Linking urban air field measurements to their chemical analysis and their effects on health: Part I

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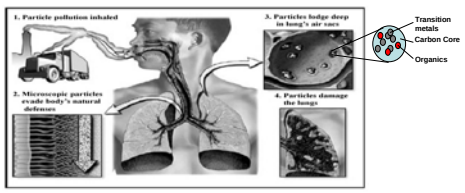
Introduction

Internationally collected data demonstrates that adverse health effects correlate with fine particulate matter (PM) both geographically and seasonally. However, major questions concerning the mechanisms by which they act remain unanswered. The aim of this study was to characterise meteorologically and spatially different $PM_{2.5}$ (particulate matter collected with 50% efficiency for particles with an aerodynamic diameter of 2.5μm) by its physicochemical and biological properties. $PM_{2.5}$ was collected at three sites representative of southern Irish atmosphere (located throughout Cork, Ireland); a central urban site (City), an urban background site (UBS) and a rural site (RS), over the four seasons. A portion of the biological component (*e.g.* fragments of pollen and spores) were identified by scanning electron microscopy exclusively in the spring and summer samples at all three sites. At the city site more than 50% of particulates were closer to the accumulation range/transient nuclei or Aitken nuclei size range (1 - 0.01μm particle mean aerodynamic diameter) irrespective of season and were comprised predominately of chain like soot aggregates. Elemental composition demonstrated spatial and seasonal variation; all samples contained varying levels of total carbon but levels were highest at the city site during the winter season. Higher transition metal concentrations were evident at the city and UBS sites, whereas higher concentrations of SO_4^{2-} were observed in the rural samples. A good agreement was noted between total sulfur and SO_4^{2} at both the UBS and rural site (r^2= 0.895 and 0.898 respectively), whereas the city site correlation (0.668) suggested that sulfur exists in other forms as well as SO_4^{2} (e.g. metal sulfides).

$PM_{2.5}$ Toxicity



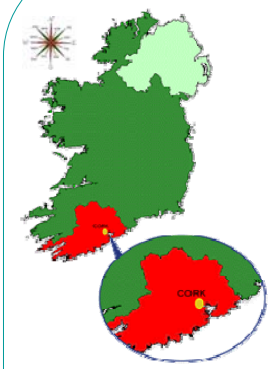
Local effects:

- Respiratory System
- Induction of inflammatory responses in the lungs

Systemic effects:

- Cardiovascular System
- Induction of systemic inflammatory responses and changes in neural control of heart function

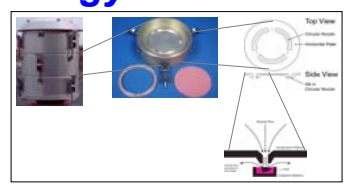
Sampling Methodology

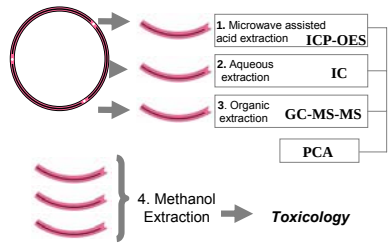


City Site

Rural

Urban Background Site





$PM_{2.5}$ was collected onto polyurethane foam filter (PUF) using a three stage high volume cascade impactor (flow rate of 900 l/ min) during a 2 year sampling campaign, collecting for 7 day periods, at three sampling sites (City, Urban background, and Rural sites). All particulates recovered from same site during the same season were pooled together to give a homogenous batch of particles representative of that site during a particular season and to avoid any day-to-day variations in composition that could be attributed to anthropogenic and meteorological activities

Objectives:

•To physicochemically characterize the geographically and meteorologically different $PM_{2.5}$ samples, along with determining a biological component (endotoxin) that have been categorised according to site and season.

•To determine any geographical/seasonal differences in both the toxicity profile and chemical composition for the $PM_{2.5}$ sampled in the urban area of Cork City.

•To use the physicochemical database to determine the toxicity profile of $PM_{2.5}$ and determine any links that different samples may have with their chemical composition

Gravimetric analysis

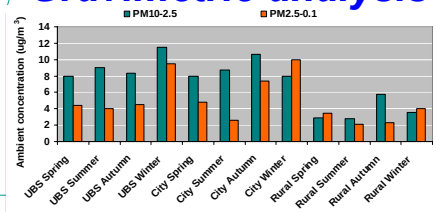


FIGURE 1 Average ambient mass concentrations (μg/m³) at the three sites during each season.

Physical Characterisation

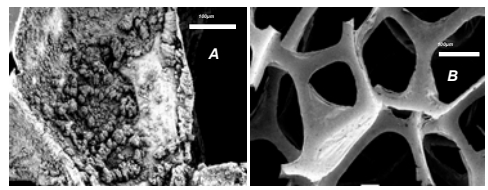


FIGURE 2 SEM image of the filter substrate after collection (A), and the same filter after the particle recovery method (B) .

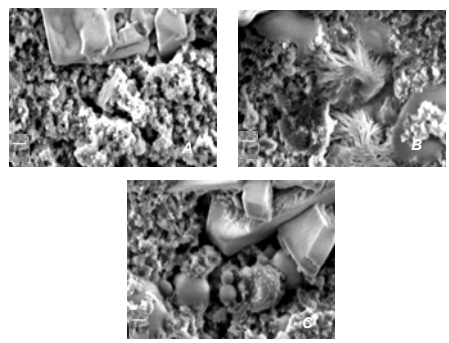


FIGURE 3. SEM images (×10,000) of the PM2.5 samples collected from ambient air in Cork during the winter season, used both for the physicochemical analysis and biological investigations. (A) City winter, (B) Rural winter, and (C) UBS winter

Chemical Characterisation

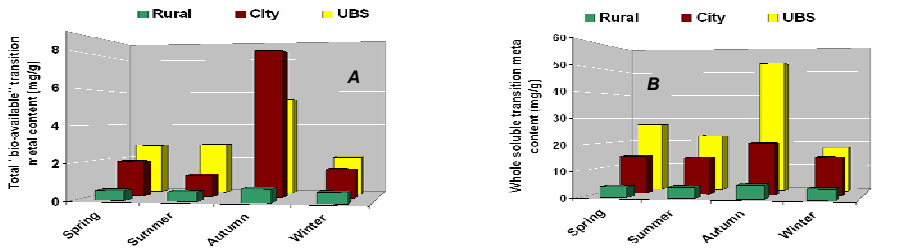


FIGURE 4 Comparison of total transition metals concentrations (mg/g) between site/season samples. (A) compares the bioavailability of the total transition metal content, determined after water extraction and analysed by ICP-OES. (B) compares the total transition metals concentrations determined after acid/microwave extraction and analysed by ICP-OES

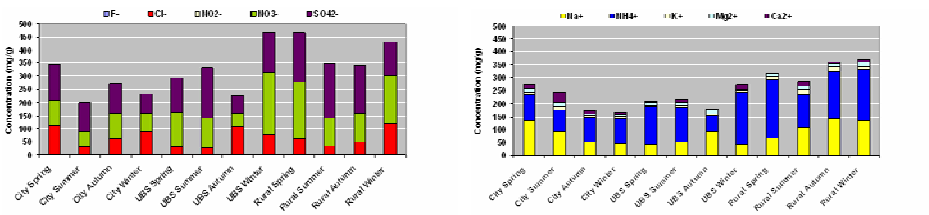


FIGURE 5 Anions and Cations determined by Ion Chromatography illustrating the anionic composition of all the site/season samples.

Conclusion

- Transition metals such as Fe, Mn, Ti and Cu were found to be more evident in the city site samples (compared to the UBS and rural site samples) whereas Zn, Pb, V, and Ni were noticeably higher at the UBS site.
- Anions such as SO_4^{2-}, NO_3^- and Cl^-; and cations such as NH_4^+ and Na^+ were found to be major components in all samples
- The influence of primary sea salt (Na^+ and Cl^-) in the Irish atmosphere was noted at all of the three sampling sites
- At the city site more than 50% of particulates were closer to the accumulation range/transient nuclei or Aitken nuclei size range (1 - 0.01μm particle mean aerodynamic diameter) irrespective of seasons,when morphologically analysed
- Relatively low concentrations (EU/mg) of Endotoxin were found on all of the $PM_{2.5}$ samples collected. (Data not shown)

Acknowledgements

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Linking urban air field measurements to their chemical analysis and their effects on health: Part II

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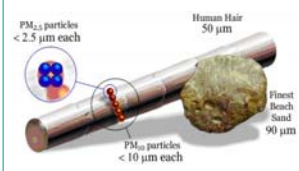
Introduction



Epidemiological studies linking particulate matter (PM) air pollution with adverse respiratory and cardiovascular effects have focused attention on the interaction of PM and lung cells both *in-vitro* and *in-vivo*. We treated human lung epithelial cells, A549, with 5.5, 11 and 22 µg/cm^2 of ambient air $PM_{2.5}$ (PM <2.5 µm aerodynamic diameter), collected from three sites over four seasons from the Irish atmosphere in the region of Munster (Cork). Production of interleukin (IL)-6, IL-8, TNF-α and reactive oxygen species (ROS) was measured. All samples (in the absence of cytotoxicity: determined by the resazurin and lactate deshydrogenase (LDH) assay) at three different concentrations (5.5, 11 and 22 µg/cm^2) induced intracellular ROS and release of IL-6 and IL-8 except TNF-α, (which after 72hrs were close to that of the control). The city site samples showed a consistent ability to increase the production of intracellular ROS irrespective of the season, measured by dichlorofluorescein acetate. $PM_{2.5}$ samples collected during the summer season at the City, Rural and Urban Background sites were the most potent stimulators for IL-6 release, reaching an average maximum of 10.06, 8.52 and 4.21 fold increase of control, respectively. Univariate regression and multivariate analysis were used to test for correlation of viability, cytokine release and the induction of intracellular reactive oxygen species (ROS) with the concentrations of 24 elements, 10 ions and endotoxin content. Principle component analysis on the elemental/biological components and toxicological endpoints database identified 4 principle components that accounted a total variance of 78.62%. Results indicate that elemental components and physical shape of the particles could play an important role in explaining the seasonal and geographical variations in PM-induced health effects related to respiratory illnesses.

Objectives

•To investigate the effect of the chemical composition of $PM_{2.5}$ on its ability to induce a toxic effect on the human lung epithelial cell line A549 and possibly identify correlations between compositional components and biological effects induced.

•To determine any geographical/seasonal differences in both the toxicity profile and chemical composition for the $PM_{2.5}$ sampled in the urban area of Cork City.

Methodology

Particle collection and extraction.
Refer to Part I for sampling methodology and particulate removal from filter substrate.
Physical, chemical and biological analysis of PM.
Refer to Part I for physicochemical analysis and endotoxin data.
Cell culture.
The human alveolar cell line A549 was used as a model system for human epithelial lung cells.
Assessment of cytotoxicity.
The resazurin assay and lactate deshydrogenase (LDH) assay.
Intracellular ROS production.
The 2', 7'-dichlorofluorescein diacetate (DCFH-DA) assay.
Cytokine release.
Levels of interleukin (IL) IL-6, IL-8 and TNF-α were determined in the culture media using commercially available enzyme-linked immuno absorbent assay (ELISA) kits
Endotoxin.
Endotoxin content was measured using the chromogenic *Limulus* amebocyte lysate assay kit with diazo modification (Cape Cod Inc., USA).

Statistical analysis.
Significance between samples and control were determined using one-way analysis of variance (ANOVA) followed by Dunnett's post test and a comparison of data from different samples (site/season) was made by ANOVA followed by the Tukey's multiple comparison post test using Graph pad software (Version 2.0.1.430; GraphPad Software Inc.). Statistically significant differences were reported when p <0.05.

Principle component analysis (PCA) was applied to the set of variables (elemental compositional data, toxicological endpoints) primarily to examine how the components vary together but also to determined whether or not differences in particle constituents can account for the variable potency of PM to induce a biological effect. Data from the three sites and four seasons were included. Components were subjected to rotation using the VARIMAX criteria for interpretation, and the individual principle components were interpreted using factor loadings. Absolute scores for each individual principle component were also obtained from this analysis and used to determine the association between site/season and toxicological endpoints/elemental composition. Linear regression analysis was also performed to determine the association between individual elemental constituents of the PM (independent variables) and the toxicological endpoints that were associated with the factors identified during PCA analysis (dependent variables).

Results

Intracellular ROS production

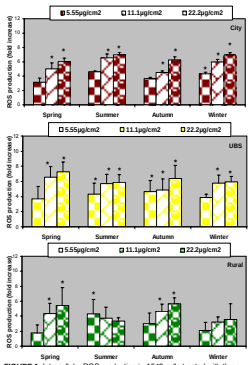


FIGURE 1. Intracellular ROS production in A549 cells treated with three concentrations of each season sample (5.55, 11.1, 22.2µg/cm2 respectively). Values are means ± SD (n=4). * Indicates statistical difference from the control (p<0.01, ANOVA Dunnett's post test)

Univariate regression analysis

	ROS (5.55µg/cm²)	ROS (11.1µg/cm²)	ROS (22.2µg/cm²)
Zn	0.577	0.301	0.429
Sb	0.643	0.535	0.525
Pb	0.726	0.551	0.627
Cd	0.816	0.681	0.749
Ni	0.894	0.540	0.803
Fe	0.751	0.525	0.639
Co	0.854	0.811	0.777
Mn	0.741	0.606	0.625
Cr	0.742	0.602	0.565
V	0.556	0.496	0.520
Ca	0.648	0.508	0.467
Cu	0.763	0.478	0.663
Ti	0.619	0.467	0.539
Al	0.831	0.557	0.614
NH_4^+	0.652	0.259	0.412
NO_3^-	0.131	0.050	0.011
NO_2^-	0.536	0.222	0.369
SO_4^{2-}	0.650	0.366	0.581

Table 1. Regression coefficient (r2) (at 95% confidence level) obtained from the plot of ROS (fold of control for the three exposure concentrations) against the concentration of individual elements in µg/g

Induction of Cytokine release

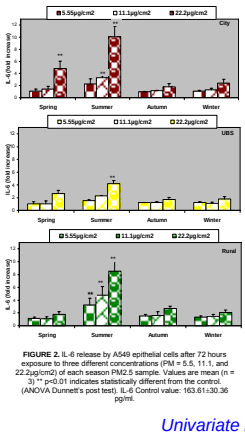


FIGURE 2. IL-6 release by A549 epithelial cells after 72 hours exposure to three different concentrations (PM = 5.5, 11.1, and 22.2µg/cm2) of each season PM2.5 sample. Values are mean (n = 3) ** p<0.01 indicates statistically different from the control. (ANOVA Dunnett's post test). IL-6 Control value: 163.61±30.36 pg/ml.

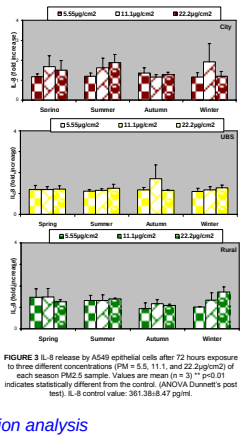


FIGURE 3 IL-8 release by A549 epithelial cells after 72 hours exposure to three different concentrations (PM = 5.5, 11.1, and 22.2µg/cm2) of each season PM2.5 sample. Values are mean (n = 3) ** p<0.01 indicates statistically different from the control. (ANOVA Dunnett's post test). IL-8 control value: 361.38±8.47 pg/ml.

Univariate regression analysis

	IL-6 (5.55µg/cm²)	IL-6 (11.1µg/cm²)	IL-6 (22.2µg/cm²)
Cd	0.62	0.63	0.65
Co	0.56	0.65	0.33
K^+	0.85	0.86	0.66
Ca^{2+}	0.57	0.46	0.83

Table 2. Univariate correlation (r^2) values between IL-6 and chemical components

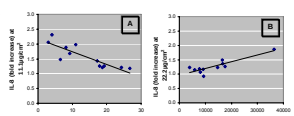


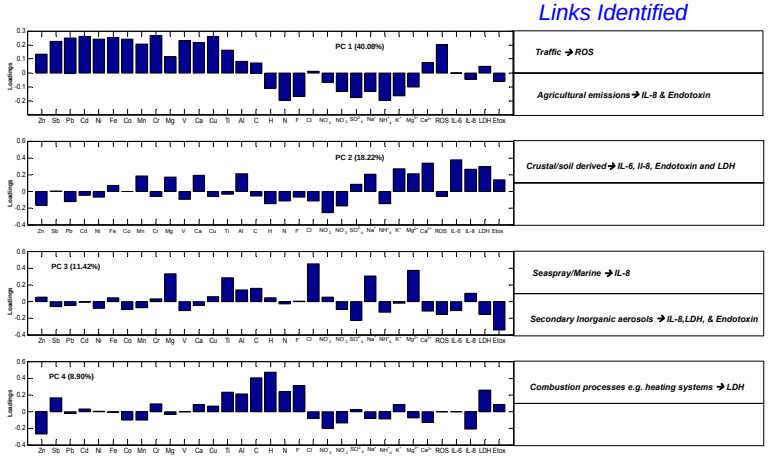
FIGURE 4. Univariate correlations between (A) IL-8 release at 11.1µg/cm² negatively correlated with water soluble Vanadium concentration (r^2 = 0.74) and (B) IL-8 release at 22.2µg/cm² showing a positive correlation with Ca^{2+} (r^2 = 0.69)

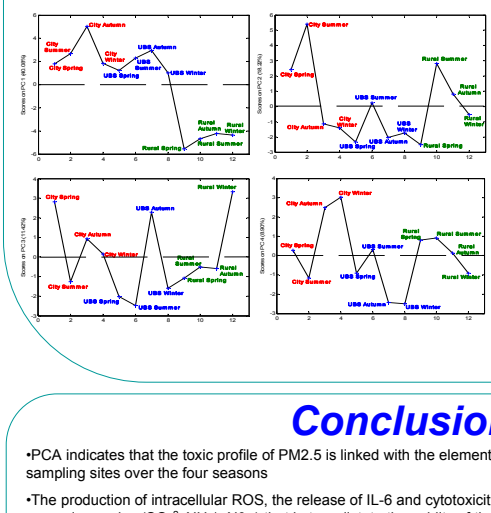
Results

Multivariate analysis:- PCA

Principle Component Analysis (PCA) was performed on both the elemental and the toxicological endpoints data to investigate multivariate correlations. FOUR principle components (PC) that accounted a total variance of 78.62%. Loadings and scores on each PC are shown below.

Links Identified





PCA indicates that the toxic profile of $PM_{2.5}$ is clearly linked with the elemental content. FOUR different components were identified: **1st PC** correlates **ROS and transition metals**, allowing the **separation of City/UBS (polluted) from the rural (unpolluted)** samples; for the **2nd PC** a correlation was noted for **crustal/soil- derived** elements (Ca, Mn, Al, K^+) and the toxicological endpoints (**IL-6, IL-8, LDH and endotoxin**); the **3rd PC** identifies a relationship between **sea-spray/marine** (Na^+, Cl^-, Mg, Ti) and **IL-8**, and between **secondary inorganic aerosols species** (SO_4^{2-} NH_4^+, $N0_3^-$) and **ROS, IL-6 and LDH**. The **4th PC** shows a positive correlation between **hydrocarbons** (C, H), Ti, Al and **LDH,** this is especially clear for the city site during winter and autumn *e.g.* heating systems.

Conclusions

•PCA indicates that the toxic profile of PM2.5 is linked with the elemental components of the samples collected at the three sampling sites over the four seasons

•The production of intracellular ROS, the release of IL-6 and cytotoxicity are someway dependant on secondary inorganic aerosols species (SO_4^{2-} NH_4^+, NO_3^-) that in turn dictate the acidity of the particle

•Results also propose new hypotheses *e.g.* endotoxin content, IL-6, IL-8 being dependent on crustal type elements within the composition of PM

•Results indicate a relationship between elements commonly found in sea-spray/marine emissions *i.e.* Na^+, Cl^-, Mg, Ti and IL-8,

•Results agree with well recognised hypotheses *i.e.* generation of ROS by transition metals

•***Our results indicate that the ability of $PM_{2.5}$ to induce a biological effect is largely dependent on elemental composition and therefore directly related to the geographical location and seasonality***