Linking urban air field measurements of particulate matter to their chemical analysis and potential effects on health

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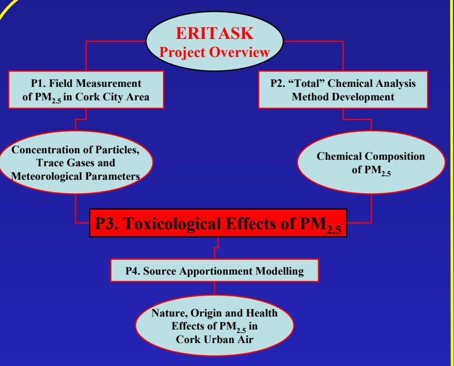
Introduction

Recent epidemiological studies have shown that atmospheric pollution caused by airborne particulate matter (PM) has a negative impact on human health. Adverse outcomes include exacerbation of respiratory symptoms, reduced lung function, chronic bronchitis, cardiovascular diseases and mortality. However toxicological studies suggest that such health effects can depend on PM chemical composition, as well as size (and possibly shape). Chemical analysis shows PM to comprise of many inorganic (Trace metal/Inorganic ions), organic, and elemental materials, several of which are toxic. For example, solubilised metal ions such as zinc have been found to be linked to lung injury. Metals on PM can be found within the matrix of an insoluble component, within a soluble salt, or complexed at a surface. Metals which can be available in more than one stable valence state can catalyse an electron transfer and therefore demonstrate some capacity to generate oxidants. Therefore it is important to determine the contribution of metals and the other chemical components, to the adverse health effects observed in PM epidemiology studies. Hence we have utilized various protocols for measuring cellular effects, which might be suitable for measuring the toxicity of PM, (e.g. assays to determine cell damage, cell defence, cell membrane damage and reactive oxygen species [ROS] production). For example, the lactate dehydrogenase (LDH) assay has been used to evaluate cell damage by measuring the enzyme activity leakage in the medium. The reduced glutathione (GSH) assay has been employed to evaluate how GSH, one of the primary biochemicals for cell defence, can be influenced by PM toxicity. Cytokine induction by PM is determined by quantitaively measuring IL-6, (a major inducer of the acute phase reactions in response to inflammation or tissue injury). Reactive Oxygen Species have been monitored by fluorimetric assay (DCF-DA), which evaluates the percent of ROS produced in exposed cells compared to control cells. To investigate the biological effects of $PM_{2.5}$ at a sub cellular level, human epithelial pulmonary A549 cell line was exposed for three days to different concentrations of $PM_{2.5}$ (0, 5.5, 11.0, 22.0 μg/cm²) and the afore mentioned assays were employed.

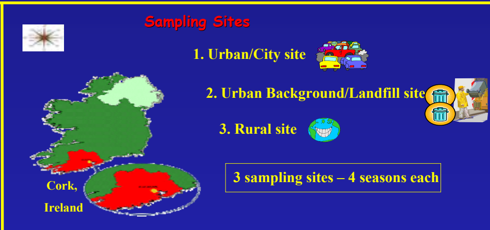
Keywords: $PM_{2.5}$, transition metals, toxicity, ROS, GSH, LDH, IL-6

Project Outline

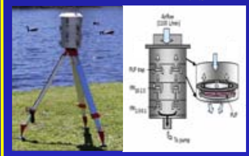


This presentation focuses only on P3 of the ERITASK project which is Toxicological effects of $PM_{2.5}$

Sampling Sites



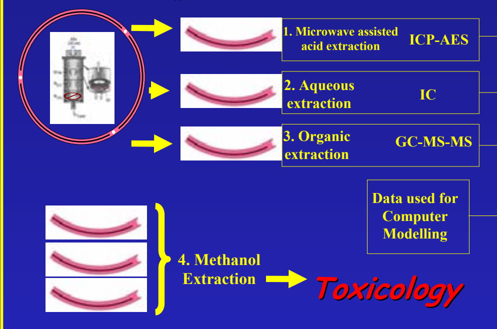
Samples are collected using a high volume cascade impactor (Fig 1). Sampling coarse ($PM_{10-2.5}$) and fine ($PM_{2.5}$) fractions.



- High flow rate of 900 l min⁻¹
- Uses polyurethane foam an inert collection substrate
- High weight of PM can be collected in a short period of time, providing an excess of sample for chemical analyses and sufficient weight for toxicological studies

Fig 1. The three stage high volume cascade impactor, collecting $PM_{10-2.5}$ and $PM_{2.5}$ onto polyurethane foam.

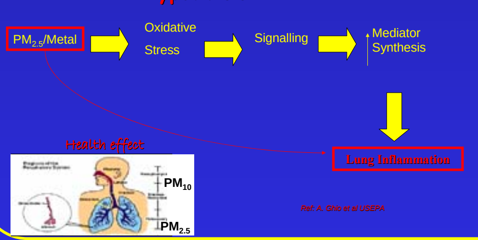
$PM_{2.5}$ retrieval from filter substrate



Samples comprising the same season and site were pooled together to be chemically analysed and compared with the results obtained with the samples used for the toxicology studies.

Methodology

Hypothesis



Experimental

- One experimental purpose of the project is to evaluate *in vitro* the variability in toxicity of PM sampled during different seasons and in different geographical sites in Cork city.

To perform the toxicology experiments, the PM was extracted from polyurethane foam collection substrate by methanol extraction: a technique based on a modified method of that by Salomen et al (2000)

The A549 cell line was chosen as a model for measuring cell toxicity exposed for 72 hours to different concentrations of $PM_{2.5}$ (0, 5.5, 11.0, 22.0 μg/cm²).

Metals: 20 metals comprising As, Sn, Hg, Mo, Zn, Sb, Pb, Cd, Ni, Fe, Co, Si, Mn, Cr, Mg, V, Ca, Cu, Ti and Al were analysed by ICP-AES after microwave assisted acid digestion and water based extraction.

Assays:

ROS
The ability of $PM_{2.5}$ to produce Reactive Oxygen Species (ROS) was determined according to the method of Tsubouchi et al. (2001), using dichlorofluorescein diacetate probe (DCFH-DA)

GSH
The reduced form of Glutathione (GSH) which provides defence against ROS by scavenging free radicals and reducing H_2O_2 was determined according to the method of Hissin & Hilf (1976) (nmol/mg prot)

LDH
As an index of cytotoxicity after $PM_{2.5}$ exposure the activity of LDH released from the cytosol of damaged cells into the supernatant was determined using the colorimetric detection kit from Roche (Absorbance units)

IL-6
Human Interleukin 6 (IL6) is a major inducer of the acute phase reactions in response to inflammation or tissue injury. IL 6 was quantitatively measured using an immunoenzymometric assay kit from Biosource.

Results

Seasonal and site variability

ROS

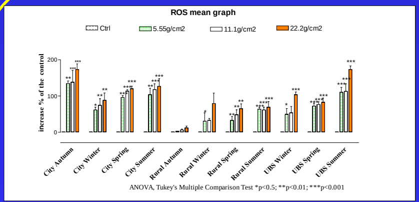


Fig 2. Reactive Oxygen Species production after 72 h exposure to different $PM_{2.5}$ concentrations expressed as an average of 4 repetitions

GSH

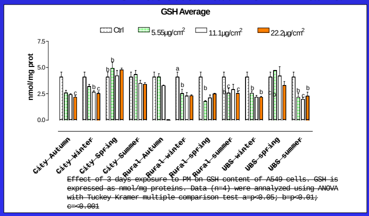


Fig 3. GSH consumption after 72 h exposure to different $PM_{2.5}$ concentrations expressed as an average of 3 repetitions.

Metals

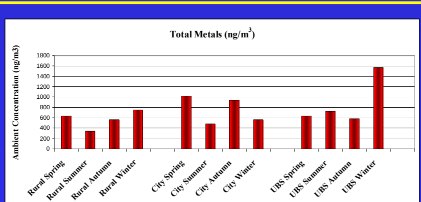


Fig 6. Seasonal average of total metal content in $PM_{2.5}$ measured by ICP-AES and expressed as ng/m³ in ambient air.

LDH

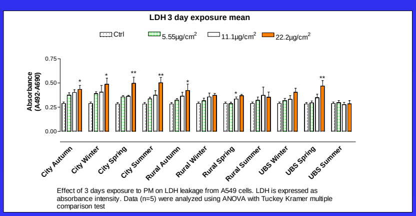


Fig 4. LDH leakage after 72 h exposure to different $PM_{2.5}$ concentrations expressed as an average of 6 repetitions.

IL-6

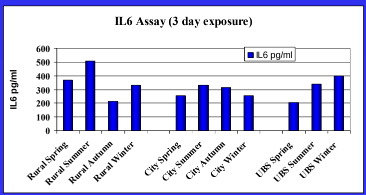


Fig 5. Human Interleukin 6 after 72 h exposure to a $PM_{2.5}$ concentration of 22.2 ug/ml.

Metals

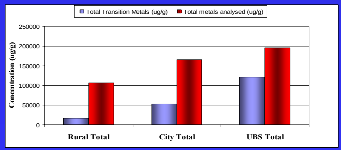


Fig 7. Total metals analysed and Total Transition Metals expressed in ug/g is shown for the 3 individual sites

(Preliminary) Statistical Analysis

Applying linear regressions to the metals and ROS results a positive correlation can be found showing a direct relation between transition metals content in $PM_{2.5}$ and ROS production (Table 1).

Correlation Coefficients	Hg	Zn	Ni	Fe	Mn	Cr	V	Cu	Ti
ROS	0.7109	0.8308	0.8553	0.7602	0.7372	0.8349	0.7460	0.7984	0.5671

Table 1. Pairwise linear correlation coefficients between selected metals and ROS production.

Conclusions

ROS
$PM_{2.5}$ from different seasons and locations showed a range of potency to produce reactive oxygen species (ROS). Both urban sites (City and UBS) were more potent in increasing the DCFH-DA fluorescence signal this is in agreement with chemical compositional analysis which also determined a higher concentration of anthropogenic compounds at these sites. In particular City autumn was found to exhibit a significantly greater ROS production along with the UBS summer sample, when compared with any other sample.

LDH
Concentration dependancy was noted for some samples for the release of cytoplasmic lactate dehydrogenase (LDH). This was mainly found for the city sites. There was no seasonal variation noted for any of the three sites.

GSH
The treatment of the A549 cell line with $PM_{2.5}$ induced a non-concentration dependent effect on the cellular GSH compared to controls. The site/seasons with the highest GSH consumption after 3 day exposure was found to be that of City Spring and UBS Spring respectively.

IL-6
The potency for $PM_{2.5}$ from different site/season samples to induce inflammatory mediators varied between the rural and UBS sites but remained relatively stable over the four seasons at the city site although rising slightly during the warmer/drier seasons of Summer and Autumn. For $PM_{2.5}$ rural summer was the most potent in inducing IL-6

Metals
The UBS site exhibited the highest total metal and transition metal concentrations shown in Fig. 7, This could be largely due to the influence of landfill and motorway activity near to this site. The order of Total and Transition metal concentration, starting with the highest concentrations was found to be: UBS site > City site > Rural site (See Fig.7.

Table 1 shows the linear correlation coefficients between selected transition metals and ROS production. It demonstrates Zn and Ni to have the highest coefficients. Both these metals were detected in the water soluble fraction and have been shown to be readily bio-available

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References

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Further Information:

- More information regarding this study can be found on our research laboratory website using the following link: http://crac.ucc.ie/research_part.html