$PM_{2.5}$ site/seasonal variability in Ireland: In-vitro toxicological effects

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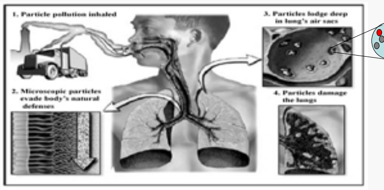
$PM_{2.5}$ Toxicity



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

1. Introduction

Samples of $PM_{2.5}$ were collected using a High Volume Cascade Impactor (HVCI) (@ 900Lmin^{-1}) located at an urban/city site, a background urban (UBS) and a rural site. Polyurethane foam (PUF) was the filter substrate. Elemental concentrations of representative suites of 20 metals were determined using ICP-OES spectrometry after microwave extraction. In addition, the aqueous extracts were analysed by ICP-OES, after sequential agitation/sonication to quantify the solubility (bioavailability) of the different metal components. This procedure was also utilised in the determination of the inorganic ion content of the $PM_{2.5}$ by ion chromatography. The % total carbon, hydrogen and nitrogen was measured using a CE440 Elemental Analyser and the endotoxin content determined for the $PM_{2.5}$ sampled. To investigate the biological effects of $PM_{2.5}$ at a sub-cellular level, human epithelial pulmonary A549 cells were exposed for 72hrs to different concentrations of $PM_{2.5}$ (0, 5.5, 11.0, 22.0 μg/cm^2) and toxicological assays were performed (Resazurin, LDH, Intracellular production of ROS, proinflammatory mediators: IL-6, IL8 and TnFα)

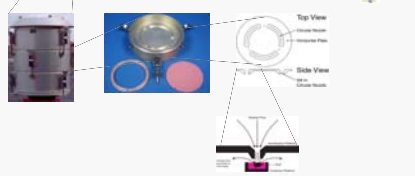
2. Objectives:

- **To investigate the toxicity profile of $PM_{2.5}$ and determine any links that different samples may have with their chemical composition**

- **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.**

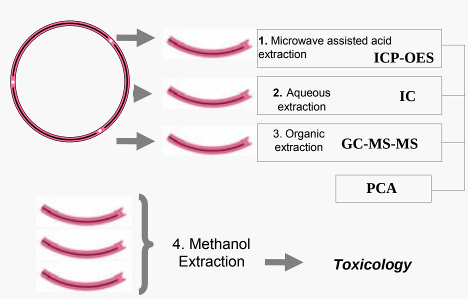
3. Methodology

Sampling



$PM_{2.5}$ 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.

Sample preparation



4. Analysis

Chemical components

	$PM_{2.5}$ (μg/g)											
	City				UBS				Rural			
Element	*Spring*	*Summer*	*Autumn*	*Winter*	*Spring*	*Summer*	*Autumn*	*Winter*	*Spring*	*Summer*	*Autumn*	*Winter*
S	25231.28	22047.31	20087.27	12390.88	25412.98	38590.83	11940.15	27786.57	33503.32	43168.55	34952.78	24296.16
As	13.62	11.62	15.14	17.17	11.80	20.37	13.23	12.37	13.00	11.45	44.15	14.29
Sn	23.77	4.70	2.65	23.30	24.54	21.61	2.31	2.16	11.27	20.96	10.33	2.50
Hg	767.98	172.43	6684.48	148.88	51.95	42.44	32.12	14.62	16.63	14.16	10.04	8.76
Mo	17.82	15.21	23.26	22.48	15.45	18.25	17.31	16.19	17.02	14.99	15.21	18.71
Zn	905.07	466.98	764.33	976.33	2386.05	2177.30	5169.17	1397.26	314.56	293.87	522.02	418.29
Sb	7.55	7.61	8.39	9.52	6.54	7.73	7.33	6.86	7.21	6.35	6.44	7.92
Pb	7.05	5.55	8.26	12.46	18.50	27.80	16.38	30.63	19.35	22.97	25.37	14.55
Cd	1.52	1.29	1.69	1.91	2.12	2.25	2.54	2.02	2.05	1.27	1.58	1.59
Ni	17.03	9.62	8.95	11.32	15.32	17.93	13.12	10.25	12.72	10.42	10.65	15.79
Fe	170.10	445.31	582.10	355.00	227.11	455.14	192.56	615.68	134.53	119.64	75.22	87.34
Co	1.37	1.17	1.40	1.66	1.21	1.99	0.86	0.80	2.10	2.73	1.60	1.60
Si	663.72	670.30	956.14	629.36	353.40	542.88	248.50	557.73	361.66	170.52	92.05	153.57
Mn	17.03	35.67	19.67	20.86	12.56	20.85	10.76	21.79	9.70	9.89	10.29	7.39
Cr	2.99	2.98	2.08	1.62	1.12	1.32	1.25	2.90	1.23	1.28	1.10	1.35
Mg	2013.26	1016.34	1113.96	822.70	630.52	898.66	1336.81	701.17	1012.15	1217.38	1275.93	1398.04
V	11.06	8.16	18.75	4.14	19.31	26.75	3.01	17.87	9.28	17.30	24.32	6.52
Ca	1397.48	1829.37	1162.74	1318.93	906.34	1073.38	1015.73	1263.82	998.51	918.02	892.61	1097.74
Cu	14.22	16.27	28.40	19.29	14.65	21.97	13.54	21.73	4.54	6.54	4.26	3.50
Ti	0.16	0.14	0.18	0.20	0.14	0.17	0.16	0.15	0.15	0.14	0.54	0.17
Al	2.90	2.47	3.18	3.65	2.51	2.97	2.81	2.63	3.61	2.43	2.47	4.48
Na+	136439.24	91559.80	53223.14	44580.33	44394.75	50327.50	90462.61	42987.99	68369.46	109562.93	143382.35	135150.33
NH4+	93967.41	83243.33	94062.46	96916.08	144804.53	135818.01	63447.23	197915.99	222377.67	120583.29	180117.48	196597.55
K+	11368.13	16302.69	8386.01	9802.79	7044.70	9819.13	9703.07	7181.26	12924.19	23478.53	18909.54	11628.24
Mg2+	16898.29	12442.66	7739.22	6968.41	5870.74	7179.31	11529.97	6161.42	10402.34	13370.73	10267.12	16768.36
Ca2+	16430.60	36442.21	9413.64	9157.38	7690.06	14686.33	5899.96	17633.21	4014.16	16684.14	8134.15	9322.55
F-	0.00	106.78	0.00	2210.54	0.00	0.00	39.47	11.31	2202.56	1530.27	614.50	1020.16
Cl-	112709.20	30843.15	61657.98	85582.72	29961.63	28972.70	109392.43	75053.26	62154.83	30910.42	50353.86	116615.08
NO2-	0.00	35.75	148.03	664.81	1484.34	51.42	1180.27	565.95	688.41	532.79	519.85	988.58
NO3-	96259.68	57973.22	100888.35	73184.85	132551.12	113627.74	50315.20	238693.41	212244.07	108527.55	107906.90	185482.34
SO42-	137888.16	110235.39	106519.55	68709.05	128109.8351	187848.3328	64385.86583	152070.1333	188689.25	206763.47	180706.12	127637.22
Total C	215657.8947	146304.6358	222760.7362	406631.2997	153360.7908	140349.0137	183755.0471	102310.9244	156367.6149	n/a	158709.6774	162840.2367
Total H	21710.52632	14940.39735	27975.46012	27294.42971	21696.86985	20303.49014	17321.66891	16890.7563	30853.39168	n/a	22935.48387	22899.40828
Total N	28750	19788.07947	43006.13497	39310.34483	49571.66392	40197.26859	20995.96231	18823.52941	88424.50766	n/a	40741.93548	61597.63314

Table 1 shows the acid digested metal concentrations (μg/g) determined by ICP-AES, inorganic ion content (μg/g) determined by Ion chromatography, and total carbon, hydrogen and nitrogen (μg/g) determined by CE440 Elemental Analyser for the 3 sites and 4 seasons. Where l.o.d./2 has replaced values that were below the limit of detection. *NA = not available; City=Urban site; UBS = Urban Background Site; Rural= rural site*

Element	Mean	Median	S.D.	Minimum	Maximum	Sum	Count
S	67725.05	67957.14	26129.35	22804.44	110538.47	812700.57	12
As	81.96	83.56	16.09	43.24	109.41	983.50	12
Sn	107.66	110.42	80.62	16.74	273.21	1291.97	12
Hg	30.51	30.12	3.93	26.29	39.44	366.11	12
Mo	22.19	13.06	31.16	7.14	120.57	266.32	12
Zn	10670.66	7008.05	12143.25	1930.60	43919.51	128047.90	12
Sb	55.93	60.69	44.72	6.31	133.51	671.15	12
Pb	241.57	263.13	116.72	90.56	424.53	2898.85	12
Cd	45.65	60.31	32.33	2.70	81.42	547.78	12
Ni	63.08	67.21	33.14	23.25	126.47	756.98	12
Fe	4827.83	5287.42	2920.25	1107.30	9309.68	57933.96	12
Co	8.40	9.92	5.74	0.99	14.94	100.77	12
Si	9589.22	7715.12	5697.70	6734.87	27164.78	115070.64	12
Mn	151.98	166.23	153.05	2.18	546.39	1823.80	12
Cr	68.55	78.87	41.71	16.97	147.67	822.65	12
Mg	5025.04	4795.90	1954.16	2358.94	9212.15	60300.53	12
V	92.82	83.96	69.84	17.72	227.46	1113.81	12
Ca	6973.50	7487.51	3377.89	1307.28	12163.51	83681.97	12
Cu	138.21	156.32	113.53	4.23	343.91	1658.47	12
Ti	454.09	451.40	169.26	265.71	833.05	5449.09	12
Al	4369.18	4303.00	1647.16	1538.38	7424.90	52430.13	12
Na+	84203.37	79416.04	39050.33	42987.99	143382.35	1010440.43	12
NH4+	135820.92	128200.65	52508.15	63447.23	222377.67	1629851.05	12
K+	12212.36	10593.63	4995.98	7044.70	23478.53	146548.28	12
Mg2+	10466.55	10334.73	3869.82	5870.74	16898.29	125598.56	12
Ca2+	12959.03	9368.09	8651.58	4014.16	36442.21	155508.41	12
F-	646.52	73.13	878.80	5.66	2210.54	7758.22	12
Cl-	66183.94	61906.40	33719.55	28972.70	116615.08	794207.25	12
NO2-	573.82	549.37	470.89	25.71	1484.34	6885.90	12
NO3-	123137.87	108217.23	59594.60	50315.20	238693.41	1477654.43	12
SO42-	138296.86	132999.00	46700.96	64385.87	206763.47	1659562.37	12
Total C	184027.61	158996.56	77147.66	102310.92	406631.30	2208331.32	12
Total H	22510.60	22304.97	4790.93	14940.40	30853.39	270127.19	12
Total N	42645.79	40469.60	20440.10	18823.53	88424.51	511749.43	12

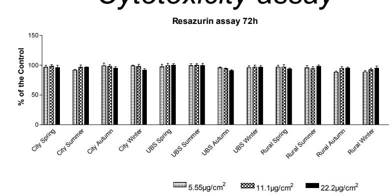
Table 2 shows the descriptive statistics of the elemental composition (μg/g) of the $PM_{2.5}$ that was used in the *in-vitro* toxicological assays

Biological component

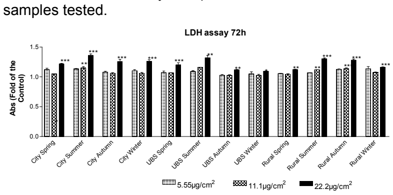
	$PM_{2.5}$ (EU/mg of $PM_{2.5}$ sample)											
	City				UBS				Rural			
	Spring	*Summer*	*Autumn*	*Winter*	*Spring*	*Summer*	*Autumn*	*Winter*	*Spring*	*Summer*	*Autumn*	*Winter*
Endotoxin	0.074	0.078	0.066	0.077	0.076	0.078	0.069	0.072	0.077	0.077	0.083	0.064

5. Results

Cytotoxicity assay

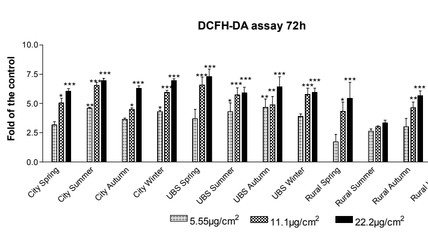


In order to evaluate the cell vitality after exposure to $PM_{2.5}$ sampled in different seasons and sites the resazurin assay was performed. A549 cells were exposed to three different doses of PM for 72h. No difference in cell vitality compared to control was noted for all the samples tested.



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 nearly all of the samples.

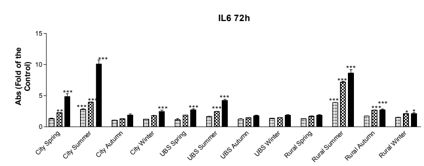
Intracellular ROS production

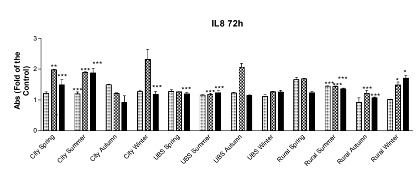


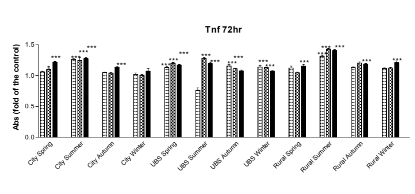
Intracellular ROS production was evaluated according to the method of Tsubouchi et al. (*Biometals*, 2001, **14**, 181-185). Cells were treated with the non fluorescent probe DCFH-DA. Once inside the cells this compound is converted to DCFH by esterase enzymes which cleave the diacetate group. DCFH is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

The assay shows that the highest ROS cell production is induced in the City and Urban background site (UBS) samples, preferentially in the summer and spring seasons. *p<0.05; **p<0.01; ***p<0.001

Proinflammatory mediators: IL-6, IL-8 and TnFα



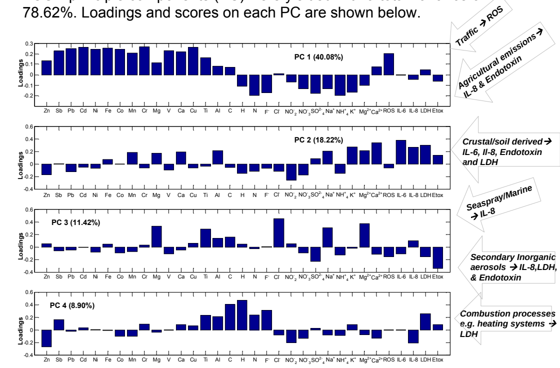




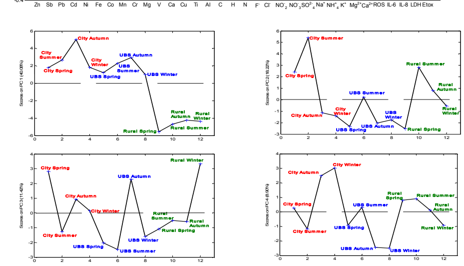
IL-6, IL-8 and TnFα were determined by ELISA kits supplied by Biosource. IL-6 and IL-8 were highly induced in cell samples exposed to City Spring and City Summer. IL-6 concentration was elevated for Rural Summer samples as well. The highest dose of PM in some samples do not correspond to the highest IL-8 concentration. Levels of TnFα in all the exposed samples were low and there was no measurable difference between seasons and sites.

Chemometrics

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



PC 1 (40.08%)
PC 2 (18.22%)
PC 3 (11.42%)
PC 4 (8.90%)
Loadings
Traffic → ROS
Agricultural emissions → IL-6 & Endotoxin
Crustal/soil derived → IL-6, Il-8, Endotoxin and LDH
Seaspray/Marine → IL-8
Secondary Inorganic aerosols → IL-8, LDH, & Endotoxin
Combustion processes e.g. heating systems → LDH



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^+, NO_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.

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6. Conclusion

Our study demonstrated that $PM_{2.5}$ from the different sites/seasons exhibit different toxicity profiles and illustrated different potencies for stimulating the production of proinflammatory mediators and ROS in human pulmonary A549 epithelial cells.