A Marie Curie TOK Project on Airborne Particulate Matter.

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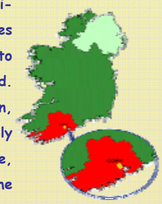
INTRODUCTION

A full understanding of the impact of atmospheric pollution on human health and the ecology of the Earth System requires a scientific approach, which links laboratory experiments to field measurements to predictive models. Therefore the ambition of ERITASK has been to recruit four expert postdoctoral researchers to University College Cork (UCC) who can combine, in a multi-disciplinary team, for the purpose of determining the impact of particles on Air Quality and Health. The main scientific objective of the ERITASK programme is to characterise the processes that produce "fine" ($PM_{2.5}$) particles in urban air, by providing appropriate chemical and biochemical analyses. Subsequent interpretation and computer analysis of the data has been used to determine the importance of the different source categories and therefore to increase our knowledge of the environmental risk associated with particulate matter in Cork, Ireland. Epidemiological studies especially have emphasised the role played by particulate matter on 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). 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. 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.

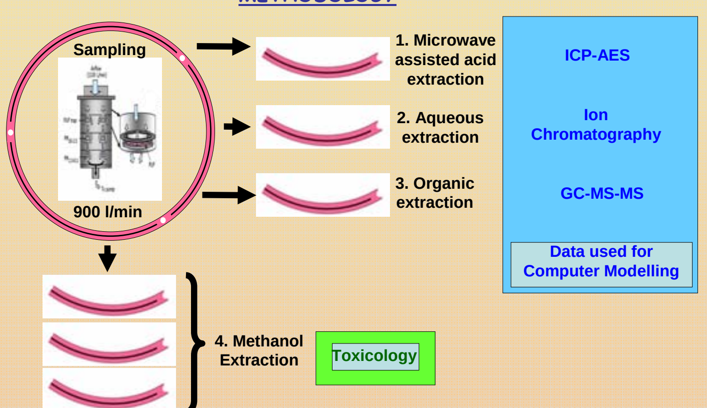
Cork, Ireland



AIMS

Samples of $PM_{10-2.5}$ and $PM_{2.5}$ have been collected using a High Volume Cascade Impactor (HVCI) in Cork City centre and also at background urban and rural sites. The collected samples have been chemically analysed for metal content using ICP-AES, organic markers have been determined using GC-MS-MS and inorganic ions have been quantified by ion chromatography. The use of this detailed chemical data is twofold: firstly it is being used to produce a computer model for the determination of the origin and distribution of $PM_{2.5}$ in Cork; secondly it is being used to find links with toxicological endpoints.

METHODOLOGY



RESULTS 2: Chemometrics

Multivariate correlations are best investigated by means of Principal Component Analysis (PCA). It is a powerful chemometrics method for identifying patterns in data, and expressing the data in such a way as to highlight their similarities and differences. When the 2 main principal components are plotted together the 36 variables comprising metals, PAHs and toxicology results group to form 2 main sets.(Fig. 6, left). The first group (orange circle) includes most of the metals and ROS results, while the second group (green circle) includes most of the PAHs, GSH and LDH results.

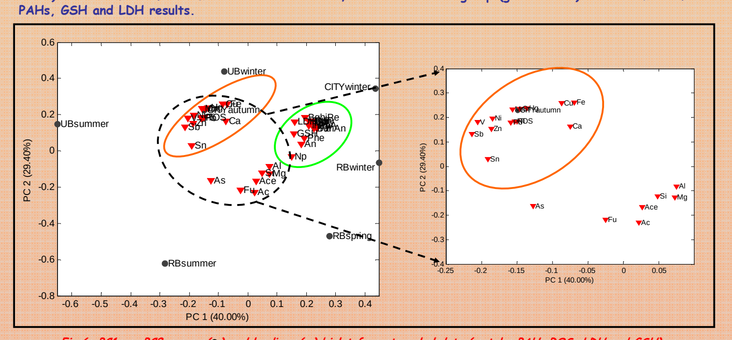


Fig 6. PC1 vs. PC2 scores (●) and loadings (▼) biplot for autoscaled data (metals, PAH, ROS, LDH and GSH)

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).

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

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

ACKNOWLEDGEMENTS

The Authors wish to thank the following bodies for their financial assistance:

- 6th Framework Program Marie Curie TOK Actions
- IRCSET Embark Initiative
- EPA-Ireland

BIBLIOGRAPHY

- Tsubouchi R, Htay HH,Murakami , Haneda M, Yoshino M, Biometals (2001) 14; 181-185.
- Hissin PJ, Hilf R, Anal. Biochem. (1976) 74; 214-226.

RESULTS 1: Seasonal and site variability

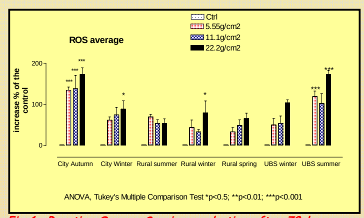


Fig 1. Reactive Oxygen Species production after 72 h exposure to different $PM_{2.5}$ concentrations expressed as an average of 4 repetitions.

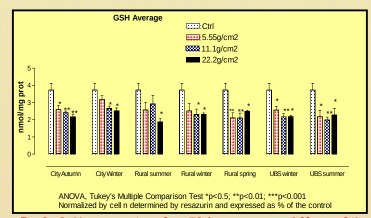


Fig 2. GSH consumption after 72 h exposure to different $PM_{2.5}$ concentrations expressed as an average of 3 repetitions.

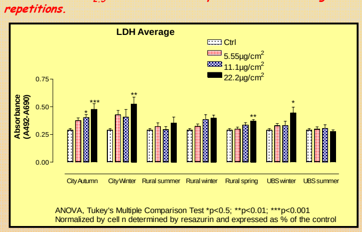


Fig 3. LDH leakage after 72 h exposure to different $PM_{2.5}$ concentrations expressed as an average of 6 repetitions.

In the present work a 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 ug/cm2).

- The ability of $PM_{2.5}$ to produce Reactive Oxygen Species (ROS) was determined according to the method of Tsubouchi et al. (2001). Results were expressed as percent of the control (Fig.1).
- 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). Results were expressed as nmol/mg proteins (Fig.2).
- Finally, 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. Results were expressed as absorbance intensity units (Fig.3).

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. For the present work we have summarised the results obtained for 2 different groups of chemicals:

- 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 a microwave assisted acid digestion. Mo, Co and Cd were excluded from the following studies because they were not detected in any sample. Results are shown in Figure 4.
- The 16 Polycyclic Aromatic Compounds (PAHs) listed by the U.S.EPA as priority pollutants were analysed by GC-MS-MS after a 24 h soxhlet extraction with DCM and pre-separation through a silica gel column. Results are shown in Figure 5.

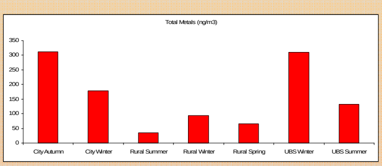


Fig 4. Seasonal average of total metal content in $PM_{2.5}$ measured by ICP-AES and expressed as ng/m³ in ambient air.

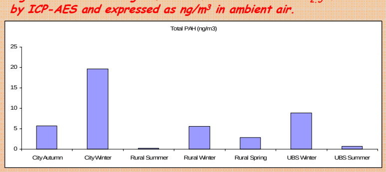


Fig 5. Seasonal average of total PAHs content in $PM_{2.5}$ measured by GC-MS-MS and expressed as ng/m³ in ambient air.

CONCLUSIONS & FUTURE WORK

- The chemical composition of $PM_{2.5}$ in ambient air shows strong location variability. Cork City centre and urban background sites are influenced by higher emissions of the key chemical groups, metals and PAHs. This fact is consistent with the higher anthropogenic emissions (industry, heating, traffic) in the urban areas compared with the rural background.
- Despite the location there is also a seasonal trend with higher emissions of metals and PAHs during the cold seasons (Autumn and Winter) due to the increase in use of domestic heating systems during these months, winter traffic (congestion and cold starts) and the special atmospheric conditions which favour less pollutant dispersion.
- $PM_{2.5}$ from different seasons and locations showed a wide range of potency to produce reactive oxygen species (ROS), along with the release of cytoplasmic lactate dehydrogenase (LDH) and the depletion of reduced glutathione (GSH). In particular for the city centre site where the chemical composition analysis determined a higher concentration of anthropogenic compounds during the cold seasons, $PM_{2.5}$ induced a significant increase in ROS production and LDH release along with a significant depletion of GSH.
- Chemometrics studies indicated 2 different groups of variables, one comprising mainly transition metals and ROS data the other group showed a different pattern and included PAHs. A direct correlation between transition metals and Reactive Oxygen Species production was found; no correlation was found with PAHs. The DCDH-DA test used for ROS determination in the present work is not able to determine ROS formation induced by PAHs, this test is more specific for species such as H_2O_2 and OH radicals, typically formed by the Fenton reaction in the presence of transition metals. PAHs would mostly lead to production of the super oxide anion, O_2^-, which requires analysis by the dithiothritol method.
- This study will be extended to include more samples, chemicals and toxicology assays so as to develop a computer model suitable for the determination of the origin and distribution of PM2.5 in Cork along with the toxicological endpoints.