacturer's instructions for an enzyme-linked immunosorbent assay (ELISA) specific for mouse IL-6 (R&D Systems, Minneapolis, MN).

2.6 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 are reported as a mean ± standard error of the mean (SEM). Data obtained for IL-6 concentrations between treatments and controls was analyzed using one-way analysis of variance (ANOVA) with Bonferroni's test for multiple post hoc comparisons where appropriate. Pearson's correlation coefficients were calculated for IL-6 concentrations to both ambient and extracted components of $PM_{2.5}$. Differences with p values <0.05 were considered significant; statistically significant findings were only observed for 24 and 48 h post-exposure.

3 Results

3.1 IL-6 Release Following $PM_{2.5}$ Exposure

Sampling locations (sites 1 to 5) were ordered lowest to highest with respect to ambient $PM_{2.5}$ concentration, without consideration of composition or extracted concentration. IL-6 release from AMs following exposure to $PM_{2.5}$ from each location was measured relative to media controls at 3, 24, and 48 h (Fig. 1). Equipment failure during the sampling period resulted in reduced collection of $PM_{2.5}$ at site 4, and IL-6 was only evaluated at 3 h post-exposure. IL-6 release following treatment with $PM_{2.5}$ from sites with the highest ambient concentrations (sites 3–5) was not significantly different from the control at any time point. In contrast, $PM_{2.5}$ from the site with the lowest ambient concentration (site 1) induced a significant increase in IL-6 release at 24 and 48 h. IL-6 release in response to $PM_{2.5}$ from site 2 was significantly higher than both the control and $PM_{2.5}$ from other sampling locations following 24 h of exposure. A significant prolonged release of IL-6 (up to 48 h) was only observed following exposure to $PM_{2.5}$ from site 1.

In order to determine the impact of ambient LPS present in $PM_{2.5}$ on the release of IL-6, the percent change between LPS-induced IL-6 and concentrations from cells treated with $PM_{2.5}$ was calculated for each time point. At 3 h, all $PM_{2.5}$-induced expression of IL-6 was below the response observed in the LPS-treated cells (expression levels ranged between sampling locations from 18.3 to 78.8% below the LPS control). At 24 and 48 h following $PM_{2.5}$, all sampling locations resulted in elevated levels of IL-6 compared to the LPS control (6.8 to 998.9% above at 24 h and 40.6 to 364.6% above at 48 h). The initial impact of LPS was evident in the pro-inflammatory release at 3 h but following a prolonged exposure (24 and 48 h), all $PM_{2.5}$ samples induced expression of IL-6 above levels due to LPS treatment alone. Sonicated blank filters that acted as an extraction methods control did not show a significant difference in IL-6 release from media controls at any of the time points.

Total cells increased for all treatments and controls over time with no visible indication of cell death except for a duplicate of AMs exposed to $PM_{2.5}$ from site 2 for 48 h. This duplicate was observed to have a reduced cell count, solely at this time point, and visual signs of cell death while the complementary sample had consistent cell counts to all other groups, IL-6 was only measured in the site 2 sample without reduced cell counts. This decision was made based on the change in cell counts from this sample being solely at the 48 h time point with no indication of death at earlier measurements of the same sample and irreproducibility, suggesting contamination of the specific well of cells treated.

3.2 Ambient Versus Extracted Comparisons

Detailed comparisons of the composition of ambient and corresponding extracted $PM_{2.5}$ have been previously reported (Roper et al. 2015) for the samples used in this study. Briefly, total percentage of $PM_{2.5}$ mass recovery was high while a significant loss of metals and organics was observed for all sampling locations, with highest losses observed as ambient $PM_{2.5}$ concentration increased. Ambient concentrations for sites 1–5 were 7.78, 13.73, 13.80, 15.01, and 16.35 $\mu g/m^3$, respectively. Ambient and extracted values for select metals (Table 2) and organics (Table 3) display the differences in contributions of components of $PM_{2.5}$ following extraction from filters. Ratios of ambient/extracted concentrations varied across locations for total measured metals (sites 1–5 = 1.18, 1.26, 1.53, 1.84, and 5.55, respectively) as well as organics (sites 1–5 = 2.03, 2.51, 8.64, 6.48, and 2.69, respectively). Loss of the contribution to $PM_{2.5}$ mass of components occurred across all sampling sites in the extracted samples compared to ambient samples for all components measured except Al, Ce, Na, 1MP, Ipyr, and BghiPer. These six components had observed mass ratios that increased at one or more sampling locations following extraction; a hypothesized rationale for these unexpected findings was that the recovery rate of these components was elevated at specific locations or in comparison to other constituents (Roper et al. 2015) and therefore these components had a greater contribution to extracted samples.

Release of IL-6 was correlated to both mass as well as specific constituents. The inflammatory marker was poorly correlated with total $PM_{2.5}$ (<0.184) across all sampling locations at 3 and 24 h for both ambient and extracted concentrations. After 48 h of exposure, IL-6 was negatively correlated with ambient (−0.592) and extracted (−0.581) concentrations ($p = 0.043$ and 0.048, respectively). The increased IL-6 concentrations

following exposure to $PM_{2.5}$ from sampling site 1, discussed above, are consistent with these results.

The trends between IL-6 release and sums of total metals (Table 4) and organics (Table 5) analyzed are reported for both ambient and extracted measurements at all time points. At 3 h, no correlation was observed for total metals or organics in either ambient or extracted samples. At 24 h, there was a negative correlation between total ambient metals and IL-6 concentrations ($p = .006$), whereas no such relationship was seen for total extracted metals. Ambient metals and organics were both observed to have a negative relationship with IL-6 release. Conversely, extracted metals and organics both showed a positive relationship with IL-6 release (Fig. 2). At 48 h, total metals were slightly positively correlated to inflammation for both ambient and extracted measurements. Total organics correlations again differed, with a slight negative relationship with ambient and a slight positive relationship with extracted measurements.

The correlations between IL-6 concentrations and the proportional mass of individual metals (ng/μg $PM_{2.5}$, Table 4) and organics (pg/μg $PM_{2.5}$, Table 5) were calculated for all sampling locations at each time point following exposure using both ambient and extracted values. Normalization to total $PM_{2.5}$ mass was utilized to eliminate the impact of differing doses between sampling locations (70–151 μg/mL).

At 3 h, specific extracted metals were positively related to IL-6 releases, including Ce, Mn, Ni, and Zn, and these relationships were not observed when using ambient measurements. Interestingly, at 24 h, significant correlations between Ni and IL-6 were observed, with inverse relationships when using ambient (− .856, $p = 0.002$) and extracted (0.857, $p = 0.002$) measurements. Positive relationships were present with extracted measurements of Al, Ce, Cl, Cs, S, and Zn ($p = 0.001$) and Cu, Fe, and Mn ($p = 0.002$, 0.045, and 0.049, respectively). These relationships were not observed in the ambient metals, with the exception of Cs ($p = 0.001$). At 48 h, negative relationships of specific ambient metals were observed: Al, Cl, Cu, Fe, Mn, and Zn ($p = 0.045$, 0.040, 0.035, 0.039, 0.040, and 0.050, respectively). Positive correlations were present for the extracted metals, Ca, Mg, Mo, and Y ($p = 0.042$, 0.035, 0.049, and 0.036, respectively).

At 3 h, positive correlations were observed for individual ambient organic components: Ace, Chr, BaAnt, and BaPyr. However, none of these constituents were present in extracted samples; Acy, 1MP, BbFl, Ipyr, and BghiPer were the only organics detected following extraction. At 24 h, individual ambient organics had positive correlations with IL-6 including Ace ($p = 0.001$), Flu ($p = 0.018$), Chr ($p = 0.003$), BaAnt ($p = 0.028$), BePyr ($p = 0.001$), BaPyr ($p = 0.004$), DBahA ($p = 0.022$), BghiPer ($p = 0.001$), and BghiFl ($p = 0.035$). These correlations were not observed for extracted organics, and a majority of the compounds were not even present in extracted samples with the primary hypothesized reason for loss due to the volatility of the compounds and loss throughout the multistep extraction process (Roper et al. 2015). In contrast to ambient associations, extracted Ipyr had a slightly negative association with IL-6. The negative association between IL-6 and ambient Acy was not observed in the extracted samples where nearly no correlation was observed. At 48 h, negative relationships were observed with ambient measurements of fluorine and

hopanes (p = 0.043 and 0.038, respectively), while these compounds were not detected in the extracted samples and therefore no correlation was observed.

4 Discussion

IL-6 release following exposure to ambient $PM_{2.5}$ has been well documented in cells involved in the respiratory inflammatory response: macrophages (Becker et al. 2005; Jalava et al. 2006), alveolar epithelial cells (Hetland et al. 2004; Shang et al. 2013), and bronchial epithelial cells (Watterson et al. 2007; Lauer et al. 2009). Our findings corroborate previous time-course experiments in which significant release of IL-6 after $PM_{2.5}$ exposure was observed at longer time points (more than 3 h) (Watterson et al. 2012). Interestingly, not all sampling locations induced release of IL-6 above controls and the locations which had significant increases in IL-6 were from sites with the lowest ambient concentrations. Based on previous epidemiology research of the impact of varying $PM_{2.5}$ concentrations on inflammatory-associated health outcomes (Ruckerl et al. 2006; Anderson et al. 2012; Villeneuve et al. 2015), these low concentration locations would be hypothesized to induce comparably reduced responses in vitro. While elevated release of a pro-inflammatory mediator with increasing ambient concentrations was not observed in this study, previous in vitro exposures have found similar results. IL-6 was not observed to have a significant increase compared to controls following exposure to $PM_{2.5}$ from an urban sampling location with high vehicular traffic (Perrone et al. 2010). Findings of mutagenic effects of $PM_{2.5}$ have been similarly unexpected: for example, Zhao et al. (2002) found that the sampling location selected as the “clean” control site, free of any major industrial sources, caused elevated mutagenic potency compared to sampling locations with vehicular and industrial sources. When interpreting these findings, an important factor to consider is that conclusions are drawn from ambient concentrations prior to extraction, and the differences in $PM_{2.5}$ following extraction are a potential cause for discrepancies with epidemiology research.

The inconsistency of these results with current epidemiological findings was also observed for specific constituents of $PM_{2.5}$. Metals established to be associated with health effects, including Ni, S, and Sr (Bernstein et al. 2004; Schwarze et al. 2006), had negative correlations with IL-6 expression when using ambient values but significant positive correlations when using extracted $PM_{2.5}$ values, highlighting the potential for misinterpretation of biological outcomes in filter-based toxicology research. Of the organics analyzed, seven were found to have significant correlations to IL-6 expression, but were not detectable in the extracted $PM_{2.5}$. Significant or complete losses in both metals (i.e., Cu, Cr, Fe, Ni, Zn) and organics (i.e., benzo[*a*]pyrene, benzo[*e*]pyrene, benzo[*ghi*]perylene, pyrene) occur when preparing ambient $PM_{2.5}$ using the extraction methods detailed (Roper et al. 2015). Ambient values of these constituents have previously been used to make associations to inflammation (Huang et al. 2003; Akhtar et al. 2014) and oxidative potential (Valavanidis et al. 2005; Janssen et al. 2014). This study demonstrated that based solely on ambient measurements, the associations between IL-6 and $PM_{2.5}$ constituents made are inaccurate and may indicate health relevancy of compounds, while associations made using data from extracted $PM_{2.5}$ do not support this conclusion. When correlating IL-6 expression to extracted values, positive correlations were observed for both total metals and total organics concentrations, as anticipated from established epidemiology research (Schaumann et al.

2004; Delfino et al. 2010). Characterization of extracted $PM_{2.5}$ solution allows for measurements that are representative of the exposure and avoids inaccurate associations between constituents and inflammatory responses.

Frequently, biological assays are performed using $PM_{2.5}$ that has not been characterized in either ambient or extracted form (Akhtar et al. 2010; Deng et al. 2013; Happo et al. 2013; Jeong et al. 2014). This practice avoids misidentification of causal constituents but disregards compositional variability altogether, despite the establishment of $PM_{2.5}$ composition as a driver of health effects, independent of concentration (Mar et al. 2000; Ostro et al. 2010). Furthermore, the use of uncharacterized $PM_{2.5}$ hinders the ultimate goal of identifying key constituents responsible for health effects. Limited toxicology studies using filter-based extraction methods have characterized samples following partial or complete extraction procedures (Gerlofs-Nijland et al. 2007; Jalava et al. 2009; Huang et al. 2014; Mirowsky et al. 2015). These studies allow for connections to be made to components or groups of components in $PM_{2.5}$ that are the predominant factors in $PM_{2.5}$-associated health outcomes. Awareness of the need for research to base associations on extracted values of $PM_{2.5}$ will facilitate accurate determination of associations to corroborate epidemiological findings and ultimately to identify the mechanisms of $PM_{2.5}$-related health effects.

While this preliminary research raised potential issues of not using properly characterized $PM_{2.5}$, there are a number of limitations that must be considered for future research. First, the dose (μg $PM_{2.5}$/mL) varied between sampling locations and cells were therefore exposed to varying concentrations of ambient $PM_{2.5}$. All concentrations were within a range commonly utilized for in vitro studies, and while direct measurements of cell death were not recorded, cell counts increased over time at all locations indicating proliferation continued and was more substantial than cell death. These doses were selected to preserve both compositional and concentration differences between sampling locations for in vitro exposures. In all data analysis of specific $PM_{2.5}$ constituents, values were normalized to total $PM_{2.5}$ to allow for comparison of constituent effects independent of $PM_{2.5}$ mass. While this study explored effects on a single inflammatory marker, $PM_{2.5}$ has been shown to induce a robust release of numerous cytokines and markers of oxidative stress (Mitschik et al. 2008; Araujo 2010; Anderson et al. 2012). Additionally, the macrophage cell line utilized is reflective of responses specific to that line, and as such is not predictive of the response by other cell types or systemic responses. Future studies using equivalent doses of $PM_{2.5}$ in other cells essential to the inflammatory response to $PM_{2.5}$ (epithelial cells or co-culture systems) or in vivo models with the measurement of multiple inflammatory markers would provide further information on the inflammatory response to $PM_{2.5}$ as well as further demonstrate the importance of well-characterized samples when making associations with these cell types.

5 Conclusions

$PM_{2.5}$ toxicology research has previously made associations between biological responses and ambient measurements, disregarding subsequent changes due to extraction methods. This study has shown that associations made to ambient $PM_{2.5}$ are not comparable to those

made to extracted $PM_{2.5}$ for the methods and marker analyzed. This misrepresentation is due to compositional alterations in $PM_{2.5}$ that occur during extraction of $PM_{2.5}$ from a filter. Recent studies have investigated the potential consequences of different filter extraction methods on biological impacts of $PM_{2.5}$; marked differences in inflammatory gene expression were observed and found to be dependent on the extraction method utilized (Van Winkle et al. 2015). In conjunction with the current study, these results demonstrate the importance of extraction methods as well as the importance of characterizing $PM_{2.5}$ composition when studying toxicological outcomes. Increased understanding of the impacts of $PM_{2.5}$ on inflammatory responses is essential to create targeted interventions relevant to human health; it is imperative that such interventions be based on accurate associations made using well-characterized $PM_{2.5}$ following extraction.

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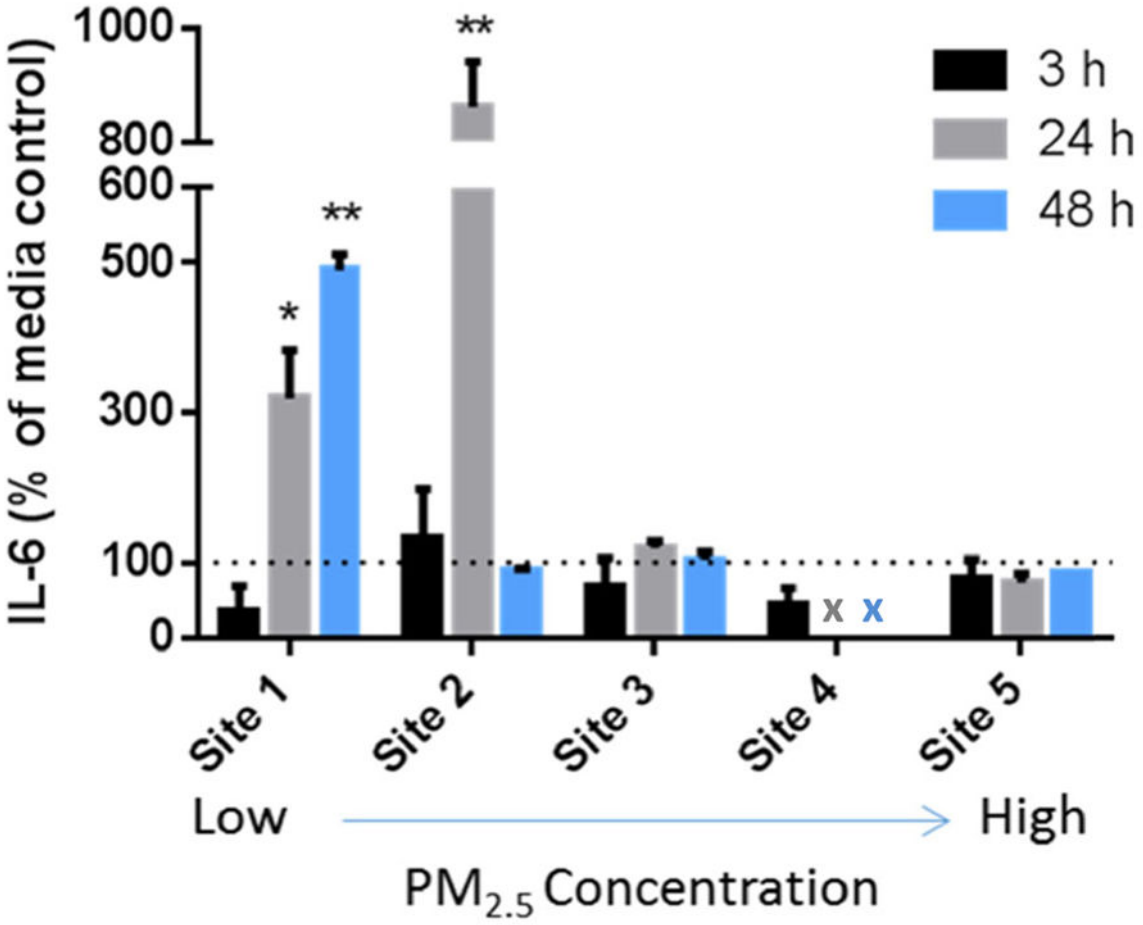

Fig. 1.

IL-6 concentrations (% of media alone control) measured by ELISA method in AMJ2-C11 cell supernatants following $PM_{2.5}$ exposure of 3, 24, and 48 h from varying ambient samples (n = 2/site and time). Sampling locations are ordered from low to high (1 to 5) ambient $PM_{2.5}$ concentrations. Doses (μg/mL) for cell exposure were 71.9 (site 1), 125.1 (site 2), 121.4 (site 3), 122.6 (site 4), and 151.2 (site 5). Results are presented as mean ± SEM with * and ** indicating a statistically significant difference from control ($p < 0.05$ and 0.001, respectively). “x” indicates time points missing from site 4 due to equipment failure during ambient $PM_{2.5}$ collection

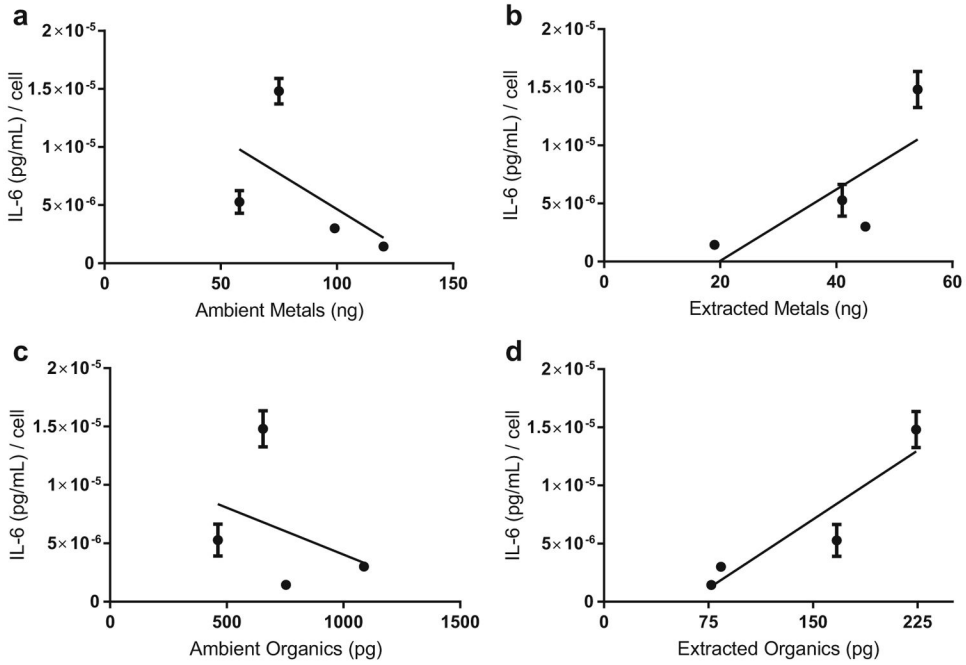

Fig. 2.

Scatterplots of IL-6 release from each sampling location by constituents following 24 h of $PM_{2.5}$ exposure. IL-6 [(pg/mL)/cell] release per total ambient (**a**) and extracted (**b**) metals (ng) or total ambient (**c**) and extracted (**d**) organics (pg). Totals refer to the sum of all constituents measured (Table 1), metals (n = 51) and organics (n = 34). Results are presented as mean ± SEM (n = 2/site)

Table 1

List of constituents analyzed

Metals				**Organics**	
Ag	Cu	Na	Sn	1-Methylphenanthrene (1MP)	Dibenzo[*a,h*]anthracene (DBahA)
Al	Eu	Nb	Sr	2-Methylphenanthrene (2MP)	Dibenzothiophene (DBT)
As	Fe	Ni	Ta	9-Fluorenone (9Flo)	Fluoranthene (Flu)
Au	Ga	P	Tb	Acenapthene (Ace)	Fluorine (F)
Ba	Hf	Pb	Ti	Acenaphthylene (Acy)	Hopanes (*n* = 10)
Br	Hg	Pd	Tl	Benzo[*a*]anthracene (BaAnt)	Indeno[1,2,3-*cd*]pyrene (Ipyr)
Ca	In	Rb	U	Benzo[*a*]pyrene (BaPyr)	Phenanthrene (P)
Cd	Ir	S	V	Benzo[*b*]fluoranthene (BbFl)	Pyrene (Pyr)
Ce	K	Sb	W	Benzo[*e*]pyrene (BePyr)	Steranes (*n* = 4)
Cl	La	Sc	Y	Benzo(*ghi*)fluoranthene (BghiFl)	
Co	Mg	Se	Zn	Benzo[*ghi*]perylene (BghiPer)	
Cr	Mn	Si	Zr	Benzo(*jk*)fluoranthene (BjkFl)	
Cs	Mo	Sm		Chrysene (Chr)	

Table 2

Ambient and extracted metals (ng/μg $PM_{2.5}$) for each sampling location

Site		Al	Cd	Ce	Cl	Cr	Cu	Fe	Mn	Na	Ni	Pb	S	Sr	Zn	Total
1	Amb	0.50	0.00	0.71	8.88	0.15	0.21	5.79	0.24	79.06	0.04	0.32	73.89	0.05	1.75	189.73
	Ext	0.60	0.00	0.00	0.00	0.00	0.07	2.49	0.06	43.78	0.00	0.10	0.00	0.00	0.00	161.18
2	Amb	2.93	0.00	0.32	38.92	0.11	0.44	15.53	0.99	48.49	0.04	0.39	45.53	0.05	2.75	151.71
	Ext	3.61	0.00	0.52	19.03	0.00	0.15	7.44	0.42	67.52	0.04	0.13	2.06	0.01	0.91	120.21
3	Amb	3.99	0.05	0.01	41.96	0.10	0.42	13.36	0.91	44.66	0.04	0.52	45.70	0.06	2.32	170.37
	Ext	0.00	0.00	0.00	0.00	0.00	0.10	5.37	0.25	57.30	0.01	0.13	0.00	0.00	0.06	111.37
4	Amb	3.65	0.00	0.17	28.50	0.14	0.32	13.68	1.37	41.11	0.04	0.28	39.37	0.04	2.32	144.26
	Ext	0.00	0.00	0.00	0.00	0.00	0.10	3.01	0.22	68.39	0.00	0.00	0.00	0.00	0.03	78.41
5	Amb	4.09	0.03	0.31	50.81	0.11	0.44	16.18	1.19	54.68	0.04	0.40	46.83	0.05	2.51	196.23
	Ext	0.00	0.00	0.00	0.00	0.03	0.06	4.71	0.25	0.00	0.01	0.06	0.00	0.00	0.03	35.38

Ambient (“Amb”) and extracted (“Ext”) metals (ng/μg $PM_{2.5}$) determined through gravimetric analysis of $PM_{2.5}$ mass and metal characterization by XRF. Ambient values measured directly from the ambient collected filter and extracted from the extraction solution that was re-deposited onto a filter. “Total” refers to sum of all components analyzed ($n = 51$)

Table 3

Ambient and extracted organics (pg/μg $PM_{2.5}$) for each sampling location

Site		Acy	Pyr	1MP	Chr	BbFl	BjkFl	BaAnt	BePyr	BaPyr	Ipyr	BghiPer	Hopanes	Total
1	Amb	955.96	20.61	3.83	31.54	30.81	69.50	13.93	16.55	11.16	11.52	54.06	3.95	1300.76
	Ext	658.20	0.00	5.75	0.00	0.00	0.00	0.00	0.00	0.00	0.00	52.48	0.00	641.26
2	Amb	784.48	25.81	2.56	49.39	33.94	65.41	24.86	25.62	18.74	18.69	73.68	20.33	1230.16
	Ext	430.50	0.00	2.67	0.00	14.69	0.00	0.00	0.00	0.00	0.00	89.79	0.00	489.91
3	Amb	1464.59	23.12	2.45	39.25	34.55	53.51	19.76	19.41	14.65	13.69	51.63	16.07	1827.10
	Ext	140.95	0.00	2.43	0.00	18.71	0.00	0.00	0.00	0.00	15.79	89.52	0.00	211.39
4	Amb	914.71	13.49	1.58	33.45	30.83	48.22	16.16	18.20	10.80	16.10	70.76	14.77	1231.76
	Ext	194.88	0.00	2.87	0.00	0.00	0.00	0.00	0.00	0.00	0.00	37.66	0.00	190.08
5	Amb	1189.79	0.00	2.36	32.47	31.32	43.89	18.24	16.02	11.38	8.90	42.13	19.42	1476.44
	Ext	489.52	0.00	2.82	0.00	10.55	0.00	0.00	0.00	0.00	0.00	74.54	0.00	548.96

Ambient (“Amb”) and extracted (“Ext”) metals (ng/μg $PM_{2.5}$) determined through gravimetric analysis of PM2.5 mass and organics characterization by TD-GC-MS. Ambient values measured directly from the ambient collected filter and extracted from the extraction solution that was re-deposited onto a filter. “Total” refers to sum of all organic components analyzed ($n = 34$)

Table 4

Pearson's correlations of metals to IL-6 release

	3 h		24 h		48 h	
	Am	**Ex**	**Am**	**Ex**	**Am**	**Ex**
Al	0.075	0.385	−0.016	0.961▲	−0.586*	−0.091
Ca	0.195	−0.118	−0.070	−0.346	−0.543	0.594*
Cd	−0.057	0.007	−0.519	−0.372	−0.395	−0.218
Ce	−0.034	0.404	−0.025	0.945▲	0.532	−0.215
Cl	0.231	0.404	0.068	0.945▲	−0.598*	−0.215
Cr	−0.226	0.007	−0.089	−0.372	0.574*	−0.218
Cs	0.404	0.239	0.945*	0.891▲	−0.215	−0.325
Cu	0.323	0.323	0.242	0.852*	−0.611*	−0.281
Fe	0.282	0.429	0.269	0.642*	−0.602*	−0.540
Mg	−0.226	−0.254	−0.749*	−0.239	0.465	0.610*
Mn	0.072	0.419	0.158	0.634*	−0.599*	−0.544
Mo	0.035	−0.223	0.129	−0.206	0.547	0.579*
Na	−0.121	0.066	−0.259	0.603	0.585*	−0.002
Ni	−0.242	0.437	−0.856*	0.857*	0.378	−0.381
Pb	0.119	0.245	−0.133	0.534	−0.456	−0.057
S	−0.145	0.404	−0.246	0.945▲	0.608*	−0.215
Sn	−0.166	−0.007	−0.489	−0.372	0.482	−0.218
Sr	−0.010	0.286	−0.590	0.739*	−0.147	0.292
Y	0.100	−0.208	0.299	−0.226	0.513	0.609*
Zn	0.386	0.409	0.490	0.934▲	−0.576*	−0.252
Total	−0.123	−0.015	−0.796*	0.198	0.323	0.470

Correlations of IL-6 release at 3, 24, and 48 h to both ambient (Am) and extracted (Ex) metal constituents. "Total" refers to the sum of all metals ($n = 51$) analyzed in Table 1.

*, ▲indicate a statistically significant correlation with $p < 0.05$ and <0.001, respectively

Table 5

Pearson's correlations of organics to IL-6 release

	3 h		24 h		48 h	
	Am	**Ex**	**Am**	**Ex**	**Am**	**Ex**
Acy	−0.153	0.064	−0.678*	0.001	−0.266	0.453
Ace	0.404	-	0.945◢	-	−0.215	-
F	0.323	-	0.279	-	−0.590*	-
P	0.158	-	0.193	-	0.516	-
Flu	0.279	-	0.722*	-	−0.001	-
Pyr	0.146	-	0.514	-	0.109	-
9Flo	−0.161	-	−0.236	-	0.608*	-
DBT	−0.063	-	−0.295	-	−0.267	-
1MP	−0.008	−0.215	−0.145	−0.220	0.607*	0.603*
2MP	0.296	-	0.536	-	−0.502	-
Chr	0.401	-	0.825*	-	−0.389	-
BbFl	0.271	0.280	0.410	0.232	−0.447	−0.557
BjkFl	0.145	-	0.412	-	0.461	-
BaAnt	0.432	-	0.687*	-	−0.519	-
BePyr	0.381	-	0.881◢	-	−0.317	-
BaPyr	0.400	-	0.814*	-	−0.387	-
Ipyr	0.235	−0.063	0.866*	−0.295	−0.211	−0.267
DBahA	0.218	-	0.709*	-	−0.406	-
BghiPer	0.172	0.329	0.923◢	0.386	−0.032	−0.569
BghiFl	0.369	-	0.667*	-	−0.528	-
Hopanes	0.337	-	0.335	-	−0.603*	-
Steranes	0.247	-	0.203	-	−0.509	-
Total	−0.085	0.124	−0.533	0.046	−0.303	0.405

Correlations of IL-6 release at 3, 24, and 48 h to both ambient (Am) and extracted (Ex) organic constituents. "Total" refers to the sum of all organics (n = 34) analyzed in Table 1. – indicates a missing value due to the constituent not being present in the extracted solution.

*, ▲ indicate a statistically significant correlation with $p < 0.05$ and <0.001, respectively