TOXICOLOGICAL EFFECTS OF INDOOR PM_{10} IN URBAN PRIMARY SCHOOLS IN BARCELONA, SPAIN

T. MORENO[1], A. WLODARCZYK[2], K. BÉRUBÉ[2], T. JONES[3], I. RIVAS[1,4], C. RECHE[1], A. TOBIAS[1], J. SUNYER[4] , X. QUEROL[1] & BREATHE participants

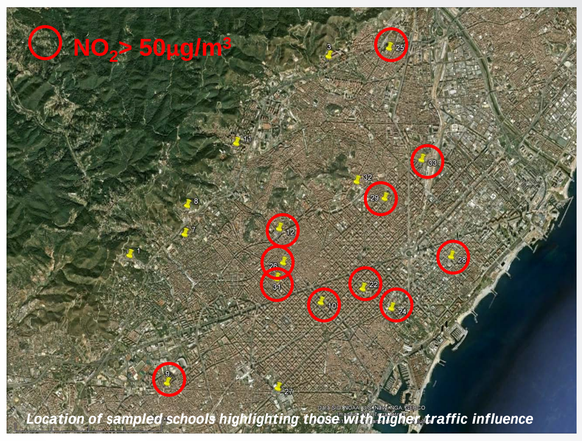
[1]Institute of Environmental Assessment & Water Research (IDÆA-CSIC), Jordi Girona 18, 08034 Barcelona, Spain;
[2]School of Biosciences, Cardiff University, Museum Avenue, Cardiff CF10 3AX, UK; [3] School of Earth and Ocean Sciences, Cardiff University, Main Building, Cardiff CF10 3YE, UK;
[4]CREAL, C/ Doctor Aiguader, 88; 08003 Barcelona, Spain; BREATHE-BRain dEvelopment and Air polluTion ultrafine particles in scHool childrEn (Advanced Grant ERC, Seventh Framework Programme).

INTRODUCTION

Background. The BREATHE project, funded by the EU and led by CREAL in Spain, has conducted a campaign measuring aerosols in primary schools in Barcelona. Monitoring sites were separated into high and low-pollution areas with the primary aim of recognizing if differences in air pollutants affect the neurological system of children and their behaviour at school.

Aims. Within BREATHE the CECAT sub-project is considering the toxicological aspect by the problem by investigating the toxicity of PM looking at its ability to induce a systemic oxidative stress which damages cells and DNA molecules.

Methods. A total of 20 schools were sampled. The sampling took place inside the classrooms for 4 consecutive days at 2 different times a year (winter/summer 2012) to take into account the changes of air pollutants in different climatic conditions. To determine particle oxidative capacity, PM10 in the classrooms was collected using an Airborne Sample Analysis Platform system (ASAP; Model 2800 Thermo) on PUF substrates (sample flow-rate 200 l/min). The toxicity of the PM10 samples are being elucidated using 4 complementary biological assays: Plasmid Scission (PSA), DCFH ROS, Haemolysis and ROTA.

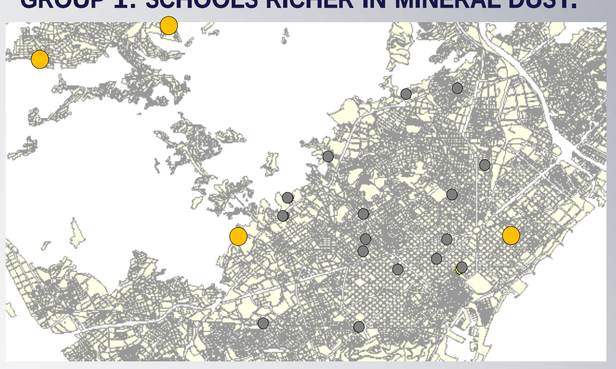


Location of sampled schools highlighting those with higher traffic influence

*Sampling is simultaneous in **indoor** and **outdoor** school environments*

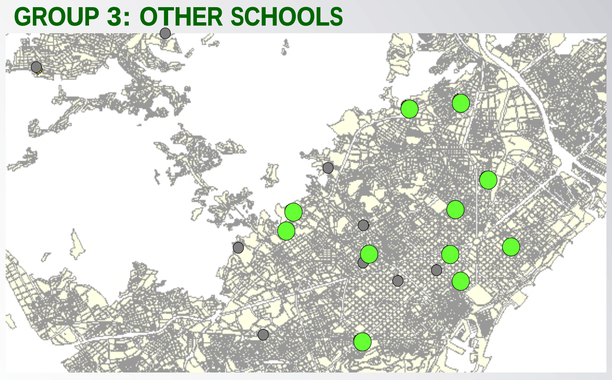
GEOCHEMISTRY

GROUP 1: SCHOOLS RICHER IN MINERAL DUST.



Al+Ca>3µg/m³ and no obvious anthropogenic metal anomaly. These samples are especially rich in natural rock-forming minerals (mostly quartz, clay minerals, feldspars, iron oxides/hydroxides). The influence of PM resuspension from gravel playgrounds is obvious, as is the "crustal" trace element signature of these samples.

GROUP 3: OTHER SCHOOLS



This leaves 15 samples as neither especially rich in minerals nor anthropogenic metals.

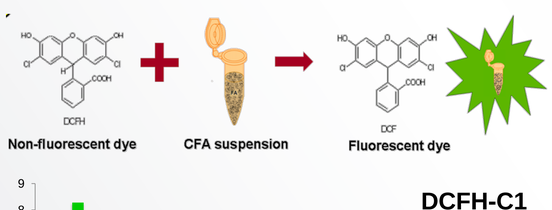
GROUP 2: RICHER IN ANTHROPOGENIC TRACE METALS

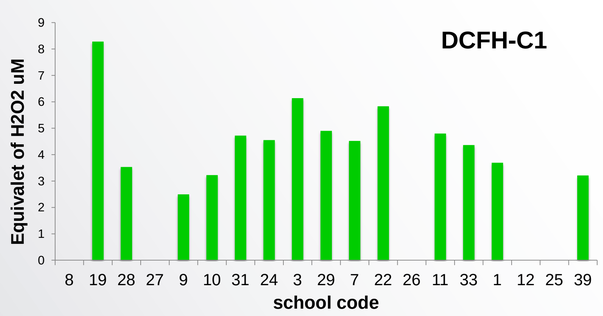
The metal chemistry of these indoor samples is complex and presumably derived from mixed sources. Different metalliferous mixtures are present, including Zn-rich sulphate-poor, V+sulphate-rich, Cr-rich, Cu+As-rich, Pb-rich, Sn-rich & Sb-rich.

TOXICOLOGICAL ASSAYS

DCFH Assay: 2',7'- dichlorodihydrofluorescein (DCFH) is oxidised to strongly fluorescent 2'7'-dichlorofluorescein (DCF) by reactive oxygen species (ROS) if present in the sample. The presence of ROS indicates likely bioreactivity and toxicity. DCFH detects a wide range of ROS, is simple and inexpensive to set up and therefore popular (used since 1940's), but lacks sensitivity and robustness and so must be treated with caution. Therefore this assay was only performed in schools from campaign 1 (C1).

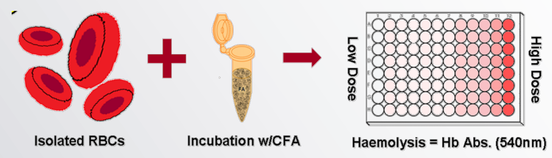
The acellular DCFH assays demonstrated classical dose-responses following challenge to increased PM mass concentrations for select school samples, indicative of a toxic response.

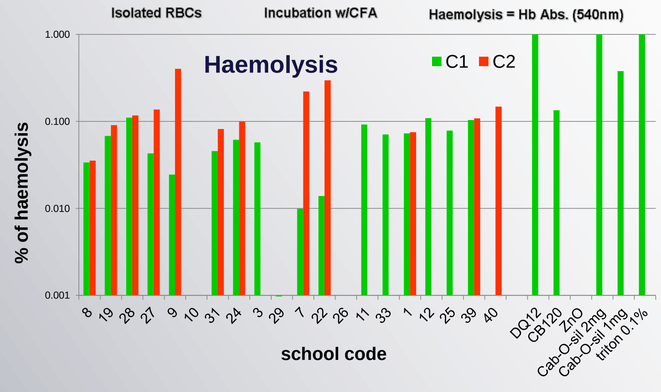




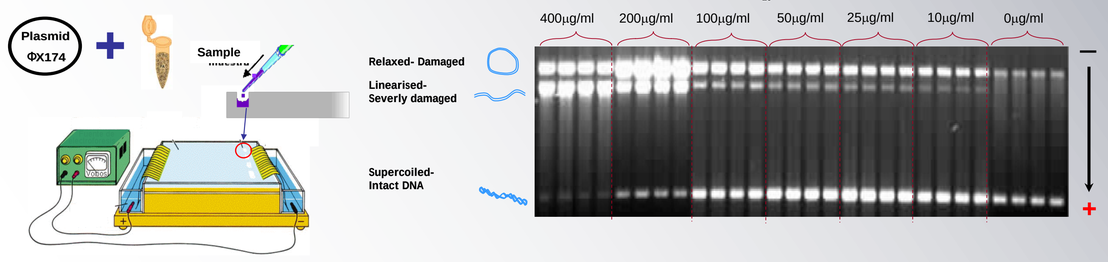
Haemolysis Assay measures the damage to erythrocyte (red blood) cell membranes resulting from oxidative stress exerted by ROS. The haemolytic assay has proved a useful preliminary test for identifying highly-irritative products (e.g. in cosmetic testing) as an *in vitro* alternative to *in vivo* studies using rabbits.

The corresponding haemolysis assays corroborated the PSA results, in that mild erythrocyte membrane lysis was achieved, in comparison to the positive control (Triton; 100% lysis) although % of haemolysis was always very low (< 1%) for all samples in campaigns 1 (C1) and 2 (C2).

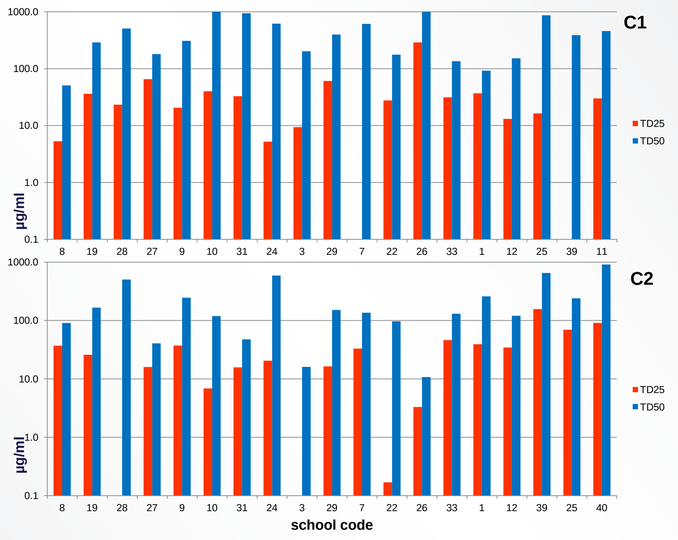


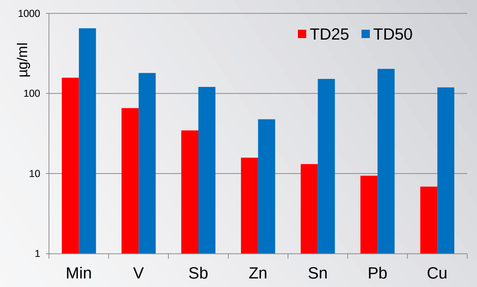


PSA: Plasmid Scission Assay quantifies the damaging effects of ROS on double-stranded plasmid DNA. All the PM10 indoor air school samples analysed via the PSA, produced mild DNA damage, i.e. single DNA strand breakage. Most samples, at the highest dose (1mg/ml), caused damage greater than 30%.



Lower PM doses were consistently needed for reaching 25% DNA total damage (TD25) than for 50% (TD50), but there is considerable variation between samples.



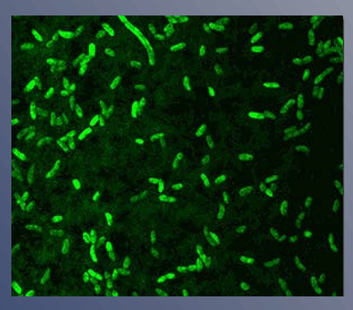


Comparing "campaign maximum" metals with crustal mineral-rich PM samples suggests that anthropogenic trace metals are inflicting more DNA damage than minerals. Thus:

TD25 damage
Cu>Pb>Sn>Zn>Sb>V>minerals
TD50 damage
Zn>Sb=Cu>Sn>V>Pb>minerals

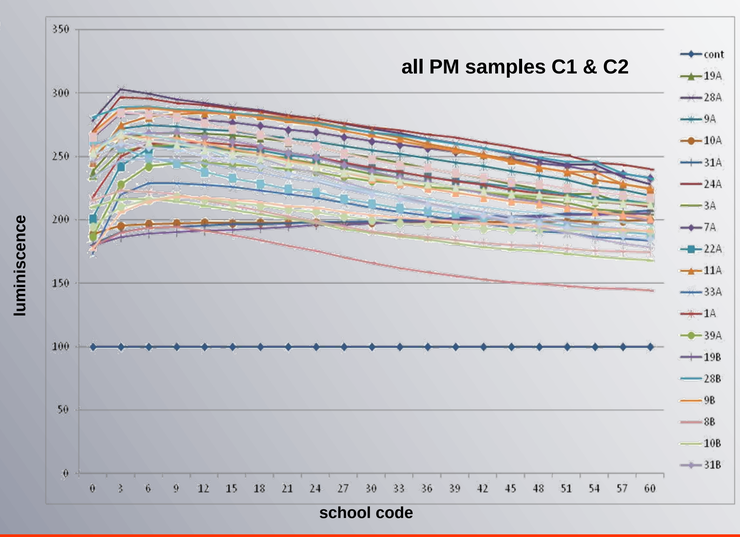
ROTAS Assay: Rapid on-site toxicity audit system. Uses marine bacterium *Aliivibrio fischeri* which luminesces as it respires. The bacteria are sensitive to toxic chemicals which inhibit growth and so cause a decrease in luminescence.

This assay has the advantage of using living organisms and directly observing the effects of potential toxins (in this case classroom PM) over a specified time (e.g. 1 hour).



Light reaction occurring within Vibrio fischeri (http://www.bmglabtech.com/db_assets/applications/downloads/applications/AN147-quality-control-ROTAS-luminescence-LUMIstar.pdf.

Most of the school PM samples tested produced an initial transient favourable response from the bacteria, which increased their fluorescence, presumably reflecting a stimulus to divide: a phenomenon known as "hormesis". After this short initial period of growth stimulation, the opposite effect set in as the number of bacteria began to decrease in response to the toxic effects of the PM. The gradual decrease in bacterial population continued steadily throughout the rest of the 1-hour experimental period, indicating mild toxicity, in contrast to the stable population maintained by the control (i.e. *Aliivibrio fischeri* in NaCl solution).



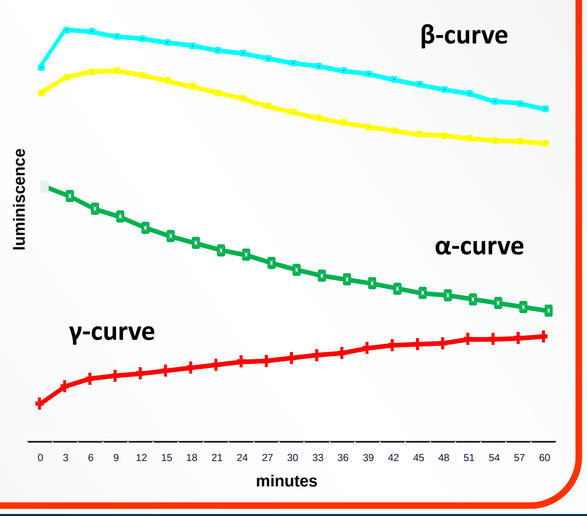
Three types of curves were observed for the PM school samples:

i) α- continuous descent of luminescence from first minute;

ii) ß- sudden bacteria stimulation (hormesis), followed by a continuous descent which confirms the fact that PM2.5 in the end turned out to be toxic to them;

iii) γ- continuous increase of luminescence, presumably reflecting a stimulus to divide.

Statistical analysis on links between curve-responses and elemental chemical composition of each sample is in progress.



SOME CONCLUSIONS so far...

1) BREATHE classroom PM10 samples are mildly toxic, but no more so than outdoor ambient air PM in Barcelona. 2) There is no immediately obvious "smoking gun" single chemical component that can be identified as much more toxic than others: the samples are complex mixtures. 3) There is, however, a strong suggestion that classroom samples richer in anthropogenic trace metals such as V, Sb, Zn, Sn, Pb and Cu are relatively more toxic than those dominated by mixtures of natural rock-forming minerals (silicates and carbonates). 4) There is also some evidence that at low doses Zn is less toxic than some other trace metals, notably Cu. 5) A majority of the samples induce the curious phenomenon of bacterial hormesis.

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