Phenotypic and metabolomic effects of atmospheric particulate matter from three different Catalonia locations on physiologically relevant human lung cell cultures

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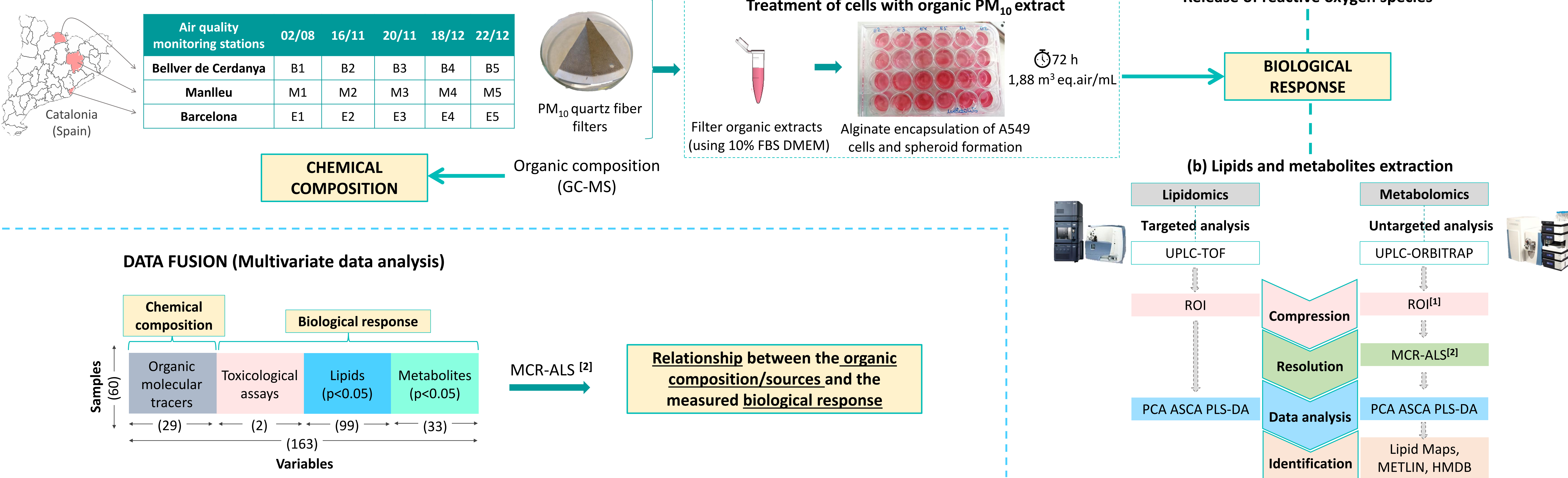p.villasclaras@gmail.com

1 BACKGROUND

Airborne particulate matter (PM) pollution has raised great concern since its exposure has been associated to adverse respiratory outcomes. However, the underlying biological mechanisms that induce lung injury remain mostly unknown.

In this work we propose the use of 3D bronchial cell cultures to study the biological effects of chemicals present in the organic soluble fraction of PM_{10} by means of the combination of toxicological assays and metabolomic and lipidomic profiling. The application of multivariate data analysis methods are useful to establish the relationship between pollutants and their biological response.
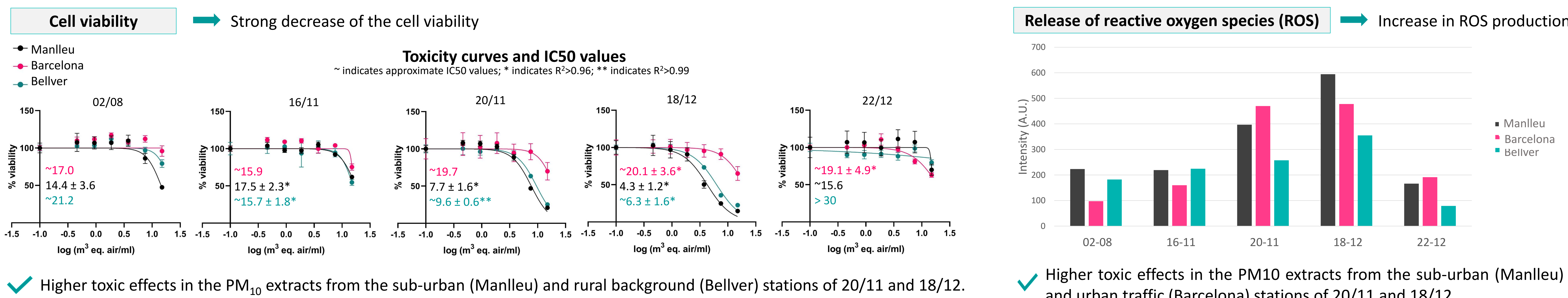

PM exposure ⟷ Health impacts

Asthma
Bronchitis
Lung cancer
Cystic fibrosis
...

Biological mechanisms

2 OBJECTIVE

→ Establishment of potential relationships between the organic composition/sources of PM_{10} pollutions and the measured biological response by toxicologic studies (cell viability and ROS), lipidomics and metabolomics.
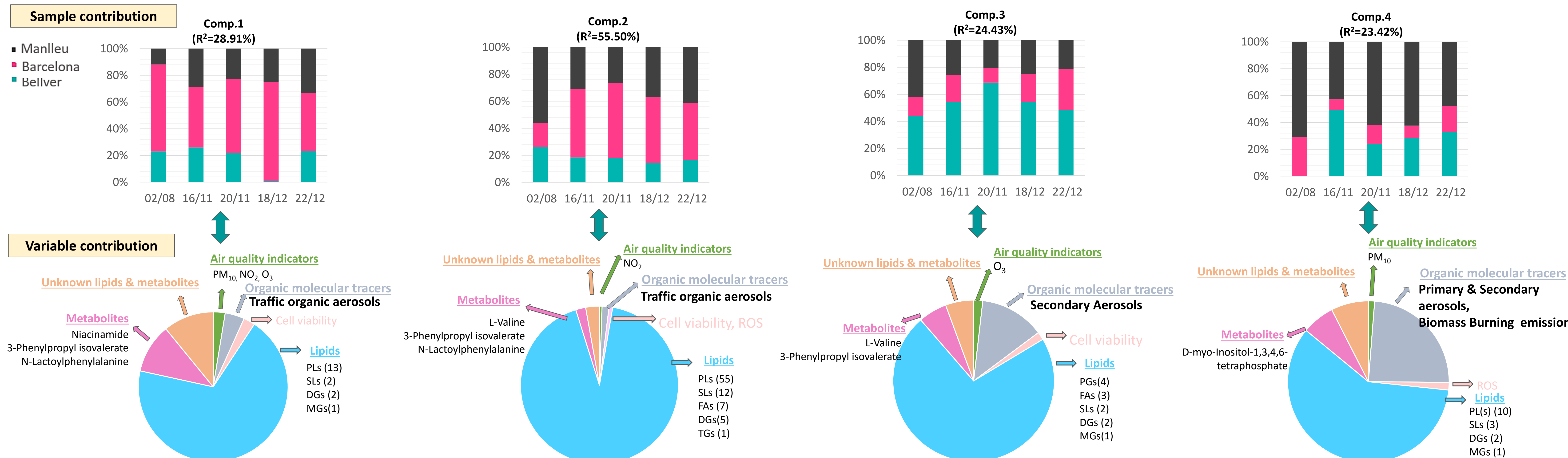
3 METHODOLOGY

Air quality monitoring stations	02/08	16/11	20/11	18/12	22/12
Bellver de Cerdanya	B1	B2	B3	B4	B5
Manlleu	M1	M2	M3	M4	M5
Barcelona	E1	E2	E3	E4	E5

Catalonia (Spain)

Treatment of cells with organic PM_{10} extract

PM_{10} quartz fiber filters

Filter organic extracts (using 10% FBS DMEM)

Alginate encapsulation of A549 cells and spheroid formation

72 h
1,88 m^3 eq.air/mL

(a) Cell viability (Resazurin assay), Release of reactive oxygen species

BIOLOGICAL RESPONSE

CHEMICAL COMPOSITION ← Organic composition (GC-MS)

(b) Lipids and metabolites extraction

Lipidomics	Metabolomics
Targeted analysis	Untargeted analysis
UPLC-TOF	UPLC-ORBITRAP
ROI	ROI[1]

Compression
Resolution
Data analysis
Identification

PCA ASCA PLS-DA	ROI[1]
	MCR-ALS[2]
PCA ASCA PLS-DA	PCA ASCA PLS-DA
	Lipid Maps, METLIN, HMDB

DATA FUSION (Multivariate data analysis)

Chemical composition / Biological response

Samples (60)

Organic molecular tracers	Toxicological assays	Lipids (p<0.05)	Metabolites (p<0.05)
(29)	(2)	(99)	(33)

(163) Variables

MCR-ALS [2] →

Relationship between the organic composition/sources and the measured biological response

4 RESULTS

Cell viability → Strong decrease of the cell viability

- Manlleu
- Barcelona
- Bellver

Toxicity curves and IC50 values
~ indicates approximate IC50 values; * indicates R^2>0.96; ** indicates R^2>0.99

02/08
~17.0
14.4 ± 3.6
~21.2

16/11
~15.9
17.5 ± 2.3*
~15.7 ± 1.8*

20/11
~19.7
7.7 ± 1.6*
~9.6 ± 0.6**

18/12
~20.1 ± 3.6*
4.3 ± 1.2*
~6.3 ± 1.6*

22/12
~19.1 ± 4.9*
~15.6
> 30

% viability vs log (m^3 eq. air/ml)

✓ Higher toxic effects in the PM_{10} extracts from the sub-urban (Manlleu) and rural background (Bellver) stations of 20/11 and 18/12.

Release of reactive oxygen species (ROS) → Increase in ROS production

Intensity (A.U.) — Manlleu, Barcelona, Bellver
02-08, 16-11, 20-11, 18-12, 22-12

✓ Higher toxic effects in the PM10 extracts from the sub-urban (Manlleu) and urban traffic (Barcelona) stations of 20/11 and 18/12.

Data fusion analysis → 4 MCR-ALS resolved components, R^2=91.85%

Sample contribution

- Manlleu
- Barcelona
- Bellver

Comp.1 (R^2=28.91%)
Comp.2 (R^2=55.50%)
Comp.3 (R^2=24.43%)
Comp.4 (R^2=23.42%)

02/08 16/11 20/11 18/12 22/12

Variable contribution

Comp.1:
Unknown lipids & metabolites
Metabolites: Niacinamide, 3-Phenylpropyl isovalerate, N-Lactoylphenylalanine
Air quality indicators: PM_{10}, NO_2, O_3
Organic molecular tracers: Traffic organic aerosols
Cell viability
Lipids: PLs (13), SLs (2), DGs (2), MGs(1)

Comp.2:
Unknown lipids & metabolites
Metabolites: L-Valine, 3-Phenylpropyl isovalerate, N-Lactoylphenylalanine
Air quality indicators: NO_2
Organic molecular tracers: Traffic organic aerosols
Cell viability, ROS
Lipids: PLs (55), SLs (12), FAs (7), DGs(5), TGs (1)

Comp.3:
Unknown lipids & metabolites
Metabolites: L-Valine, 3-Phenylpropyl isovalerate
Air quality indicators: O_3
Organic molecular tracers: Secondary Aerosols
Cell viability
Lipids: PGs(4), FAs (3), SLs (2), DGs (2), MGs(1)

Comp.4:
Unknown lipids & metabolites
Metabolites: D-myo-Inositol-1,3,4,6-tetraphosphate
Air quality indicators: PM_{10}
Organic molecular tracers: Primary & Secondary aerosols, Biomass Burning emissions
ROS
Lipids: PL(s) (10), SLs (3), DGs (2), MGs (1)

5 CONCLUSIONS

→ Phenotypic, metabolomic and lipidomic characterization of the PM_{10} samples effects on A549 lung cells has been performed. Significant differences were found between locations and sampling days.

→ The joint analysis of chemical composition and omics data fusion enabled the identification of the most relevant relationships between PM_{10} pollution sources and biological responses.

6 REFERENCES

[1] Pérez-Cova, M., Bedia, C., Stoll, D. R., Tauler, R., & Jaumot, J. (2021). MSroi: a pre-processing tool for mass spectrometry-based studies. *Chemometrics and Intelligent Laboratory Systems*, 104333.
[2] Tauler, R. (1995). Multivariate curve resolution applied to second order data. *Chemometrics and Intelligent Laboratory Systems*, 30(1), 133–146.