Association of ultrafine fresh urban particles with OP and human oxidative markers

M. Gualtieri – 18th october 2025

Outline

- Introduction and experimental approach

- Results

- Perspectives

- Conclusions

The experimental approaches

Aerosol Characterization

The RHAPS (Redox-Activity And Health-Effects Of Atmospheric Primary And Secondary Aerosol) experience

Atmosphere 2022, 13, 704. https://doi.org/10.3390/atmos13050704

Where: Bologna urban background, 44°31’29’’N, 11°20’27’’E

When:

	Winter	**Summer**	
IOPs	21 January 2021–18 March 2021 26 January 2021–3 January 2021	8 June 2021–14 July 2021	IOP: Intensive observational period
SIOPs	2 February 2021–6 February 2021 16 February 2021–20 February 2021	29 June 2021–2 July 2021	SIOP: Super intensive OP

What:

Non-Refractory PM_1 Chemical Components (AMS)

Elemental Composition (STRAS)

Particle Number Size Distributions (CPC, model 3772 TSI) and a commercial aerodynamic particle sizer (APS, TSI)

OC-EC Model-4 Semi-Continuous Field Analyzer—Sunset Laboratory

Oxidative potential – OP (semi-continuous) measurements 2’ 7’-dichlorofluorescein (DCFH) assay (OPDCFH) with 2-h time resolution

Micrometeorology

The experimental approaches

Toxicological Characterization

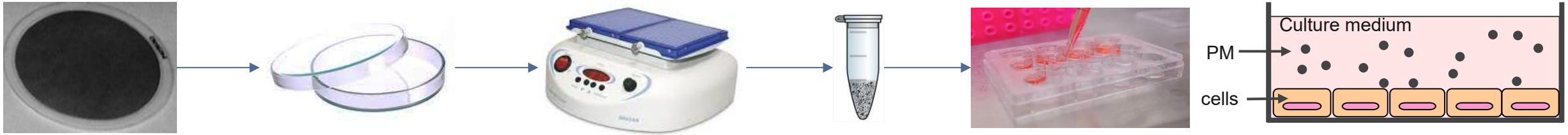
Offline – from sampled filters and extracted particles

Online – direct exposure to the ambient aerosol

Offline approach:

PTFE filters for particles extraction: 1.5 mL in contact with filters and mechanical agitation for 30 min at 300 rpm.

Cell exposure and OP measurements



Problem with the extraction of particles from filters, issues on the OP validity after days post sampling and after extraction form filters… these issues may hinder the overall understanding of the real relevance of OP measurements and potential toxicological and health outcomes

Data and results on: Toxicology and Applied Pharmacology 485 (2024) 116913

The experimental approaches

Toxicological Characterization

Offline – from sampled filters and extracted particles

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Offline approach:

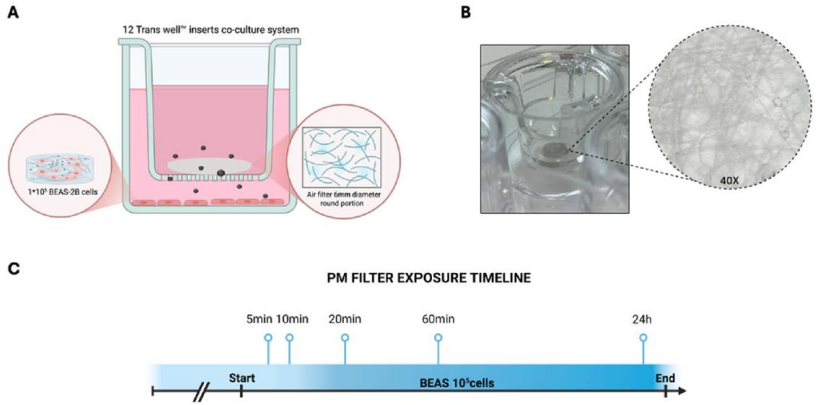
Filters punch direct in contact with the cell culture medium but not with cells to mimic the OP procedure

Cell exposure and OP measurements

Only the components soluble in the cell culture medium are released from the filter punch, issue on the representativity for real human exposure.

Problem with the definition of the concentration of exposure

To be validated in terms of relevance for human risk evaluation



Data and results on: Environmental Toxicology and Pharmacology 120 (2025) 104853

The experimental approaches

Toxicological Characterization

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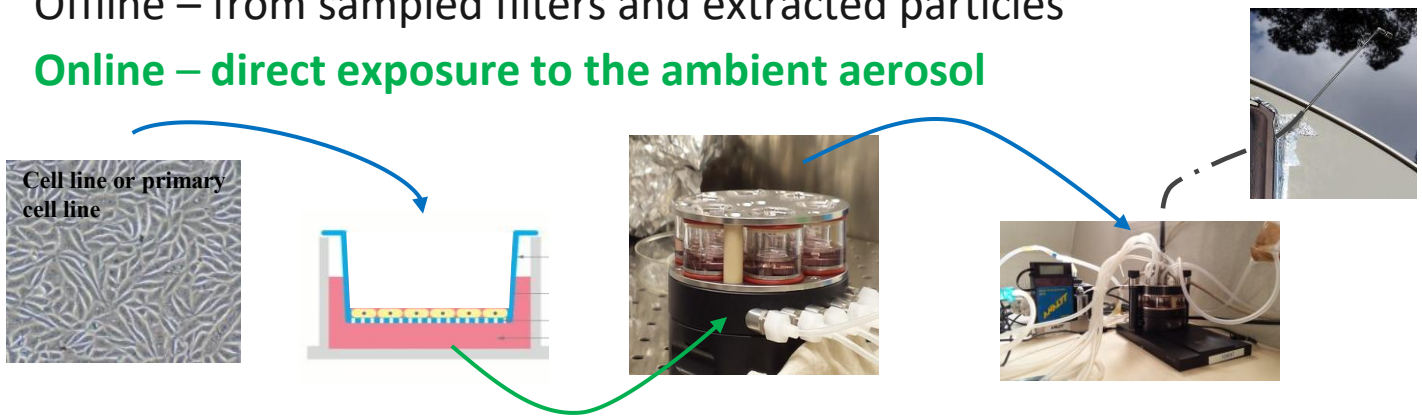
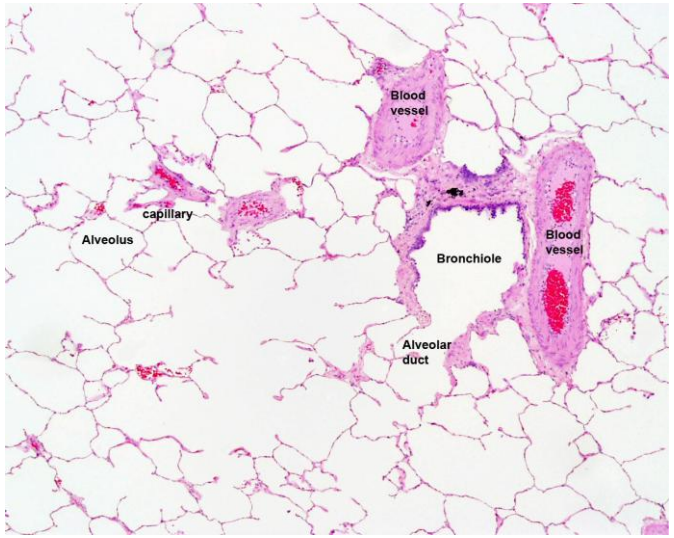


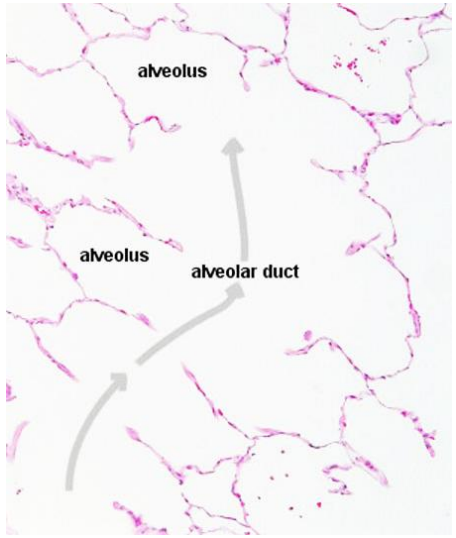
1) Selection of the *in vitro* model “representative” of the lung epithelium – mono or co-culture, cell line, immortalized or primary cells
2) Air liquid interface culturing of the *in vitro* model
3) Preparation of the exposure module
4) Direct exposure of the *in vitro* model to ambient aerosol

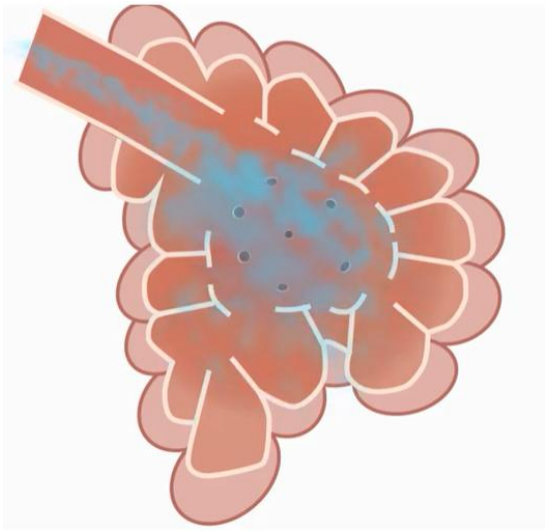
The experimental approaches

Why online exposures are promising:

The mimic the real interaction between particles and the lungs i.e. at the air liquid interface







The lungs can be considered as a series of empty “ducts” and, finally, “sacs” in which the air (and all the components that can travel in it) can flow. The “walls” of these ducts and sacs are formed by an epithelial tissue (which cell types differs in the different regions of the lungs). Underneath there is a connective tissue (ducts) or endothelial cells (sacs – the alveoli).

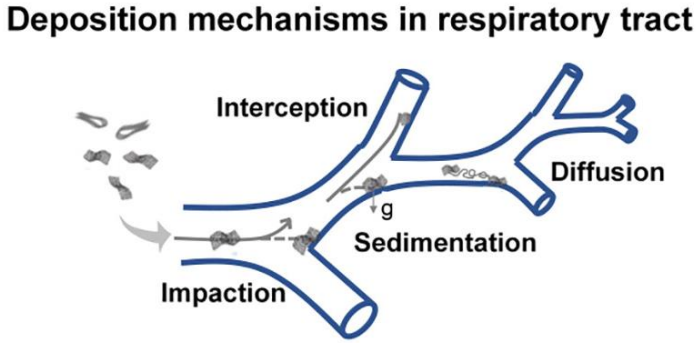
The experimental approaches

Why online exposures are promising:

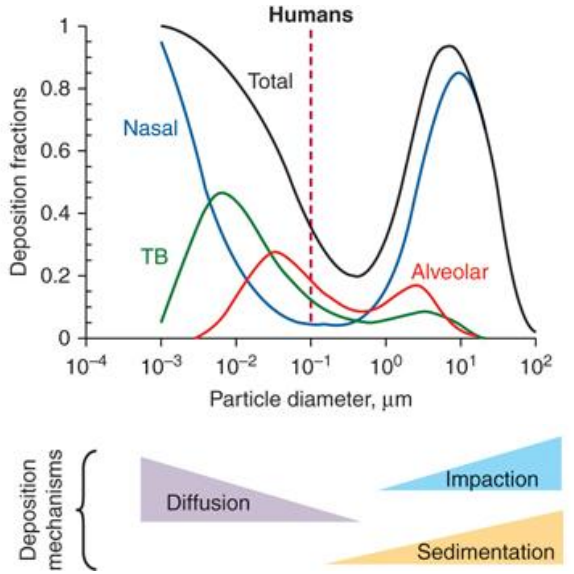
The particle losses are “only” related to the length and composition of the sampling/expsosure line. Stainless stell, short enough exposure lines should be always preferred.

Given this assumptions then the online exposure methods allow to replicate the expected lung deposition of particulate matter fractions as from reference in silico models. Particles deposition is determined by physical and chemical properties which are long studied and defined.

The “where” the particles deposit to determines the potential effects.



Gao et al., NanoImpact 21 (2021) 100292



From: Toxicology, Cassarett and Douls 8th edition

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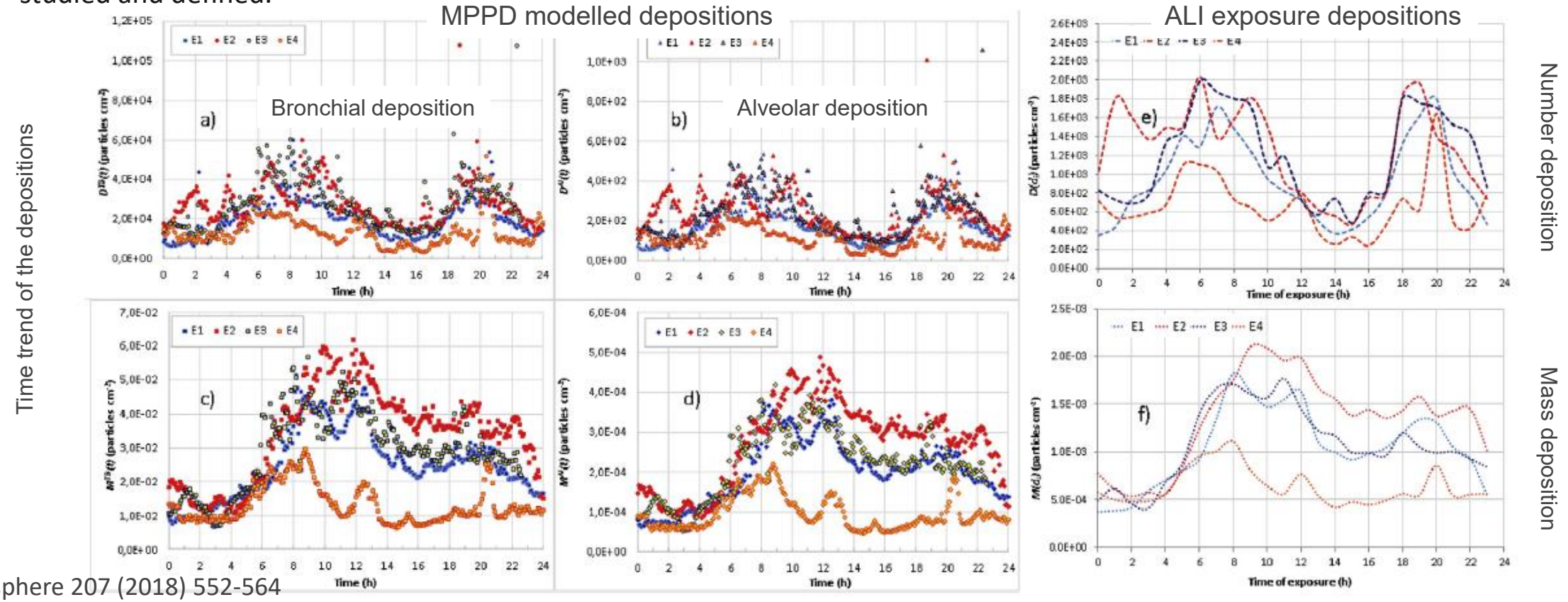

Chemosphere 207 (2018) 552-564

The experimental approaches

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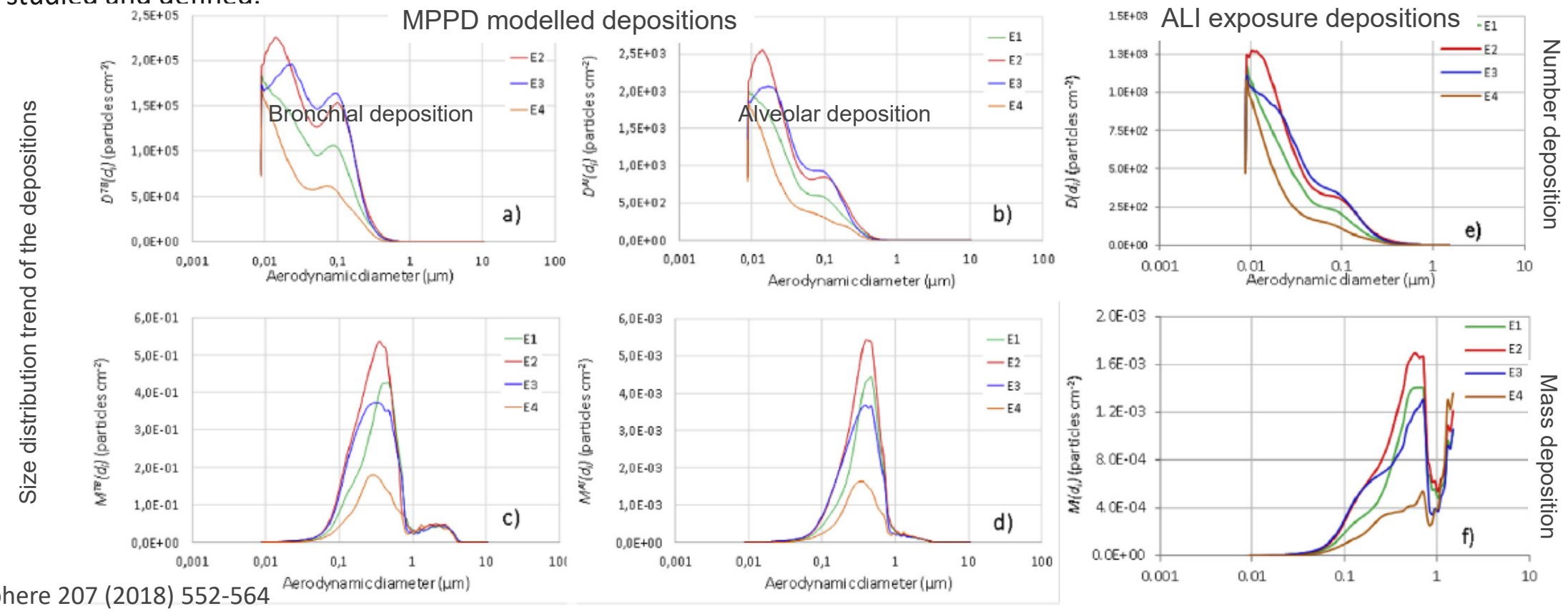
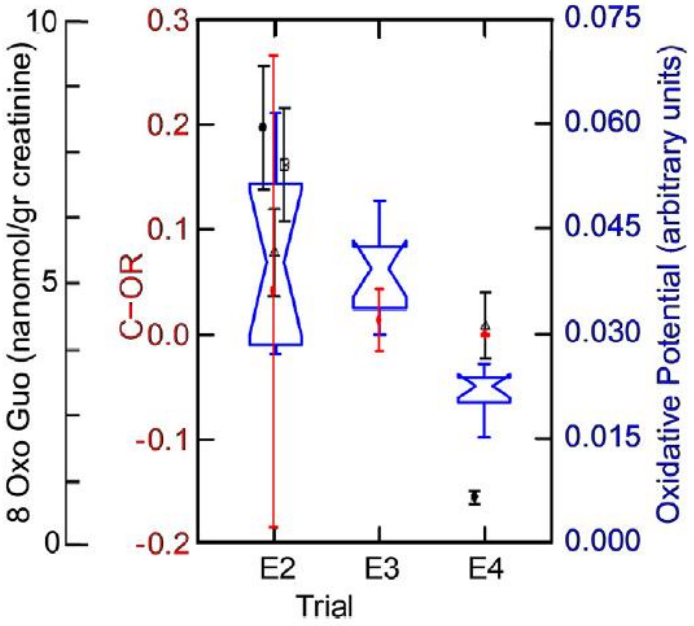


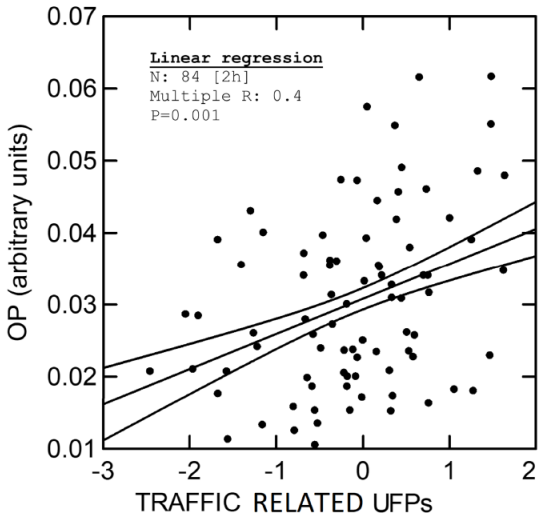
Chemosphere 207 (2018) 552-564

Results

First results combining online exposures and online OP The aerosol oxidative potential (OP) was determined by the 2’ 7’-dichlorofluorescein (DCFH) assay (OPDCFH) with 2-h time resolution in the soluble fraction of PM2.5 (more details in Atmosphere 2017, 8, 249; doi:10.3390/atmos8120249)



The relation between a human biomarker of oxidative damage (RNA oxidative damage Oxo-7,8-dihydroguanosine 8-oxoGuo); the oxidative effects on the ALI exposed in vitro models (C-OR) and the OP measured online are all in agreement, with higher effects observed during the day (E2) with the higher OP. This day was characterised by higher combustion emissions compared to E3 and E4. In general OP have fair relation with traffic UFP emissions.



Atmospheric Environment 213 (2019) 444–455

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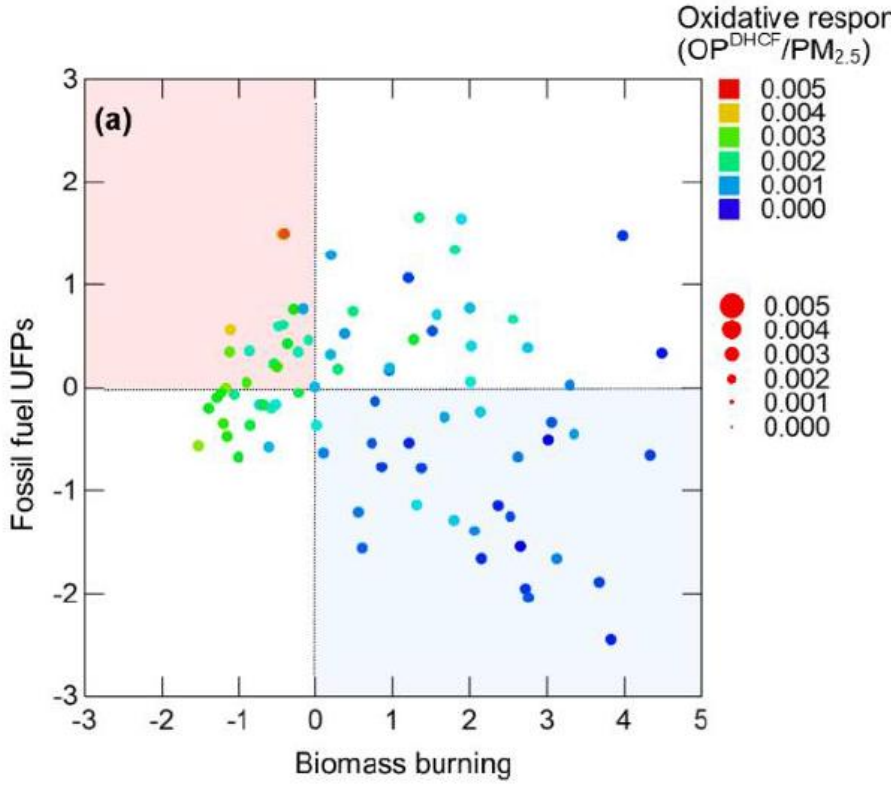
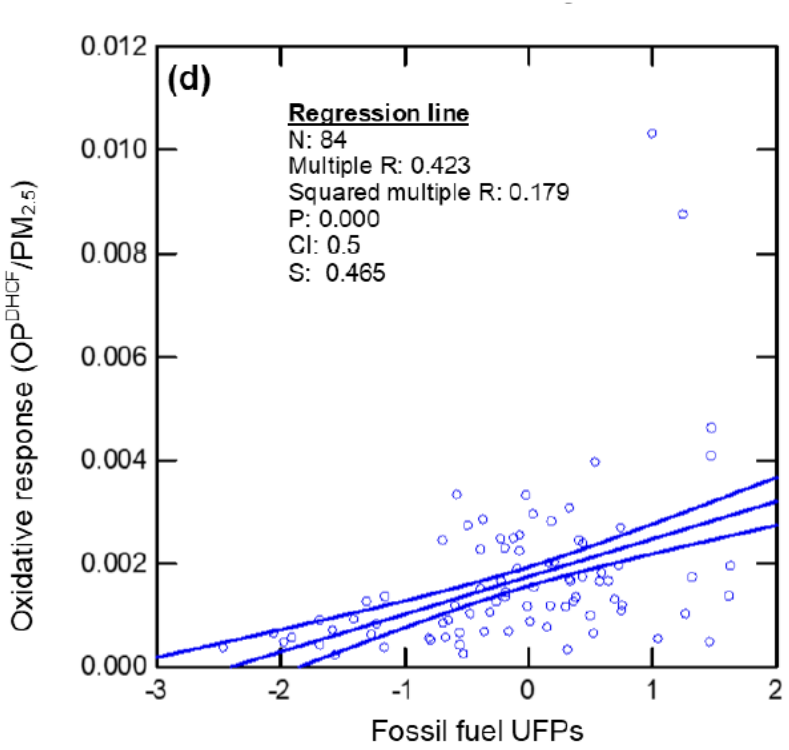


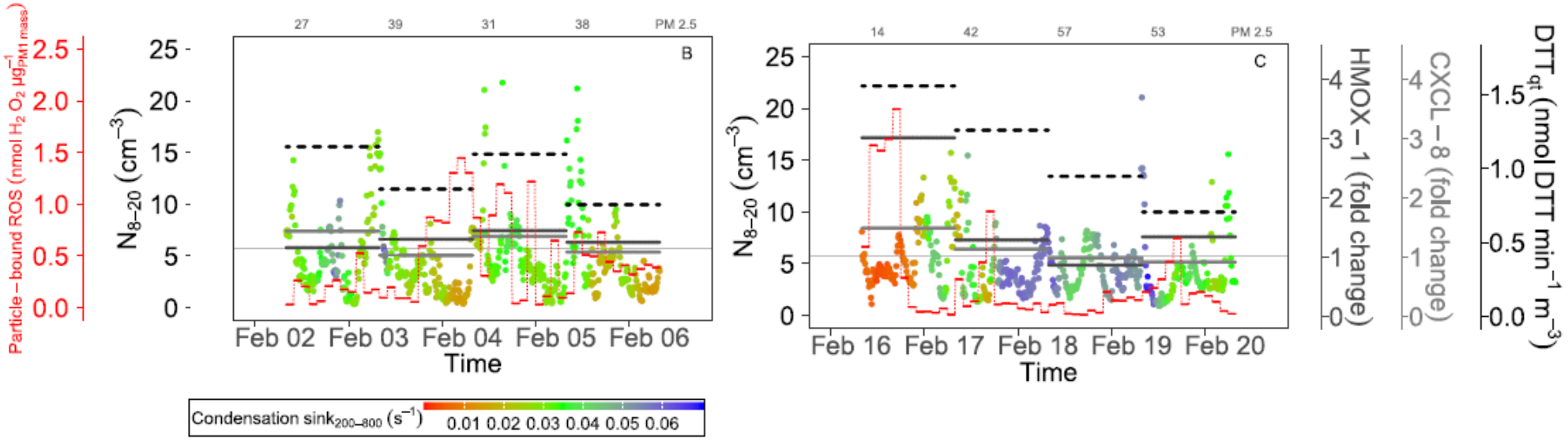


The relation between OP and traffic UFP emissions was further explored and analysed also in comparison with biomass burning emissions. Days with higher enrichment in fossil fuels emissions were characterized by higher OP values

Atmosphere 2020, 11, 414

Results

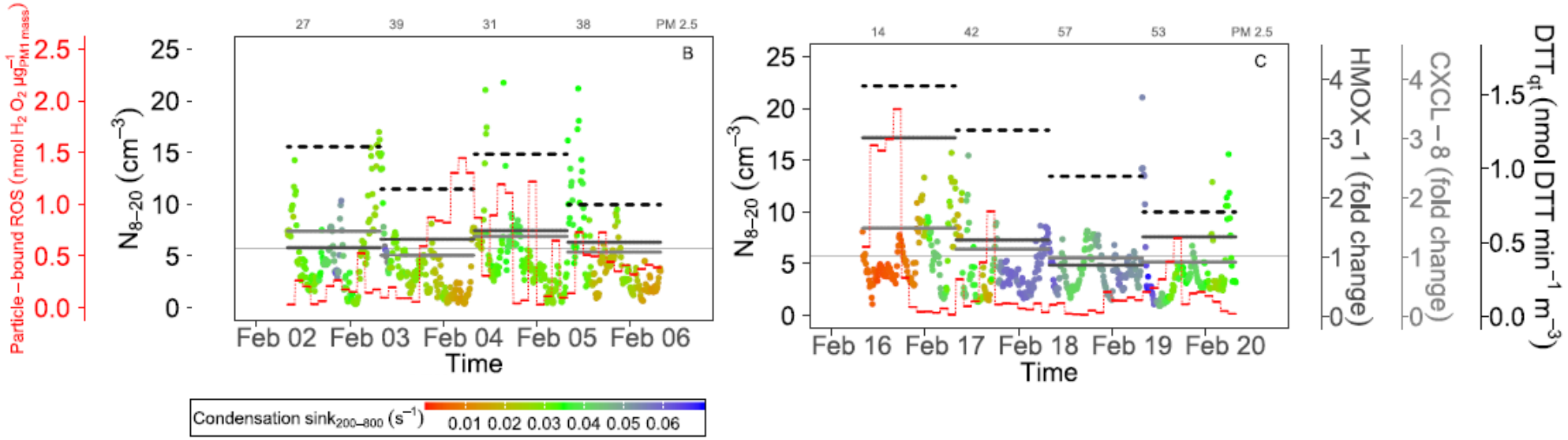
Results from the RHAPS campaigns showed a better relation of the online OP with the number of ultrafine (8-20 nm) particles. The filter based DTT OP measure (dotted lines in the figure) failed to properly catch the inter days variations. Moreover, highest online OP values were recorded during days with lower condensation sink (i.e. a measure to commonly used to determine of how rapidly molecules condense onto pre-existing aerosols)



Scientific Reports (2023) 13:18616 https://doi.org/10.1038/s41598-023-45230-z

Results

Results from the RHAPS campaigns showed a better relation of the online OP with the HMOX-1 gene expression (dark grey lines). This gene is related to the cellular defences against oxidative hazard, and it is upregulated (increased expression of the genes copies) when intracellular levels of oxidative molecules are increased.



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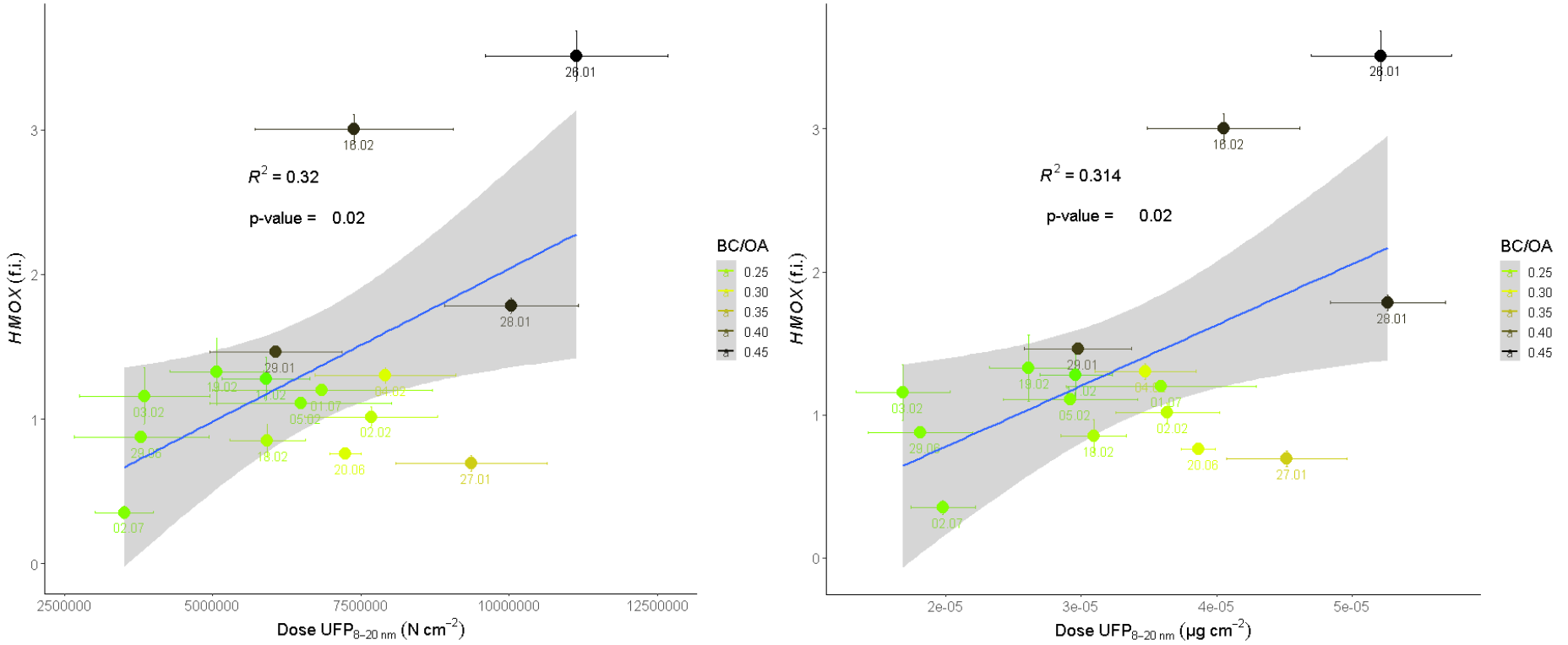
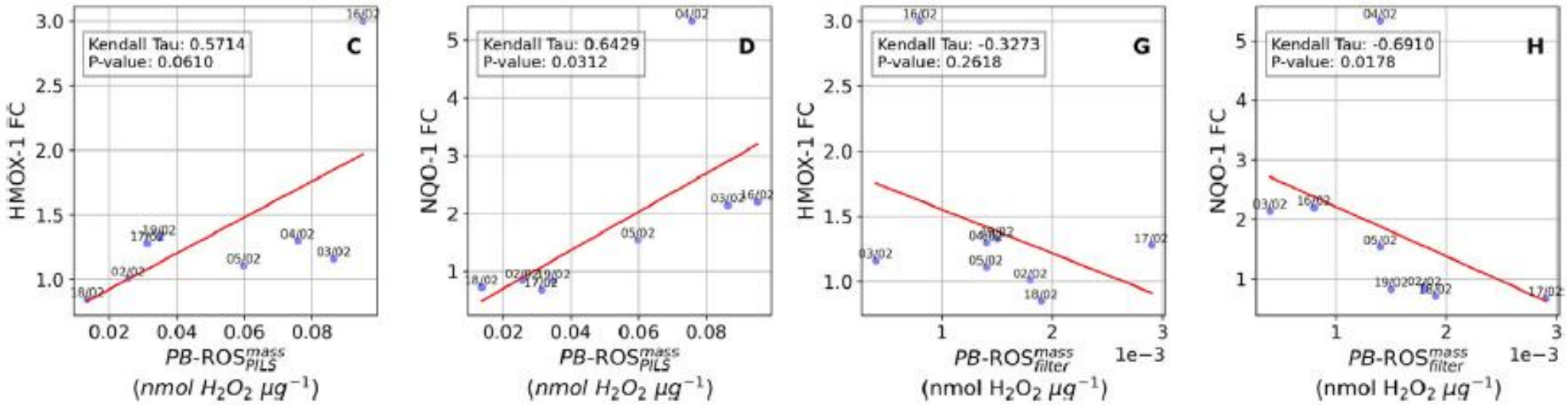
Results

Since the ultrafine particles combined with a low condensation sink showed better correlation with the oxidative damages, we explored specifically this relation. Positive significant relation is observed between ultrafine (8-20 nm) particles (expressed as delivered number or mass) and the expression of the HMOX gene. The samples with higher BC to OA ratio (fresh emitted particles) showed higher expression of the gene.



Results

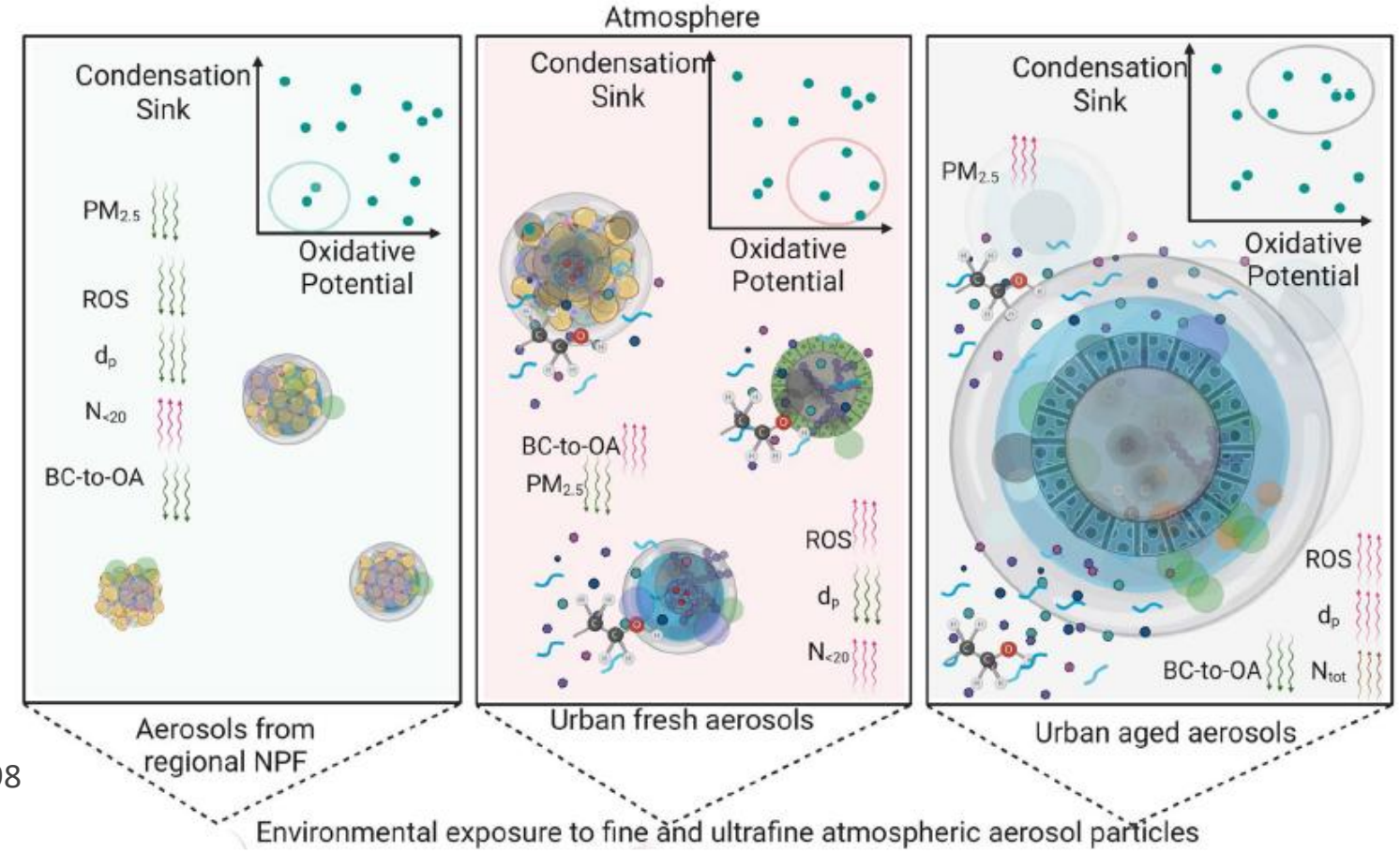
The relation between online OP and offline OP is further analysed showing a clear difference. While online OP and oxidative gene responses (HMOX-1 or NQO-1) positively relate with the online OP values, using the same procedure offline (on sampled filters) gives negative relation.



Environmental Pollution 385 (2025) 126874

Results

To sum-up: fresh urban aerosol with higher OP, lower Dp and higher BC to OA ratio is determining higher oxidative responses in human cells and human samples. The increase in the condensation sink lower the oxidative properties and the observed effects. Small particles from regional sources (secondary) are minimally contributing to OP and effects.



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Perspectives

- Combined analysis of human biomarkers, online exposure and online OP measurements could provide better insight and more robust information on the effects of fresh combustion particles with dimension lower than 100 nm, usually addressed in most of the literature.

- The possibility to measure online the oxidative responses in biological samples could further improve our understanding of the contribution of different sources that vary during the day in terms of relative contribution.

- Novel online OP techniques should be developed to analyse the complexity of this parameter, as from the knowledge gained from filters analyses.

- Dedicated human biomonitoring experiments, addressing also the personal exposure to, at least PM1 and quasi ultrafine particles during the time and space (personal monitors) could also help in pinpointing to the most relevant source of oxidative damage.

Conclusions

- Over 10 years the relation between combustion emissions and the potential oxidative effects is explored showing the importance of the finest particles.

- The limitation of filter OP measurements is more evident when comparing these parameters with data obtained from online exposure systems, that replicated the real human exposure.

- The combination of different expertise is pivotal to explore such complex relation (aerosol scientists, chemists, toxicologists…. are necessary to explore these intricated relations)

- The 8-20 nm particles should receive special attention or at least an indication of how fresh is the particulate matter (BC to OA ratio), if the protection of human health is priority.

Acknowledgments

- All the results here presented derives from collaborations with other researchers and institutions. Among the number of researchers special thank to Francesca Costabile, Gabriele Zanini, Milena Stracquadanio, Gianluca Di Iulio, Stefano Decesari and Silvia Canepari.

- The institutions that mostly contributed to this research are:

Thank you for your attention !!!

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