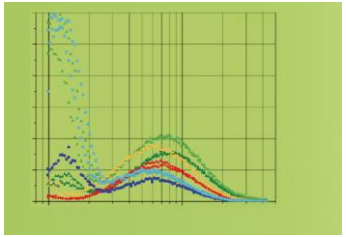


25th ETH-Conference on Combustion Generated Nanoparticles

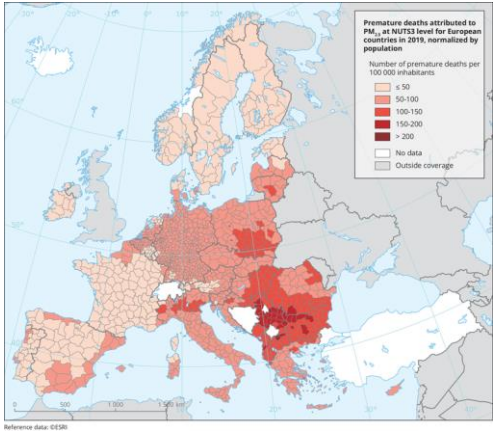
# On the toxicological effects of primary vs secondary aerosols: implication for human health

Maurizio Gualtieri, Francesca Costabile, Milena Stracquadanio, Teresa La Torretta, Ettore Petralia, Matteo Rinaldi, Emanuela Corsini, Gloria Melzi, Emma Nozza, Roberta Vecchi, Stefano Decesari, Gabriele Zanini

Zurich, June 23rd 2022

Maurizio Gualtieri
ENEA SSPT-MET-INAT & University of Milano-Bicocca - DISAT

# Introduction – PM exposure

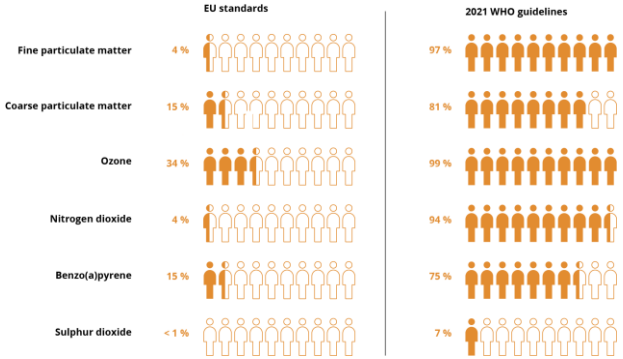


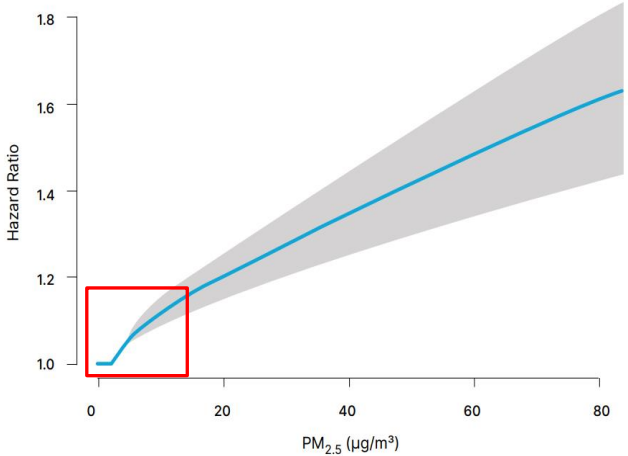

EEA 2022 https://www.eea.europa.eu/data-and-maps/figures/premature-deaths-attributed-to-pm2

Percentage reduction on 2005 premature deaths that reaching different EU limit values and WHO guidelines across the EU-27 could deliver


EEA Air quality report 2021

Percentage of exposed population in 2019




*Notes*: The lowest observed $PM_{2.5}$ concentration was 2.4 µg/m³.
*Source*: Burnett et al. (2018), Fig. 1.

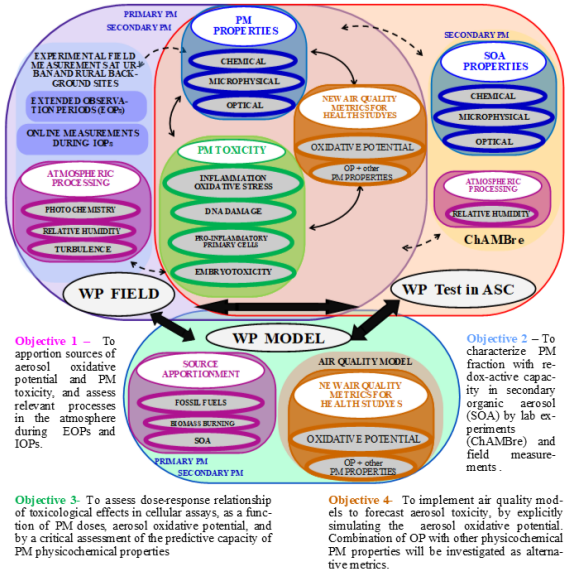
Annual global burden of deaths attributable to air pollution: long-term exposure to ambient fine particle air pollution ($PM_{2.5}$) caused 4.2 million deaths in 2015 Cohen et al., Lancet 2017; 389: 1907–18

# Introduction – PM exposure

Too little is known about how **different components** contribute to the overall **toxicity of particulate matter**. Such information could inform policy strategies to reduce deaths [..]. Techniques for detecting highly reactive and short-lived hazardous compounds [..] could be combined with **toxicological experiments**. These might include **exposing cell cultures to ambient air.** Lelieveld and Pöschl Nature 551 (2017) , 291-293

To obtain a more relevant assessment of health effects in vitro, the UFP and NM **dose per cell number or area** should reflect **real inhalation**. The initial use of high UFP or NM doses may be justified [..] but such high doses need to be accompanied or followed up with studies using **realistic doses in relation to current information** concerning occupational and ambient exposures to UFPs or to specific NM types. Stone et al., Environ Health Perspect. 2017 Oct 10;125(10):106002. doi: 10.1289/EHP42

# Introduction – The RHAPS project (in a nutshell)




IOPs 21 January 2021–18 March 2021 8 June 2021–14 July 2021

**SIOPs:**

26 January 2021–31 January 2021 (Exposure 1 – W1)

2 February 2021–6 February 2021 (Exposure 2 – W2)

16 February 2021–20 February 2021 (Exposure 3 –W3)

30 June 2021–3 July 2021 (Exposure 4 – S1)

Institute of Atmospheric Sciences and Climate, National Research Council, Department of Physics, Università degli Studi di Milano, INFN-Milan,
Department of Physics and Astronomy, Università degli Studi di Firenze, National Institute of Nuclear Physics, Istituto Nazionale di Fisica Nucleare—INFN-Florence,
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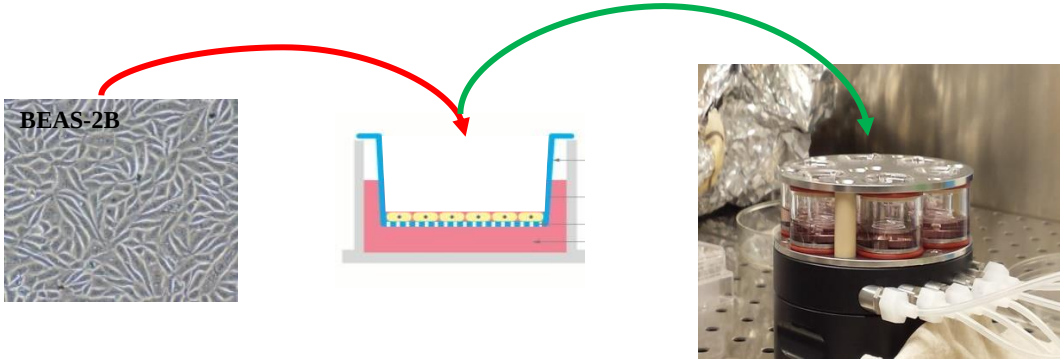
Department of Environmental Science and Policy, Università degli Studi di Milano,
Department of Environmental Biology, University of Rome Sapienza,
Institute of Air Pollution, National Research Council

More details in Costabile et al., Atmosphere 2022, 13, 704. https://doi.org/10.3390/atmos13050704

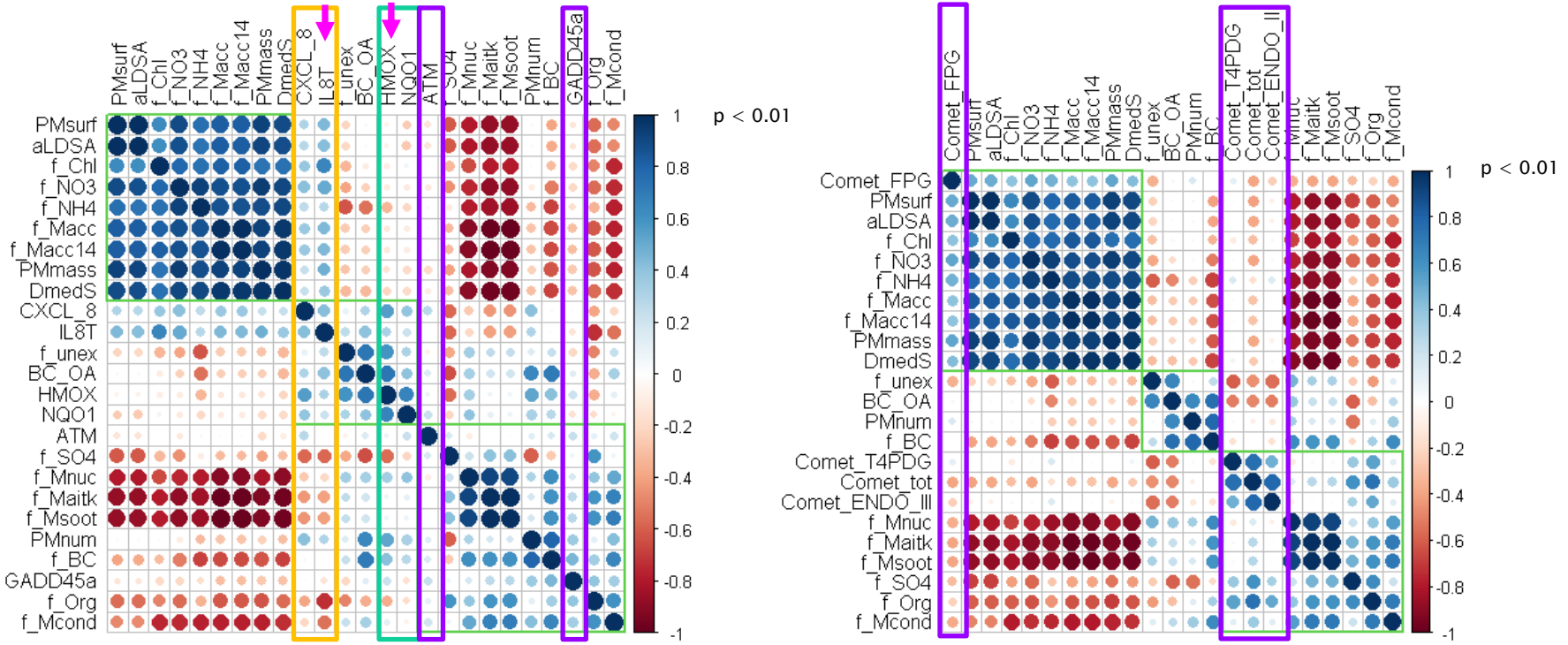
# Introduction – The RHAPS project (toxicological methods)




- BEAS-2B cells (lung bronchial cells) cultured and differentiated at air-liquid interface 24 hours before exposure
- Exposure by means of a Cultex$^{(R)}$ RFS-1 for 24 hrs to $PM_1$ @ the site of monitoring
- Real time PCR of genes (*HMOX, NQO1*, *Cxcl-8*, *ATM, Gadd45α*), release of IL-8 (inflammatory protein) and DNA damage assay (Comet, also with Fpg, EndoIII and T4PDG for specif DNA damages)

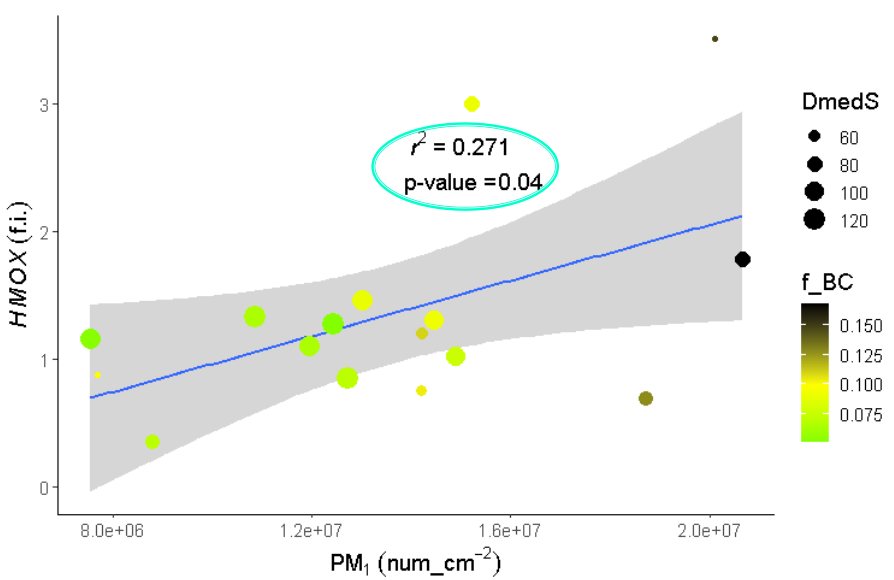
More details on the technical approach in Gualtieri et al. 2018 Chemosphere 207 552 – 564.

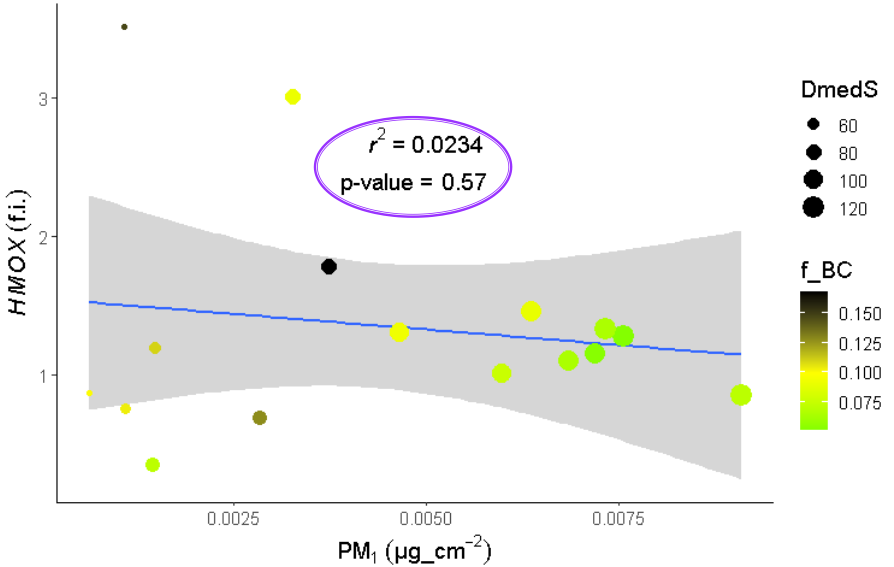
# Results – Correlation analyses




Significant positive correlation of *HMOX* and *NQO1* with **# of deposited particles, f_BC and negative correlation with DmedS,** $f\_NH_4$, $f\_SO_4$. **IL-8 and *Cxcl-8* correlate** with **inorganic f_masses** (Chl, $NO_3$ and $NH_4$), **DmedS, PM mass** and #, and negatively with $f\_SO_4$ and f_Org. Comet outcomes (but the FPG) correlate with the f_Org and $f\_SO_4$

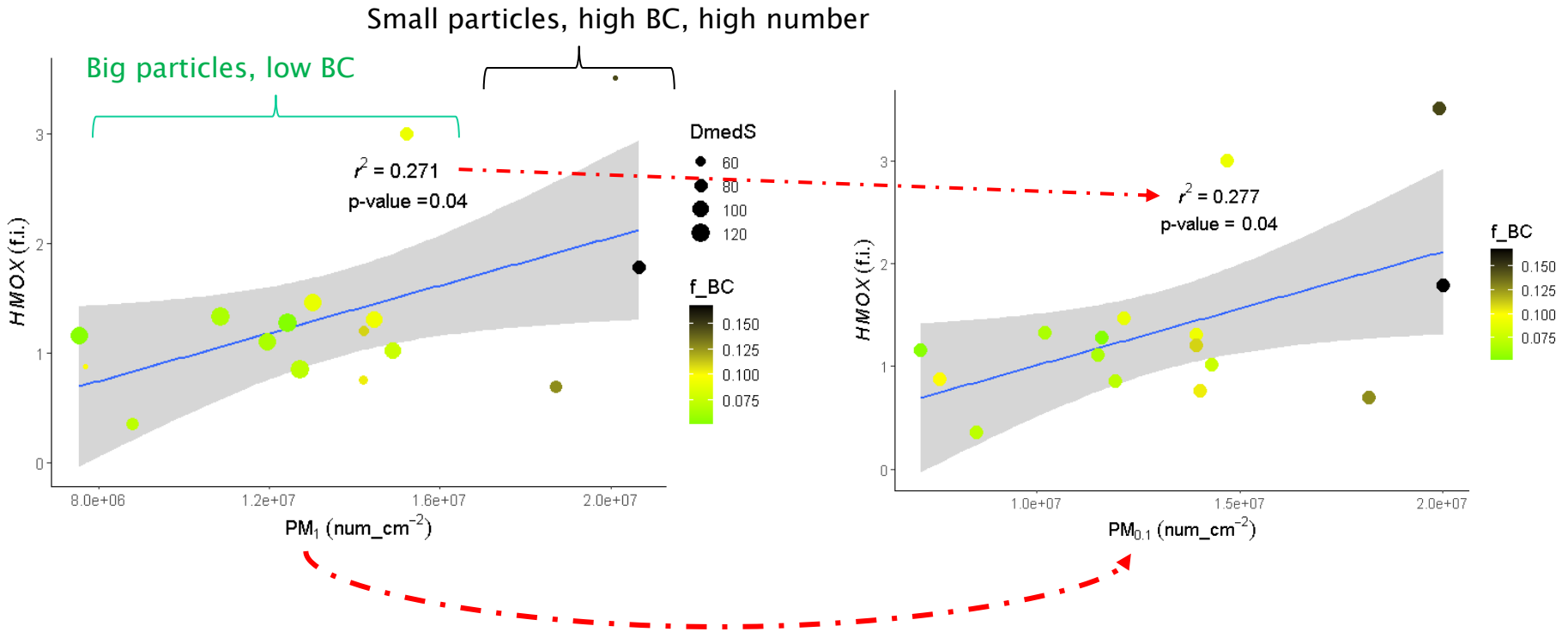
# Results – Correlation analyses of oxidative marker *HMOX*



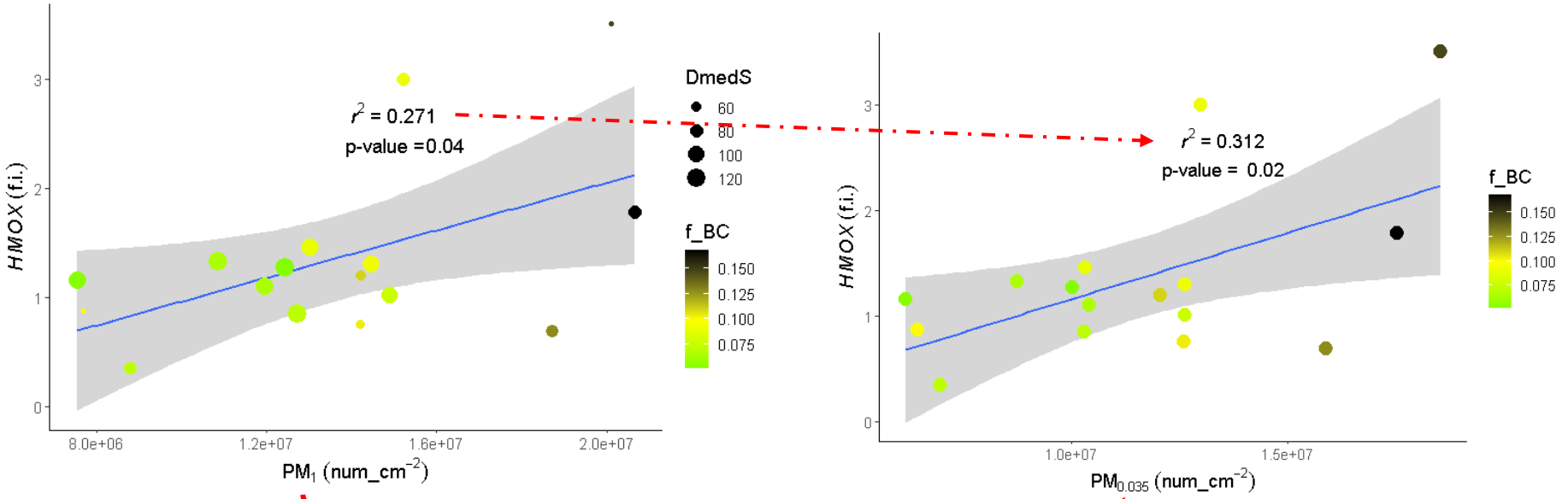

PM mass deposition is not predictive for oxidative responses ($R^2 = 0.023$). Interestingly higher deposited mass has lower effects on *HMOX* expression and are characterized by lower f_BC. On the contrary the number of deposited mass seems to be a more interesting parameter to evaluate the oxidative effects of PM ($R^2 = 0.271$, $p < 0.05$).

# Results – Correlation analyses of oxidative marker *HMOX*



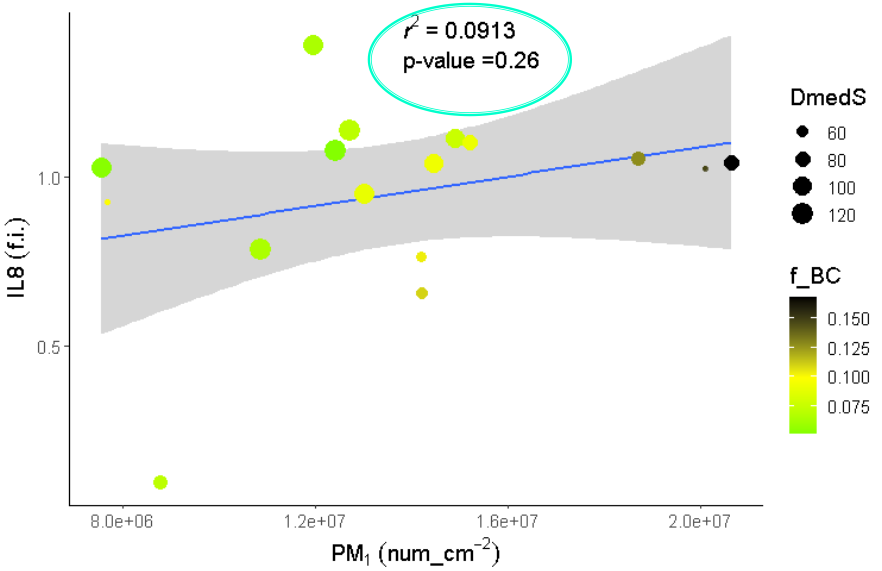

Sligthly higher correlations were foud considering the ultrafine fraction of PM ($PM_{0.1}$)
$R^2 = 0.277$, we therefore explored other ultrafine fractions…

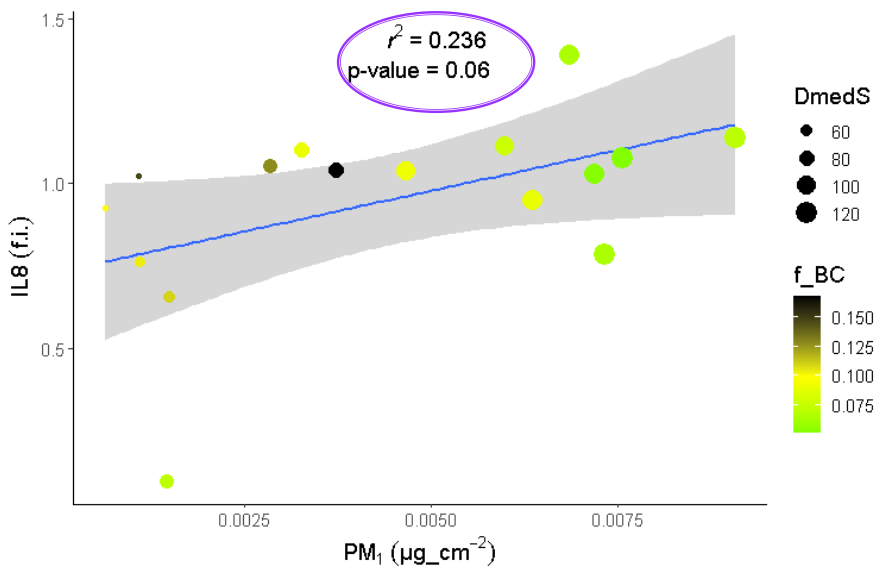
# Results – Correlation analyses of oxidative marker *HMOX*




Stronger correlations ($R^2$ = 0.312, p = 0.02) were foud considering the number of nanoparticles (nucleation mode). Primary fresh ultrafine particles may have a stronger pro-oxidant effect on lung epithelia. Ongoing analyses on the importance of the chemical composition.

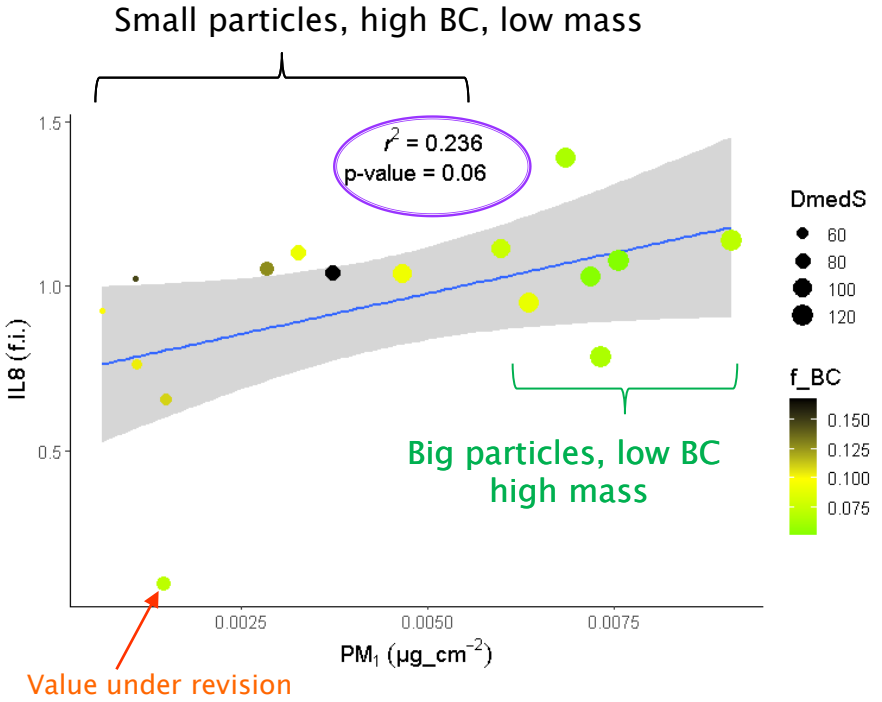
# Results – Correlation analyses of the inflammatory marker IL8

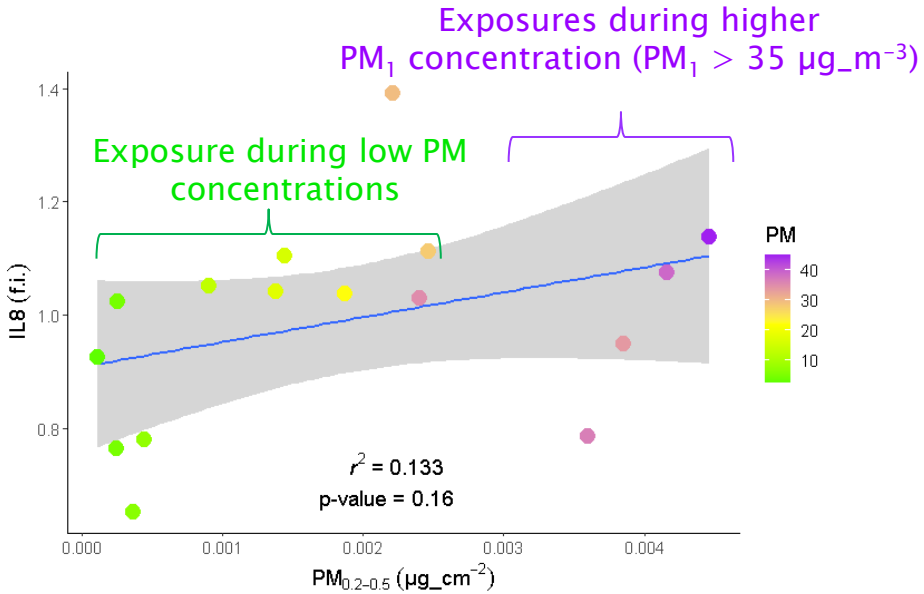



PM mass deposition is instead predictive for the inflammatory response ($R^2$ = 0.236). Interestingly higher deposited mass characterized by lower f_BC induces higher response. On the contrary the number of deposited mass is not predictive ($R^2$ = 0.0913).

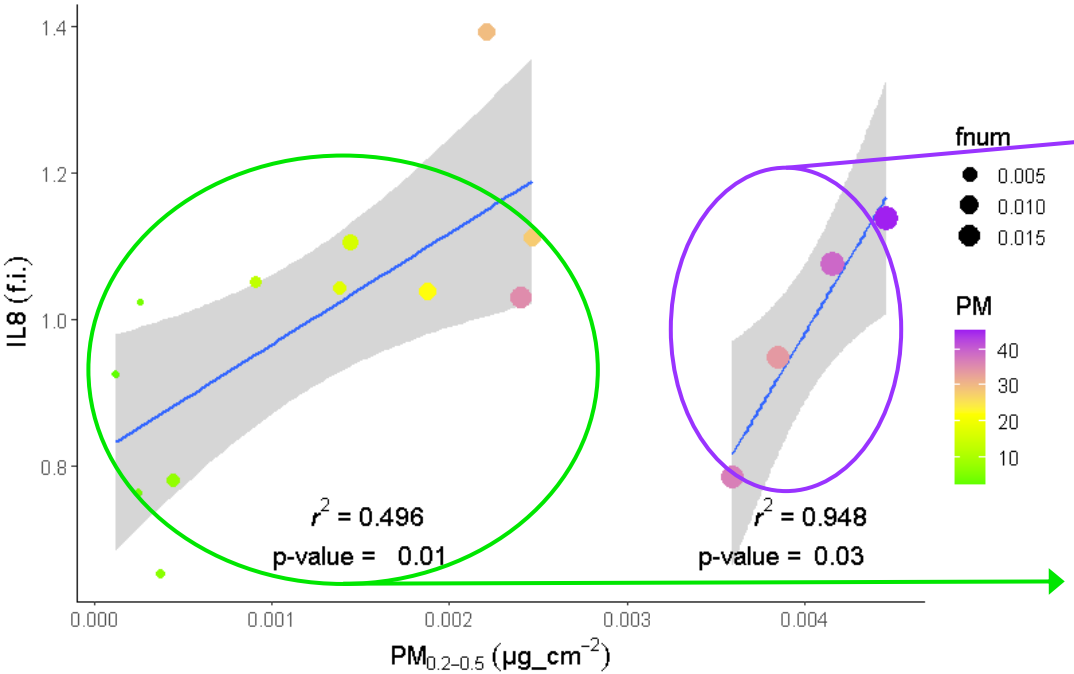
# Results – Correlation analyses of the inflammatory marker IL8




Again we explored how different fractions of $PM_1$ deposited on the cells correlates with the release of the inflammatory mediator IL8. We found interesting correlation with the accumulation mode of PM1 (200 – 500 nm)...

# Results – Correlation analyses of the inflammatory marker IL8




Higher exposure doses during accumulation periods (higher f_num of the accumulation mode over the whole $PM_1$ number of particles)

Lower relative exposure doses during absence of aging processes (low contribution of the accumulation mode, higher contribution of primary fresh emissions f_BC)

These results suggests that beside mass, the chemical properties of the particles, enrichment in secondary particles in the accumulation mode, may activate proinflammatory responses differently. In particular, relative enrichment in secondary aerosol may be a trigger for specific inflammatory responses. Analyses on specific chemical markers are ongoing.

# Conclusions

- The possibility to expose cells directly at air pollution and at doses representative of human exposure should be considered as a pivotal asset for the upcoming toxicological data on PM since…

- These first results clearly show that the **mass metric *per se* is not informative** for the toxicological potency of airborne PM since..

- Oxidative markers correlate with the number of nucleation mode particles (size matters!) rather than the mass of fine $PM_1$ deposited on cells (i.e. on lungs) or the total number of $PM_1$ particles and..

- Inflammatory markers correlate with the mass of the accumulation mode rather than the total mass of fine PM, but with possible different mechanisms of action considering the chemical composition of the deposited particles (i.e. sources and aging)

- To protect human health the reduction of the mass metric (new WHO guideline of 5µg/$m^3$) may be not sufficient if not irrelevant. New metrics based of specific diameter class or on proxy variable may be a more powerful tool to develop sustainable and effective measures to reduce air pollution-related adverse health effects.

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# Nano and Ultrafine Particle Toxicology and Exposure Assessment

IMPACT FACTOR 4.146

Indexed in: PubMed

## Special Issue Introduction

The increasing number of data available for nanoparticles and nanomaterials shows the necessity to define standardized and homogeneous toxicological approaches to test adverse effects. At the same time, the development of approaches based on adverse outcomes pathways (AOPs) frameworks is gaining relevance in understanding and describing the effects of nanomaterials. On the contrary, despite the increasing attention devoted to nanomaterials, efforts in understanding ultrafine environmental particle adverse effects need to increase to also support the development of new standards for human protection.

We, therefore, encourage you to submit original research papers or reviews concerning the toxicological effects and hazard posed to humans and the risk evaluation of nano and ultrafine particles..

The Special Issue aims to report high-novelty results on innovative approaches in vitro or in silico models that go beyond the present toxicological understanding of ultrafine or nanoparticles. Additionally, the identification and proposal of novel AOPs is strongly recommended in combination for the exposure of assessment evaluations or modelling. Manuscript submissions based on innovative exposure systems for, but not limited to, inhalation studies are highly encouraged, possibly in combination with “omics” approaches. Research or review papers dealing with the description or definition of hazard and risk assessments from exposure to nano and/or ultrafine particles in the framework of new metrics for human health protection are also encouraged.

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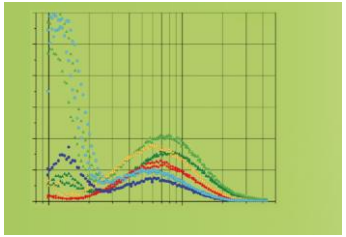

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