Seasonal and geographical variation in ambient air particulate matter-induced oxidative stress and cytotoxicity for Cork City (Ireland)

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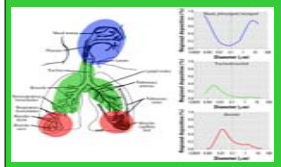
1. Toxicity of Particulate Matter (PM)



Local effects:
Respiratory System

- Induction of inflammatory responses in the lungs

Systemic effects:
Cardiovascular System

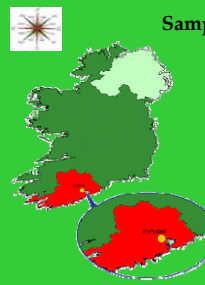
- Induction of systemic inflammatory responses and changes in neural control of heart function

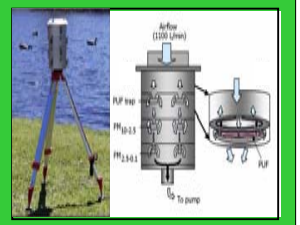
2.

The aim of the work is to evaluate the *in vitro* toxicity of $PM_{2.5}$, collected in three different areas of Cork City (City centre, urban background and rural site) in relation to the seasonal and geographical variation of its chemical composition.

A human epithelial pulmonary A549 cell line was exposed for 72 h to different concentrations of PM. Cytotoxicity of PM as well as its potency to produce Reactive Oxygen Species (ROS) and release proinflammatory mediators was evaluated. Principal component analysis (PCA) was applied to determine any possible correlation between PM chemical composition, cellular cytokines and ROS.

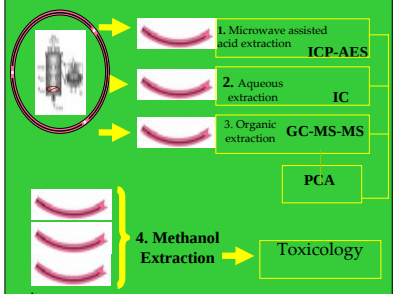
3. Sampling campaign





$PM_{2.5}$ was collected onto polyurethane foam filter (PUF) with a three stage high volume cascade impactor (flow rate of 900 l/ min) during a year long sampling regime.

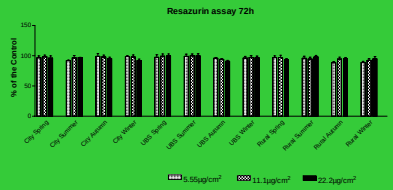
4. Sample preparation for chemical and toxicological analysis



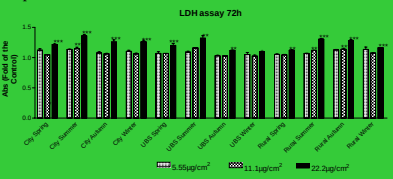
Metals: 20 metals comprising As, Sn, Hg, Mo, Zn, Sb, Pb, Cd, Ni, Fe, Co, Si, Mn, Cr, Mg, V, Ca, Cu, Ti and Al were analysed by ICP-AES after microwave assisted acid digestion and water based extraction.

The methanol-extracted samples were used for the *in vitro* experiments

5. Cytotoxicity assay

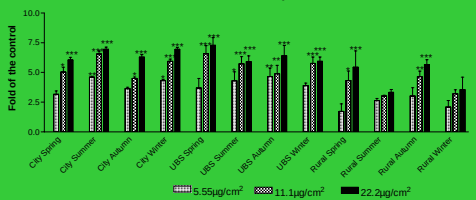


In order to evaluate the cell vitality after exposure to PM 2.5 sampled in different seasons and sites the resazurin assay was performed. A549 cells were exposed to three different doses of PM for 72h. No difference in cell vitality compared to control was noted for all the samples tested.



The LDH assay was performed to evaluate cell membrane damage after exposure to PM 2.5. Leakage in LDH was significant for the highest dose of mostly all the samples.

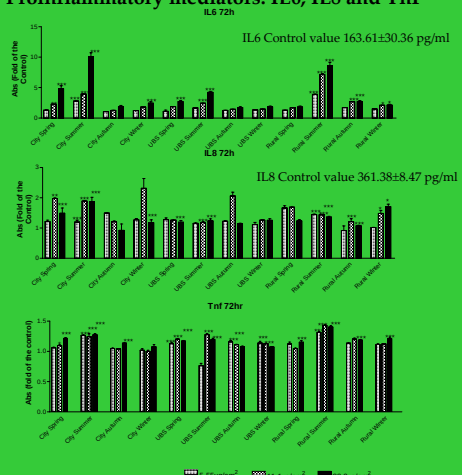
6. Intracellular ROS production



Intracellular ROS production was evaluated according to the method of Tsubouchi et al. (*Biometals*, 2001, **14,** 181-185). Cells were treated with the non fluorescent probe DCFH-DA. Once inside the cells this compound is converted to DCFH by esterase enzymes which cleave the diacetate group. DCFH is then oxidized to the fluorescent DCF by ROS (preferentially H_2O_2).

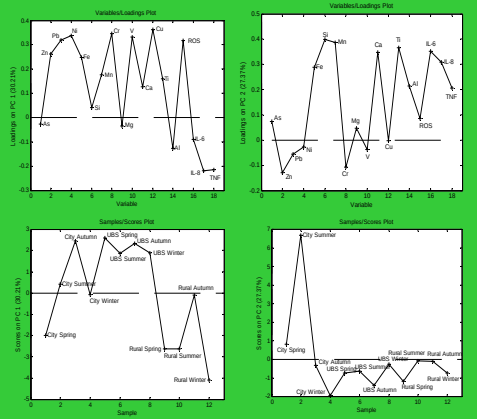
The assay shows as the highest ROS cell production is induced in the city and UBS samples, preferentially in the summer and spring seasons. *p<0.05; **p<0.01; ***p<0.001

7. Proinflammatory mediators: IL6, IL8 and TnF



IL6, IL8 and TnF were determined by ELISA kits supplied by Biosource. IL 6 and IL8 were highly induced in cell samples exposed to City Spring and City Summer. IL6 concentration was elevated for Rural Summer samples as well. The highest dose of PM in some samples do not correspond to the highest IL8 concentration. Levels of TnF in all the exposed samples were low and there was no measurable difference between seasons and sites.

8. Principal Component Analysis (PCA).



Multivariate correlations between PM chemical composition, cytokines and ROS production were investigated by means of PCA. PCA is a chemometrics tool able to identify patterns in data, and expressing them in order to highlight their similarities and differences.

9. Conclusion

Seasonal and geographical variation in PM toxicity was evaluated analyzing different end points. Toxicity was then related to PM metal composition using PCA. Two principal components (PC) explained the metals and toxicological end points data. First PC was loaded with most of the transition metals and ROS while second PC was characterized by crustal metals and IL-6 and IL-8, with the City Summer sample being the most dominant of all the sites.

10. Acknowledgments

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