MIND-BB - AIR QUALITY AND HEALTH RISK MEASURED IN DOMESTIC BIOMASS BURNING HEATING DEVICES

ATMO-ACCESS Programme – MIND-BB Activity REPORT

1 - Project Partners

- ENEA - Italian National Agency for New Technologies, Energy and Sustainable Economic Development
- UNIMIB - University of Milano "Bicocca"
- CEAM Foundation - Mediterranean Center for Environmental Studies (hosting institution)

2 - Access period and comprehensive operations period

- Trans-National Access (TNA): 02 - 22.11.2024
- Experimental operations: 04.11.2024 - 13.01.2025

3 - Personnel involved

- ENEA Team: Ettore Petralia (physical access), Ilaria D'Elia (physical access), Laura Caiazzo (physical access), Teresa M.G. La Torretta (remote access), Giandomenico Pace (remote access), Antonio Piersanti (remote access), Milena Stracquadanio (remote access);
- UNIMIB Team: Maurizio Gualtieri (physical access), Rossella Bengalli (physical access), Sara Marchetti (physical access), Giulia Motta (physical access);
- CEAM Team: Amalia Muñoz, Mila Ródenas, Teresa Vera, Rubén Soler, Esther Borrás, Beatríz Domínguez, Tatiana Gómez, Maria Luisa Martinez.

4 - General framework and the MIND-BB Project

Biomass burning (BB) represents a global concern due to its impact on air pollution, health, and climate change resulting in 2.3 million premature deaths yearly. BB in the residential sector is one of the main sources of primary particulate matter (PM) emissions.

BB emits a significant amount of black and brown carbon (BC, BrC), Polycyclic Aromatic Hydrocarbons (PAHs) and contributes to secondary formation of ozone (O_3) by photochemical reaction by emitting volatile organic compounds (VOCs) and nitrogen oxides (NO_x). On the other side, BB is an alternative green energy source to fossil fuels reducing carbon dioxide (CO_2) emissions with lower costs, whose use is even increased due to the last energy crisis and natural gas cost.

The use of domestic appliances for heating is still widespread in Europe with relevant impact on both rural and urban areas. Nevertheless, BB is affected by a wide range of uncertainties attributed to emission factors (EFs), air pollutants contribution and their formation, and health estimates.

An almost complete chemical characterization of primary and secondary emissions, combined with the understanding of its potential health effects, requires dedicated experiments to assess a proper offset of BB. Most of the studies are focused on characterizing the composition of the primary emissions, only scarce works have considered the aging of particles and gas-phase precursors and products.

In our project (MIND-BB - air quality and health risk Measured IN Domestic Biomass Burning heating devices), primary gaseous and particulate emissions will be analysed within the outdoor EUPHORE (EUropean PHOto REactor) chamber, highly instrumentalized, representing the best suitable facility that can simulate experiments under near-real conditions given its large size and the use of natural light thanks to its Teflon™ fluorinated ethylene propylene (FEP) cover and its related opening-closing dome. EUPHORE has been recently adapted to introduce fumes from stoves and open fire, allowing the analysis of the aging of the primary emissions under both day (sunlight) and night conditions.

EFs as primary or secondary emissions per mass of burned fuel used will be defined.

In parallel, we exposed lung epithelial cells to the primary and secondary emissions to evaluate the toxicological hazard of particle and gas phase pollutants. Direct exposure at the EUPHORE chamber allowed a proper estimation of the risk of inhalation of the emissions. All this knowledge will offer a complete picture of the BB impact improving its emission inventory estimates, its toxicological hazards and its contribution to air quality models.

The present Report describes the Trans-National Access (TNA) experimental activity within the context of ATMO-ACCESS Programme, giving some preliminary scientific results.

The more complete and exhaustive results from this TNA, needing more time to analyse all the data collected from experiments, will be disseminated in scientific congress and in peer-reviewed papers. Then all the data will be delivered as well to the ACTRIS database. Informative brochures on best practices to household use of woody biomass and a guide with measures to reduce BB harmful emissions and to mitigate their impacts will be realized. Several meetings at different scales, a workshop and educational programs are planned.

This work was supported by the EC under the Horizon 2020 – R&I FP through the ATMO-ACCESS Integrating Activity under GA N. 101008004.

5 - Experimental activity

An innovative methodology is proposed that integrates high resolution chemical characterization, not only of primary emissions (as usually reported) but also their chemical transformation and aging, combined with toxicological hazards, and that will provide scientific impact in a field still affected by large uncertainties. Significantly, the direct exposure of human lung cells to primary and aged emissions to define their toxicological hazard is a procedure never tested in a well-controlled environment such as EUPHORE and will provide new and so far, unavailable data.

The use of high temporal resolution instruments allows to characterize and study the rapid chemical transformation of primary BB emissions during different combustion phases and real aging conditions.

All the results serve to understand and develop effective mitigation policies and allow a more reliable evaluation of BB impacts on air quality and climate change models studies.

The results will produce a trans-disciplinary improvement in understanding the impacts of BB primary vs secondary emissions on air pollution and its toxicological risk, allowing to improve the estimates of emission inventories and scenarios and to offset the pros and cons of using woody biomass as energy source and its effects on air quality, climate change and health.

During experimental activity, 2 types of domestic heating devices (pellet stove and traditional wood stove) and different BB fuels (pellet, pine logs and oak logs) were tested, with evaluation of primary emissions during flaming (FP) and smouldering (SP) phases, and their aging in day (chamber's dome open) and night (chamber's dome closed) conditions, by monitoring BB chemical and physical properties.

As for chemical elements and compounds, our attention was focused on the characterization of primary and aged emissions for VOCs, PAHs, O_3, NO_x, Total Carbon, Brown Carbon, Elemental Carbon, Organic Carbon, water-soluble Inorganic Ions (e.g. SO_4^{2-}, NO_3^-, NH_4^+, K^+, Na^+, Cl^-), Levoglucosan, Metals-Trace Elements, Particulate Matter (PM) expressed in mass concentration and particles number concentration.

In parallel the exposition of lung epithelial cells (BEAS-2B or A549) to pollutants (by a Cultex RFS module) was performed, to define the BB toxicological hazard as cell viability and gene expression (oxidative and inflammatory genes).

6 - Equipment

- TEOM - Tapered element oscillating microbalance, for continuously measure concentrations of air particles;

- SMPS / DMA 3081 (TSI) - Scanning Mobility Particle Size Spectrometer, for measuring the size distribution of airborne submicron particles;
- PTR-ToF-MS (IONICON)- Proton Transfer Reaction Mass Spectrometer Time of Flight, for real-time monitoring of volatile (organic) compounds (VOCs);
- FTIR NICOLET 6700 (Thermo Scientific) - Fourier transform infrared spectroscopy, used to obtain concentration of organic and inorganic gas-phase compounds;
- O_3 Ozone Analyser Serinus 10 monitor (Ecotech);
- NO, NO_2 T200UP monitor (Teledyne);
- NO_2 T500UP CAPS monitor (Teledyne);
- CO monitor (TE48C);
- CO_2 Li-850 monitor (LICOR)
- SO2 Ecotech (Serinus) monitor;
- API-ToF-CIMS - (Aerdodyne Research) Time of Flight Chemical Ionization Mass Spectrometer, for online monitoring of gas-phase compounds and particle-phase compounds (FIGAERO module);
- LV37, Low-volume filter sampler;
- PM1 Quartz filter sampling line, for Particulate Matter < 1 μm sampling;
- PM2.5 Quartz filter sampling line, for Particulate Matter < 2.5 μm sampling;
- Aethalometer AE33 (Aerosol MAGEE Scientific) Aethalometer, for on-line Black Carbon and Brown Carbon measures;
- TCA, Aerosol Magee Scientific, for total carbon (Aethalometer + Carbonaceous Aersosol Speciation System).
- Optical particle counter (GRIMM, AMOF), environmental dust monitor 107, for on-line PM mass concentration (PM10, PM2.5, PM1) and particle number (from 0.25 to 32 μm) measures;
- Cultex RFS module to perform cells exposition

7 - <u>Experiments' schedule</u>

The first day of the campaign was dedicated to install the instruments and to have a meeting to discuss details of the experiments. Then, a total of 12 single-day experiments were performed during the whole experimental session and an additional experiment to characterize the background conditions of the chamber. Table 1 reports the characteristics of the different tests performed: Number of Experiment, Date, Stove typology (Traditional; Pellet), Fuel typology (Pine, Oak, Pellet), Burning phase (Flaming, Smoldering), Oxidation condition (Night-time, Day-time), Emissions (Primary; Aged), Upshot (Valid; NOT Valid; Doubtful).

The 1st (06.11.2024, PN_FL_DK) and 7th test (18.11.2024, OK_SM_LT) was immediately considered as NOT Valid (according to the instrumental feedback) and so repeated respectively

on 07.11.2025 (2nd test) and 13.01.2025 (12th test); the 9th test (20.11.2024, PN_SM_DK) was preliminary considered as NOT Valid since concentration values were too low; the 10th test (21.11.2024, OK_SM_DK) was preliminary considered as Doubtful since values seem too high compared tests under similar conditions. Further analysis will evaluate the validity of these two latter tests.

EXP.	CODE	DATE	STOVE TYPOL.	FUEL	BURNING PHASE	OXIDATION CONDIT.	EMISS.	UPSHOT
1st a	PN_FL_DK	06.11.2024	Traditional	Pine	Flaming	Night-time	Primary	NOT Valid
1st b	PN_FL_DK_AG	06.11.2024	Traditional	Pine	Flaming	Night-time	Aged	NOT Valid
2nd a	PN_FL_DK	07.11.2024	Traditional	Pine	Flaming	Night-time	Primary	Valid
2nd b	PN_FL_DK_AG	07.11.2024	Traditional	Pine	Flaming	Night-time	Aged	Valid
3rd a	PN_FL_LT	08.11.2024	Traditional	Pine	Flaming	Day-time	Primary	Valid
3rd b	PN_FL_LT_AG	08.11.2024	Traditional	Pine	Flaming	Day-time	Aged	Valid
4th a	PE_FL_LT	11.11.2024	Pellet	Pellet	Flaming	Day-time	Primary	Valid
4th b	PE_FL_LT_AG	11.11.2024	Pellet	Pellet	Flaming	Day-time	Aged	Valid
5th a	OK_FL_LT	12.11.2024	Traditional	Oak	Flaming	Day-time	Primary	Valid
5th b	OK_FL_LT_AG	12.11.2024	Traditional	Oak	Flaming	Day-time	Aged	Valid
6th a	OK_FL_DK	13.11.2024	Traditional	Oak	Flaming	Night-time	Primary	Valid
6th b	OK_FL_DK_AG	13.11.2024	Traditional	Oak	Flaming	Night-time	Aged	Valid
7th a	PE_FL_DK	15.11.2024	Pellet	Pellet	Flaming	Night-time	Primary	Valid
7th b	PE_FL_DK_AG	15.11.2024	Pellet	Pellet	Flaming	Night-time	Aged	Valid
8th a	OK_SM_LT	18.11.2024	Traditional	Oak	Smoldering	Day-time	Primary	NOT Valid
8th b	OK_SM_LT_AG	18.11.2024	Traditional	Oak	Smoldering	Day-time	Aged	NOT Valid
9th a	PN_SM_LT	19.11.2024	Traditional	Pine	Smoldering	Day-time	Primary	Valid
9th b	PN_SM_LT_AG	19.11.2024	Traditional	Pine	Smoldering	Day-time	Aged	Valid
10th a	PN_SM_DK	20.11.2024	Traditional	Pine	Smoldering	Night-time	Primary	NOT Valid
10th b	PN_SM_DK_AG	20.11.2024	Traditional	Pine	Smoldering	Night-time	Aged	NOT Valid
11th a	OK_SM_DK	21.11.2024	Traditional	Oak	Smoldering	Night-time	Primary	Doubtful
11th b	OK_SM_DK_AG	21.11.2024	Traditional	Oak	Smoldering	Night-time	Aged	Doubtful
12th a	OK_SM_LT	13.01.2025	Traditional	Oak	Smoldering	Day-time	Primary	Valid
12th b	OK_SM_LT_AG	13.01.2025	Traditional	Oak	Smoldering	Day-time	Aged	Valid

Table 1 - Characteristics of the different performed test

8 - **Experiments' general protocol**

In general, the daily protocol followed to perform each trial was:

1. chamber flushing for cleaning (PM = 0.0 - 0.1 μg/m^3);
2. chamber conditioning up to 50% RH;
3. ignition of the stove (with about 200 – 250 gr of woody biomass fuel) and introduction of smoke (for 1 min) into the chamber;
4. waiting (few minutes) for fumes mixing within the chamber;

5. sampling fumes (on-line hight-time resolution monitors and filters for post analysis) for 2 hours to catch fresh emissions: subsequently referred to as PERIOD 1;
6. stop filter sampling; continuation with on-line monitors sampling; opening the chamber's dome (for aging in day-time conditions) or addition of O_3 and NO_2 to generate NO_3 radicals (for aging in night-time conditions);
7. monitoring for 4 hours during the aerosol aging processes: subsequently referred to as PERIOD 2;
8. closing the chamber's dome, if it was open to simulate day-time conditions;
9. sampling fumes (on-line hight-time resolution monitors and filters for post analysis) for 4 hours to catch aged aerosols: subsequently referred to as PERIOD 3;
10. end of the experiment.

9 - Preliminary results

This report presents the very preliminary results of the experiment conducted at the EUPHORE chamber.

Most of the data is still under analysis and will be object of further deliverables, reports and scientific papers, as well as their inclusion in dedicated European databases (e.g. ACTRIS database) available for the scientific community.

This work presents some preliminary results of the different tests regarding the PM1 mass concentration and the Number of particles for fractions 0.25-0.28 µm, 0.28-0.30 µm, 0.30-0.35 µm, 0.35-0.40 µm, 0.40-0.45 µm, 0.45-0.50 µm, 0.50-0.58 µm, 0.58-0.65 µm, 0.65-0.70 µm, 0.70-0.80 µm, 0.80-1.0 µm measured (1 minute time-resolution) by Optical Particle Counter (OPC) GRIMM environmental dust monitor 107 (raw data) and preliminary results on toxicological outcomes.

The presentation and discussion of mass concentration and particle number results will be done considering the next experimental PERIODS (e.g. Figure 8), outlined in Table 2:

PERIOD	DURATION	EMISSION KIND	LOCAL TIME-FRAME (approximately)
1	2 hours	Primary emissions	10:30 – 12:30
2	4 hours	Aging process	12:30 – 16:30
3	4 hours	Aged/secondary compounds	17:00 – 21:00

Table 2 - Characteristics of the three different periods composing each test

9.1 - PM1 Mass Concentration

9.1.1 - Primary Emissions (Period 1)

Figure 1 reports the box-plot graph of PM1 mass concentration values measured during the PERIOD 1's 2-hours sampling, referred to PRIMARY emissions for all the fuel species and stove

typology. In that PERIOD 1 the conditions within the chamber were the same (whether the figures report the acronym LT or DK in the test's label) since the chamber was closed.

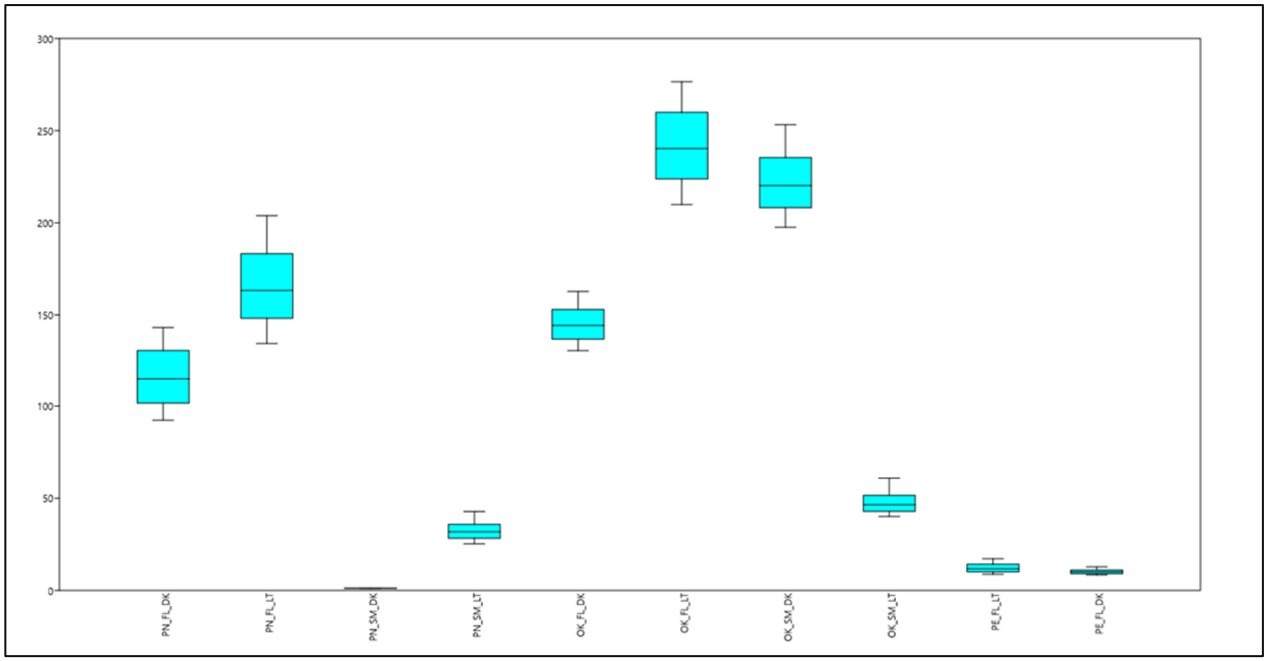


Figure 1 - Box-plot graph of PM1 mass concentration measured during the Primary emission's period. Concentrations in μg/m^3

Not considering PN_SM_DK (values apparently too low) and OK_SM_DK (values apparently too high), all the FLAMING tests presents values not always comparable between the same traditional fuel typology (pine and oak logs respectively), but highly comparable in the case of the two Pellet tests: that seems to highlight that Pellet fuel response is more stable and replicable and that also produces remarkably lower PM1 mass concentration than the other woods tested.

The SMOLDERING phases, both for "valid" Pine (LT) and Oak (LT) tests, show values considerably lower than the FLAMING phases. However, at a first general consideration for the overall smoldering combustion data observed, we can hypothesize that smoldering is more difficult to segregate and sample, apparently presenting more variability than flaming.

The experiment PN_SM_DK was removed for analysis shown in Figure 2 and Figure 3.

The modeling by classical Hierarchical clustering (Figure 2) pairs the two Pellet tests as very close, within a macro-cluster including also the two "valid" SMOLDERING tests (Pine LT and Oak LT). Then, a second macro-cluster groups the Pine and Oak FLAMING phases (and the ambiguous OK_SM_DK).

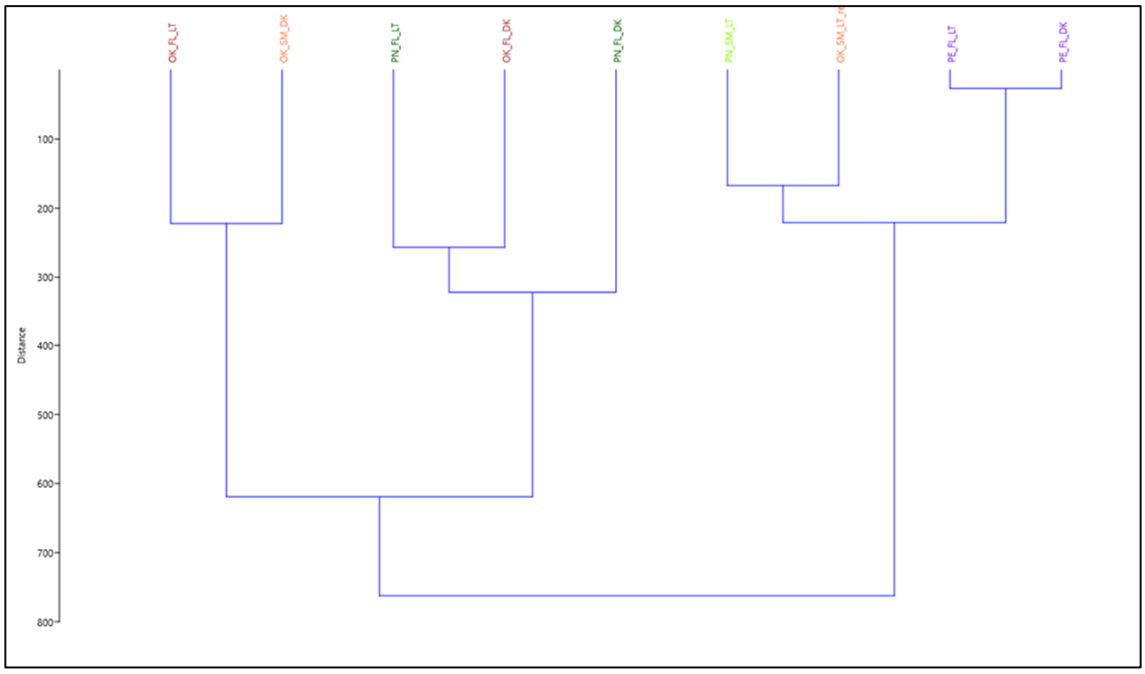


Figure 2 - Hierarchical clustering graph of PM1 mass values measured during the Primary emission's period

The spatial relationship of non-metric Multi-dimensional Scaling (Figure 3) confirms the close similarity of two Pellet tests, and their proximity with the two "valid" SMOLDERING tests (Pine LT and Oak LT). Then, the two Pine FLAMING phases are close together, and the two Oak FLAMING phases are positioned together in a third spatial area but less close reciprocally as is for the other fuel typology couples.

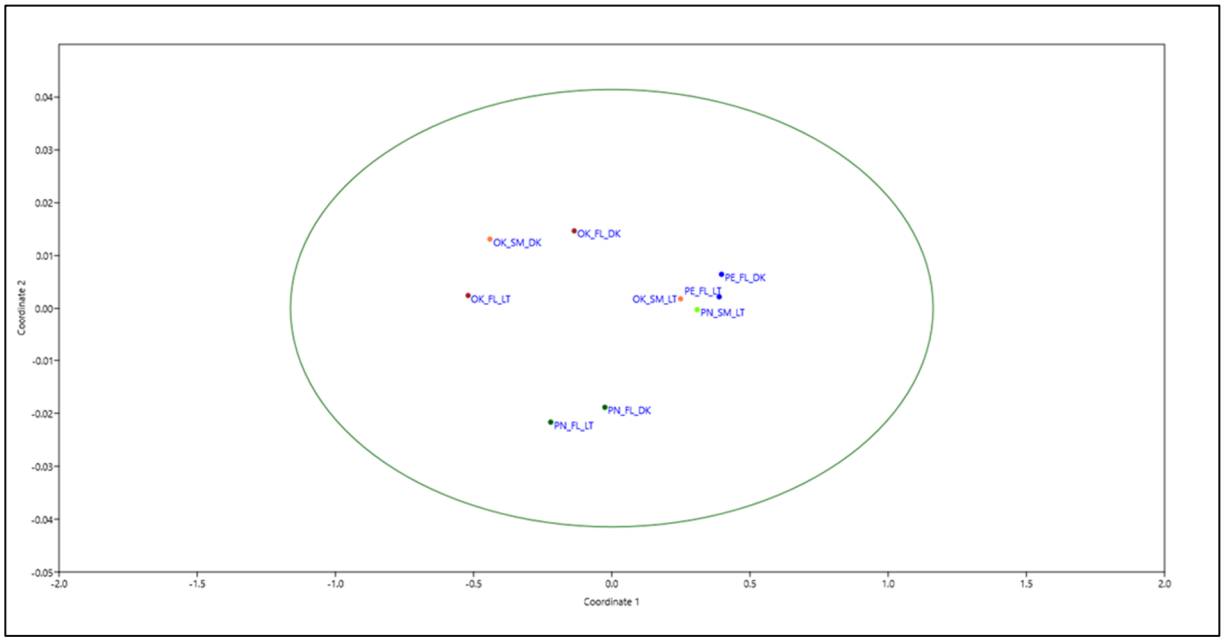


Figure 3 - Non-metric Multi-dimensional Scaling graph of PM1 mass values measured during the Primary emission's period

9.1.2 - Aging process (Period 2)

Figure 4 reports the box-plot graph of PM1 mass values measured during the PERIOD 2's 4-hours sampling, referred to as AGING PROCESS of aerosol for all the fuel species and stove typology. In that PERIOD 2 the chamber covering was opened to let the emissions evolve under day-time conditions (acronym LT in tests' labels), or it was kept closed with addition of O_3 and NO_2 to generate NO_3 radicals, for aging in night-time conditions (acronym DK in tests' labels).

The PM1 mass concentration is dependent on the emission values in the PERIOD 1, therefore, FLAMING tests in the PERIOD 2 also present values not always comparable between the same traditional fuel typology (Pine and Oak logs respectively), but highly comparable in the case of the two Pellet tests.

The SMOLDERING phases, both for Pine (LT) and Oak (LT) tests (not considering OK_SM_DK because values apparently too high), show values considerably lower than the FLAMING phases.

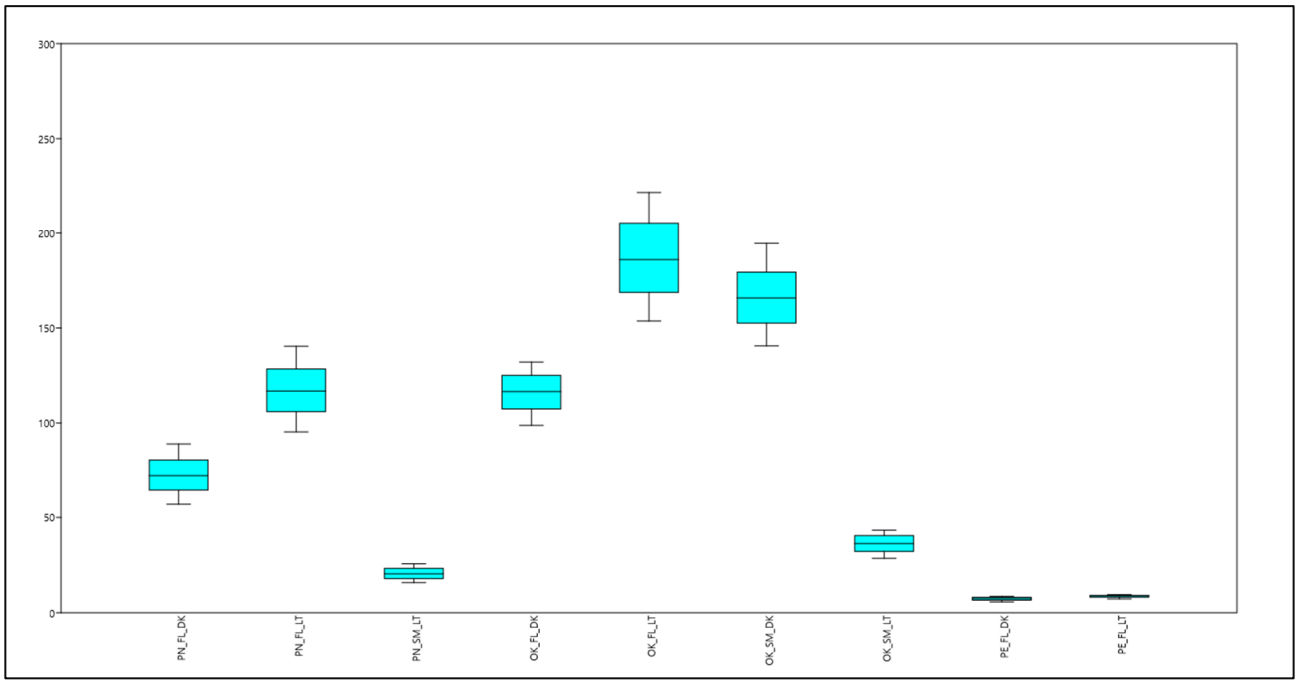


Figure 4 - Box-plot graph of PM1 mass values measured during the Aging process period. Concentrations in $\mu g/m^3$

The modeling by classical Hierarchical clustering (Figure 5) pairs the two Pellet tests as very close, within a macro-cluster including also the two "valid" SMOLDERING tests (Pine LT and Oak LT). Then, the second macro-cluster emphasizes the differences between the Pine FL tests and the Oak FL tests.

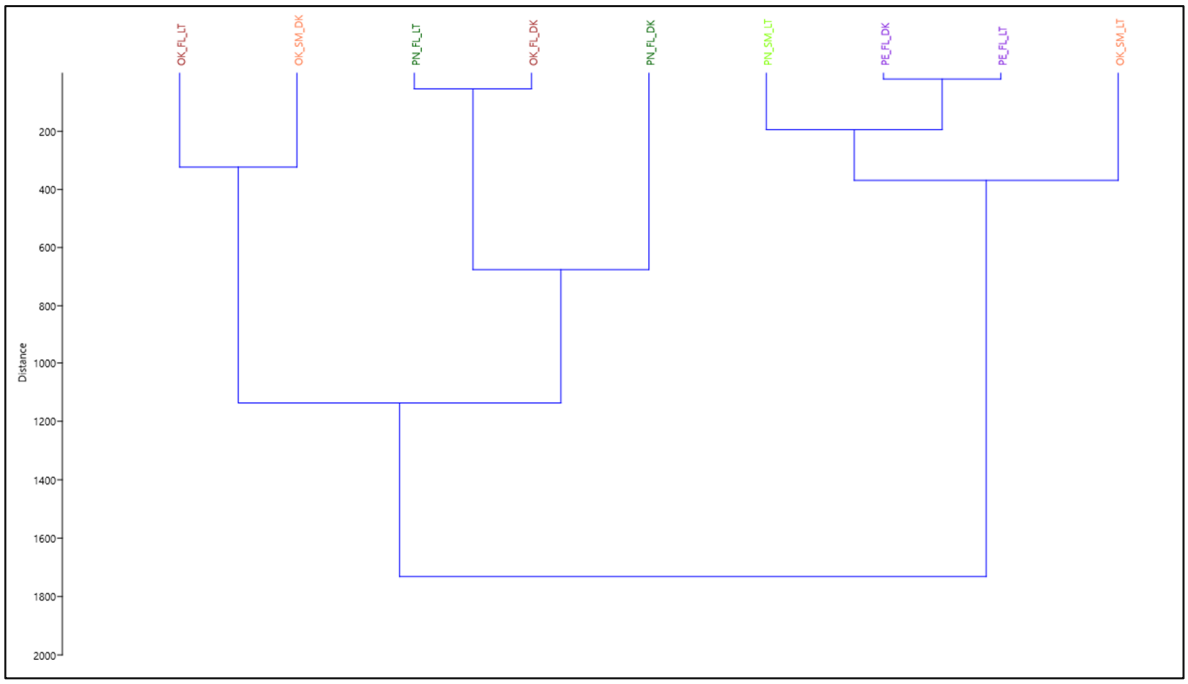


Figure 5 - Hierarchical clustering graph of PM1 mass values measured during the Aging process period

The spatial relationship of non-metric Multi-dimensional Scaling (Figure 6) confirms the close isolated similarity of two Pellet tests. The two “valid” SMOLDERING tests (Pine LT and Oak LT) are close together. Then, the two Pine FLAMING phases are grouped together but not very close, and the two Oak FLAMING phases are scattered: the aging phase presents complex processes, more difficult to interpret.

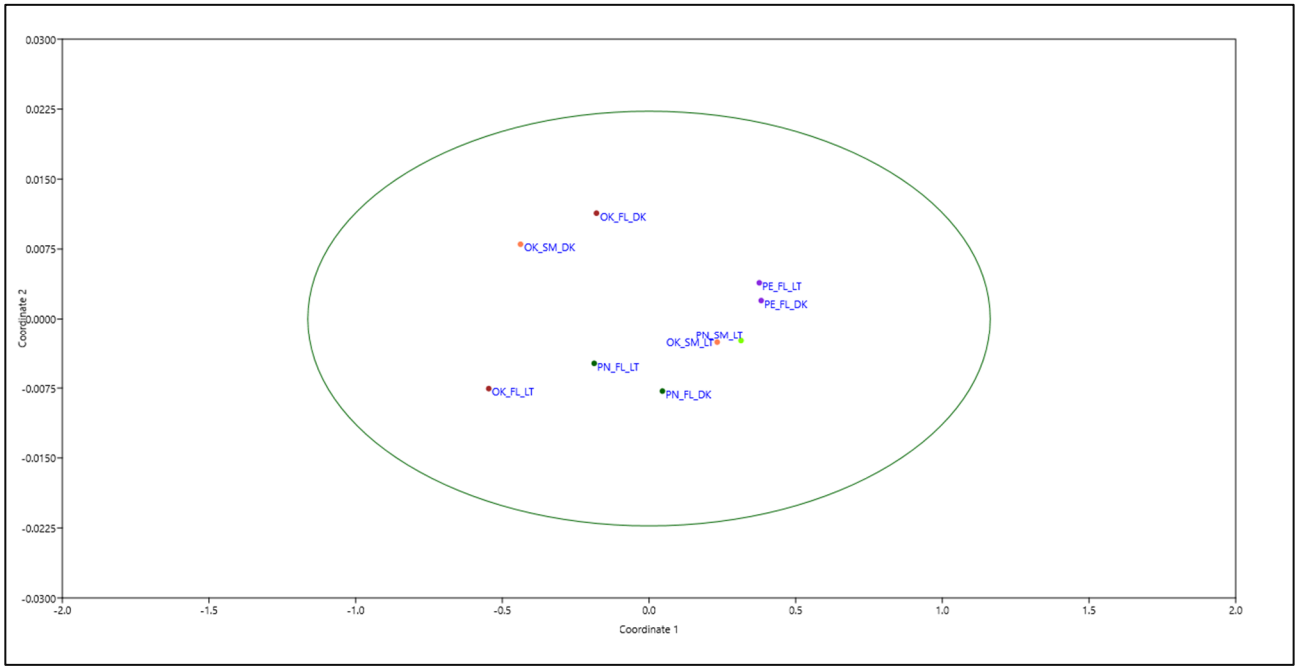


Figure 6 - Non-metric Multi-dimensional Scaling graph of PM1 mass values measured during the Aging process period

9.1.3 - Aged/secondary compounds (Period 3)

Figure 7 reports the box-plot graph of PM1 mass values measured during the PERIOD 3's 4-hours sampling, referred to as AGED and SECONDARY COMPOUNDS of aerosol for all the fuel species and stove typology. In that PERIOD 3, the chamber was closed, i.e. the conditions were the same for all the tests (whether the figures report in the name of test the acronym LT or DK) since the chamber was closed, with no additions.

As for PERIOD 1 and 2, all the FLAMING tests present values not always comparable between the same traditional fuel typology (Pine and Oak logs respectively), but highly comparable in the case of the two Pellet tests.

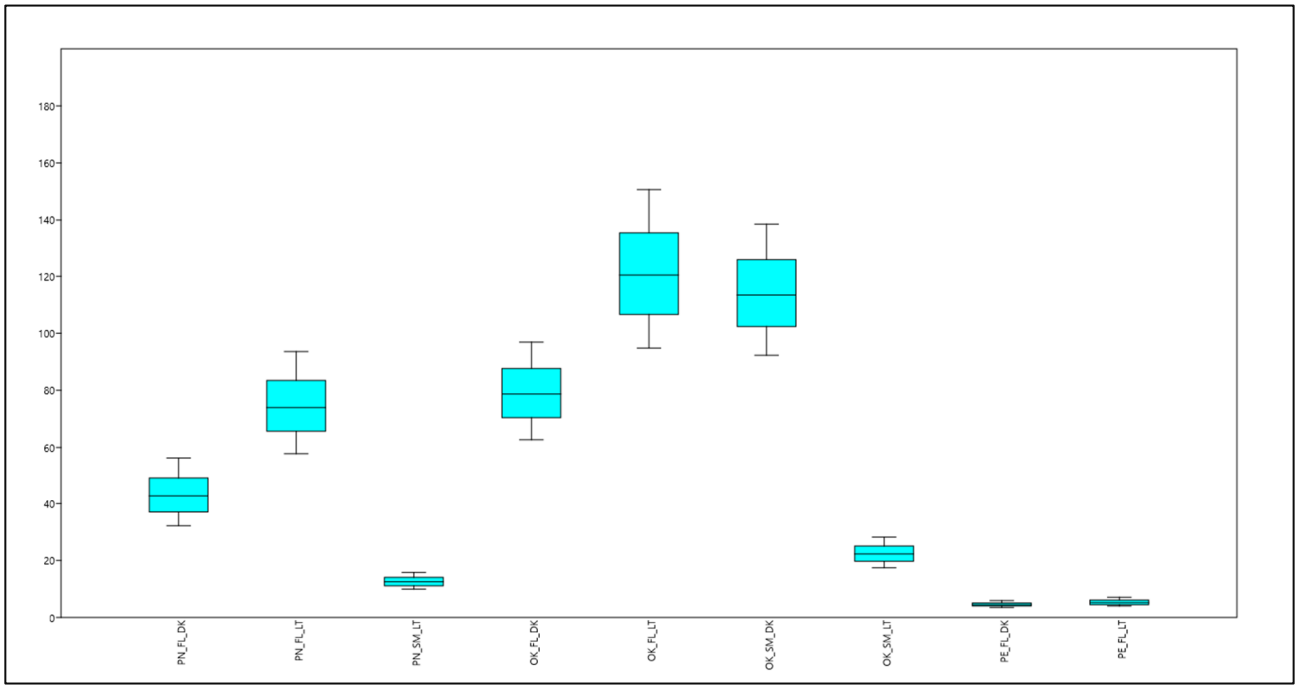


Figure 7 - Box-plot graph of PM1 mass values measured during the Aged/secondary compounds period. Concentrations in $\mu g/m^3$.

The modeling by classical Hierarchical clustering (Figure 8) pairs the two Pellet tests as very close, within a macro-cluster including also the two "valid" SMOLDERING tests (Pine LT and Oak LT). Differences between the two Pine FL tests and the two Oak FL tests are highlighted by their reciprocal distance and segregation.

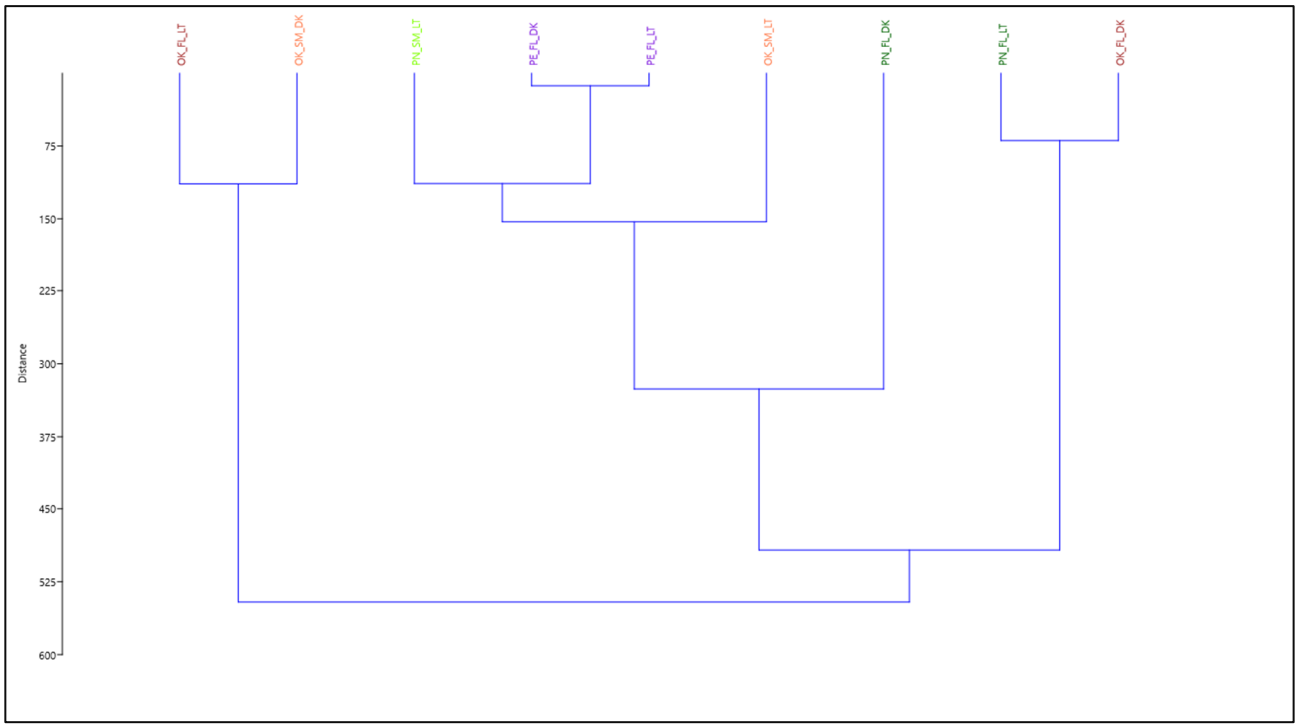


Figure 8 - Hierarchical clustering graph of PM1 mass values measured during the Aged/secondary compounds period

The spatial relationship of non-metric Multi-dimensional Scaling (Figure 9) confirms the close isolated similarity of two Pellet tests, placed near the two "valid" SMOLDERING tests (Pine LT and Oak LT). Then, the two Pine FLAMING and the two Oak FLAMING phases are dispersed, demonstrating most likely the greater variability during and after the chemical transformation phase of primary compounds.

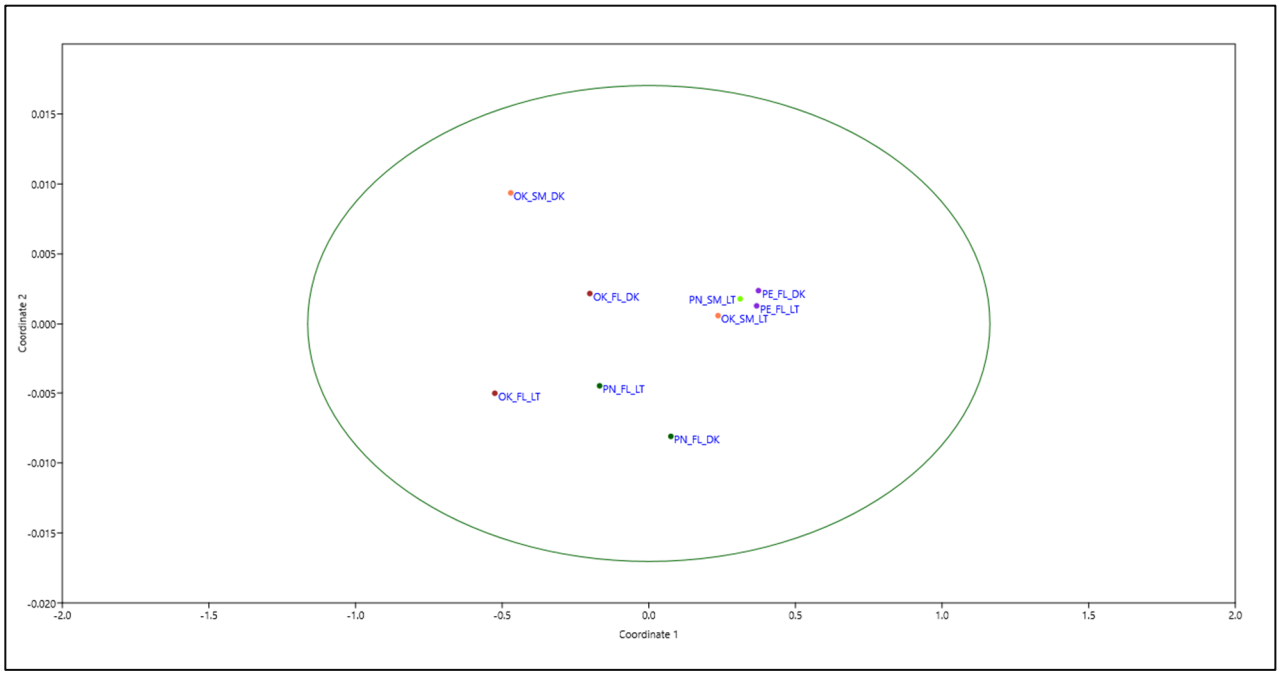


Figure 9 - Non-metric Multi-dimensional Scaling graph of PM1 mass values measured during the Aged/secondary compounds period

9.2 - Particles Numbers Concentration

The evolution in terms of Particles Number, per dimensional class, observed during the three periods (as in Table 2) aggregated is summarized in Figures 10, 12, 14, 16 (where vertical black lines isolate the different periods). Then, Figures 11, 13, 15, 17 display the particle number distribution in the three separated periods.

Regarding the FLAMING phases, Pellet (Figure 10 and 11) shows a considerably small number of particles in comparison to Pine and Oak, and again a more stable behaviour comparing the two homologous tests.

The most represented particles classes for Pellet fuel are that from 0.25 to 0.35 μm: these decrease in number more rapidly than the larger ones in the primary emission phase; then an increase is observed during the aging phase. Finally, a slower decrease is observed. In general, by a preliminary consideration valid also for the other tests, the 2nd period is involved in particle formation and the 3rd period shows less decrease than the 1st one since chemical evolutions lead to aged particle formation.

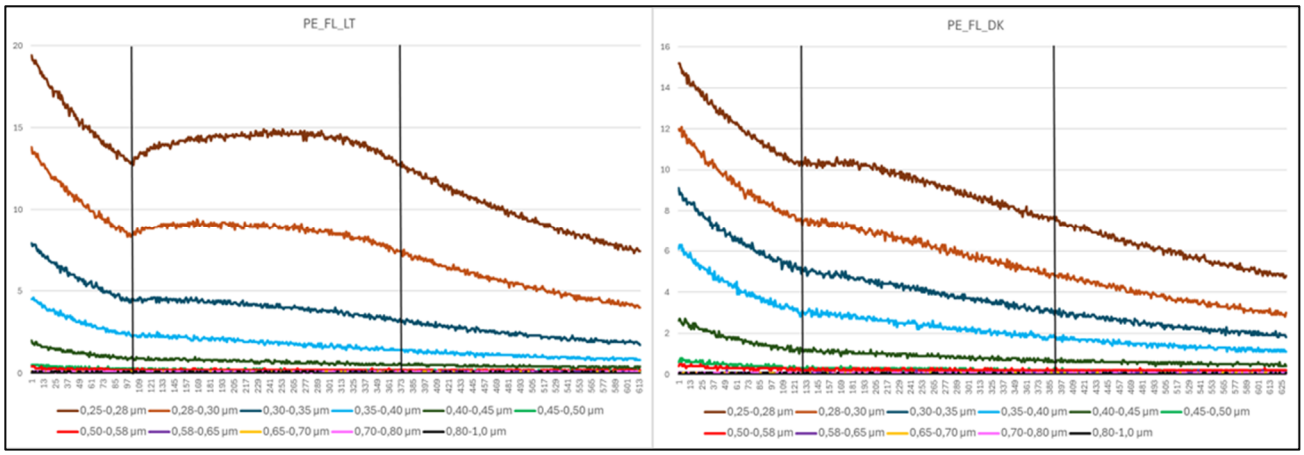
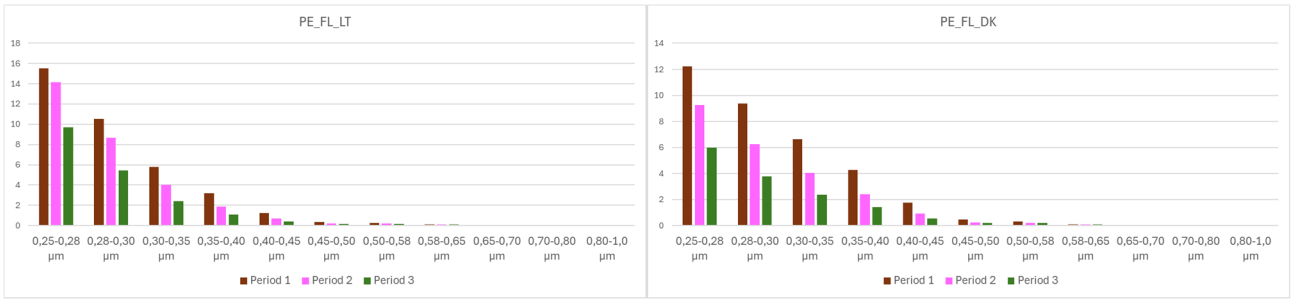


Figure 10 – Particle number trend for the entire Pellet Flaming Day-time (LT) and Night-time (DK) tests



Figures 11 - Particle number distribution in the three separated periods for Pellet Flaming Day-time (LT) and Night-time (DK) tests

Both the traditional fuels, Pine (Figure 12 and 13) and Oak (Figure 14 and 15) show more variability between their relative couple of tests. Anyhow the most represented particles classes are that from 0.28 to 0.40 µm, followed by 0.25-0.28 µm. That from 0.28 to 0.40 µm decrease more rapidly than the 0.25-0.28 µm in the primary emission phase; then a little increase is observed during the just beginning of aging phase, followed by a slow decrease.

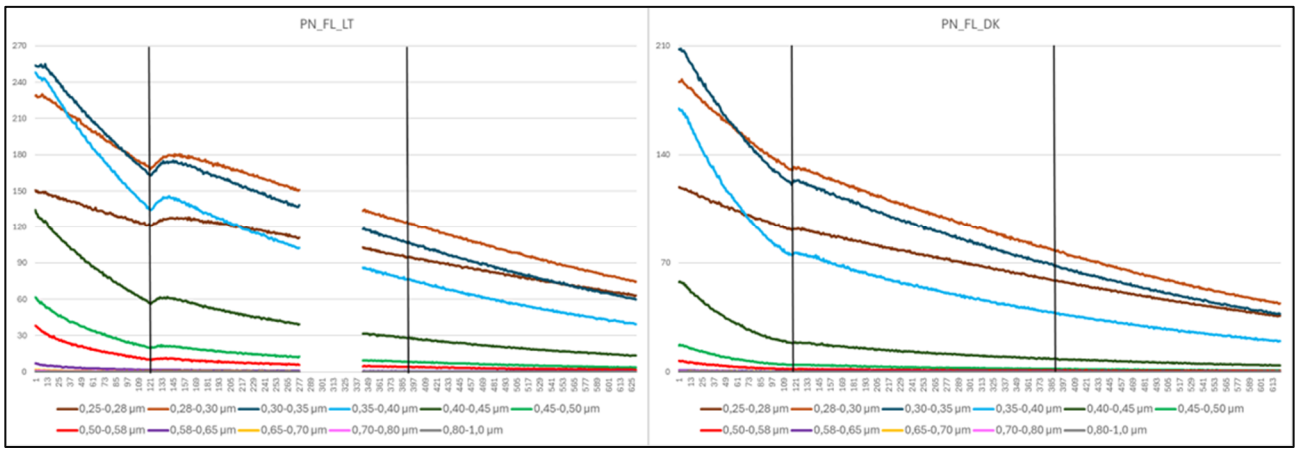
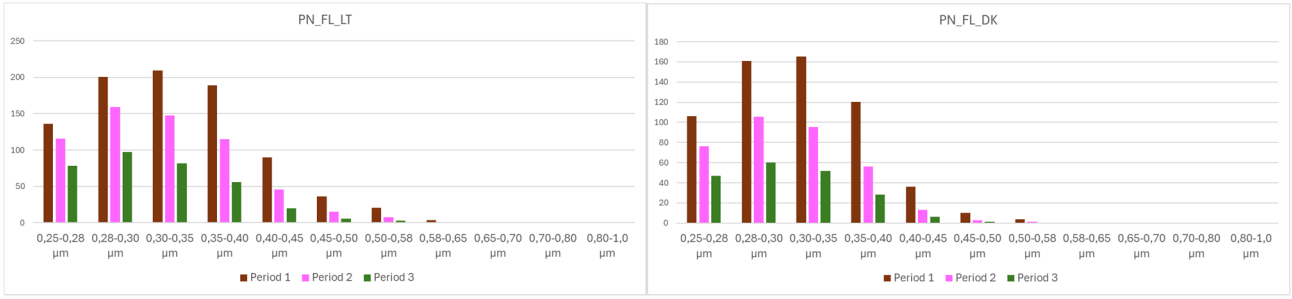


Figure 12 – Particles number trend for the entire Pine Flaming Day-time (LT) and Night-time (DK) tests



Figures 13 - Particle number distribution in the three separated periods for Pine Flaming Day-time (LT) and Night-time (DK) tests

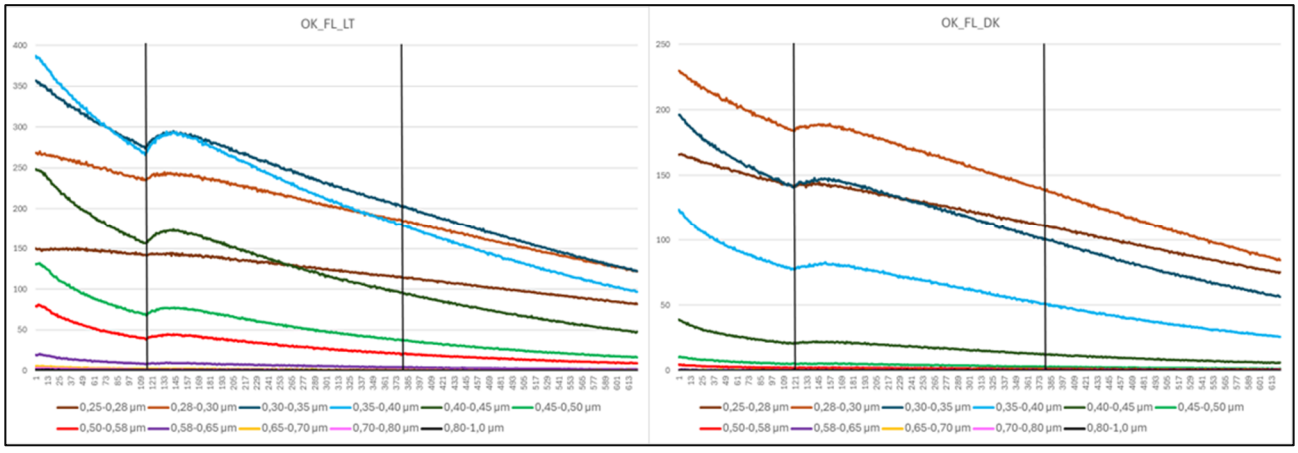
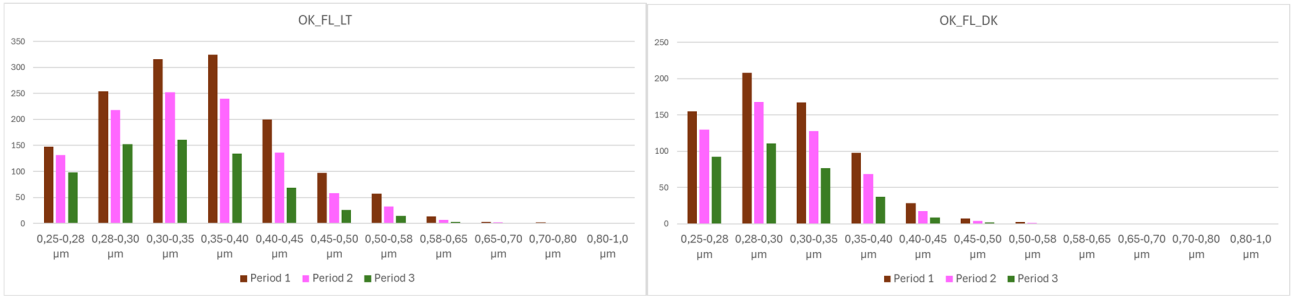


Figure 14 – Particles number trend for the entire Oak Flaming Day-time (LT) and Night-time (DK) tests



Figures 15 - Particle number distribution in the three separated periods for Oak Flaming Day-time (LT) and Night-time (DK) tests

As expected, the particle number concentration for aerodynamic diameters (d_{ae}) < 0.4 µm, in Pine and Oak logs fuel combustion, revealed higher values during the Day-time tests compared to Night-time tests. Higher values could be probably related to secondary particles formation in Nucleation Mode (d_{ae} 0.003 ÷ 0.02 µm) and Aitken mode (d_{ae} 0.02 ÷ 0.1 µm) particles and their later growth in the Accumulation Mode (d_{ae} 0.1 ÷ 0.41 µm), due to following coagulation and condensation processes of Ultrafine Particles (UFPs d_{ae} < 0.1 µm).

The difference in Particle Number concentration between pellet and woody logs combustion could be explained by higher combustion efficiency for pellet fuel regard to woody logs fuels.

As already underlined, the SMOLDERING phase (Figure 16 and 17), where we have available the particles number data just of one Pine test and one Oak test, presents some criticality which does not allow a correct interpretation here.

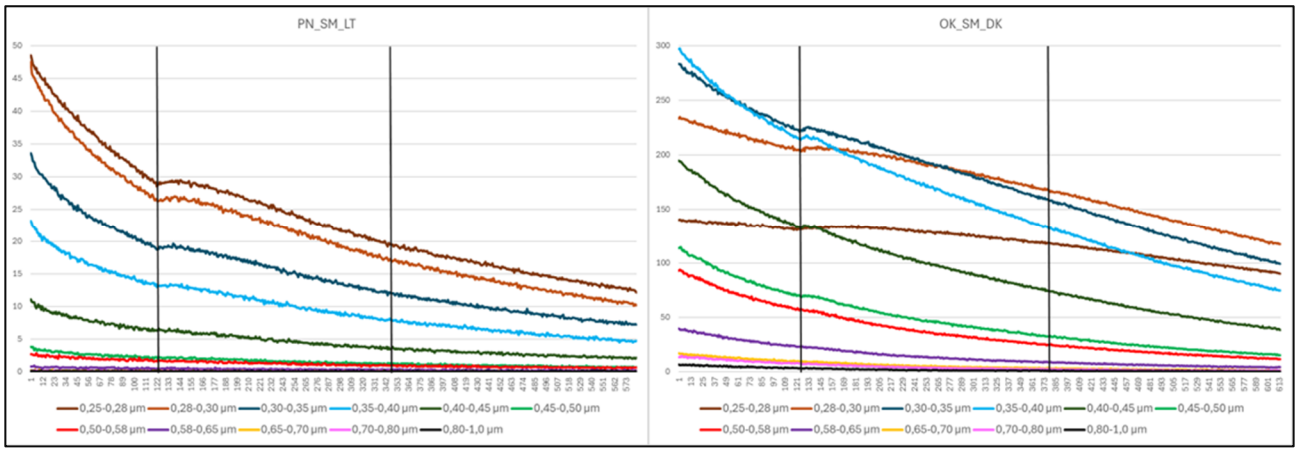
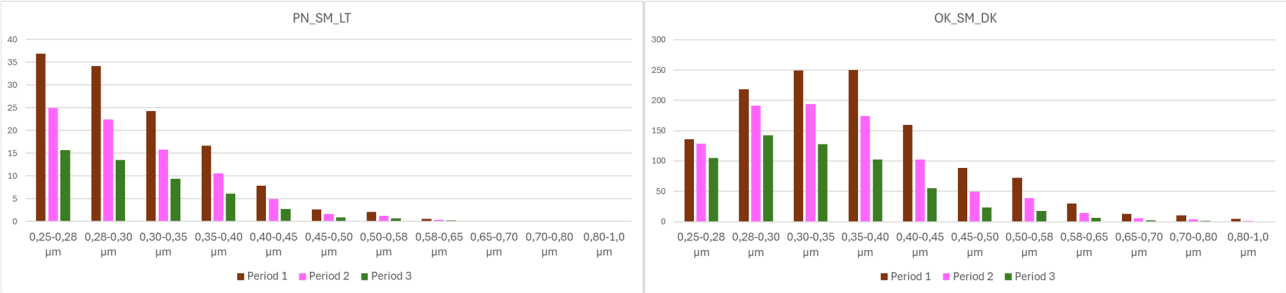


Figure 16 – Particles number trend for the entire Pine Smoldering Day-time (LT) and Oak Smoldering Night-time (DK) tests



Figures 17 - Particle number distribution in the three separated periods for Pine Smoldering Day-time (LT) and Oak Smoldering Night-time (DK) tests

9.3 - Preliminary results on toxicological outcomes.

Cell culture used for the direct exposure of A549 cells to BB combustion fumes were prepared in the Cell Biology laboratory of the University of Valencia. The A549 cells are representative of the alveolar space, and they are largely used for toxicological analyses of airborne pollutants.

Cells were maintained in DMEM plus 10% fetal bovine serum and 1% antibiotics. The medium was changed every three days. For direct exposure experiments the cells were seeded on Teflon transwell insert with 0.4 µm pores (Corning, NY, USA). 24 hours before the exposure the medium above the cells was removed from 8 inserts to allow adaptation of the cells to the air-liquid interface condition. Six inserts were placed into the exposure module Cultex RFS compact under a sterile bench. Two inserts were kept in the laboratory incubator as reference controls (i.e. cells not exposed to the flux of air operating in the exposure module). The exposure module was then transferred from the laboratory to the CEAM Center.

Direct exposure to BB combustion fumes was performed during the following conditions:

Exposure 1, E1– Pellet Light

Exposure 2, E2 – Oak Light

Exposure 3, E3 – Oak Dark

Exposure 4, E4 – Pellet Dark

Each exposure lasted for six hours; at the end of the exposure time the module was transferred back to the biology cell lab to collect the biological samples.

The following samples were recovered:

medium under the cells for pro-inflammatory cytokines measurement (IL-6 and IL-8) and for cell viability assessment (LDH release).

Cell lysate for gene expression measurement by RT-qPCR of CYP1b1, AhR, NQO1, NFkB and IL-8.

The results obtained were analyzed comparing the outcomes between control and exposed cells with the exposure module and the cells maintained in the CO_2 incubator. Unfortunately,

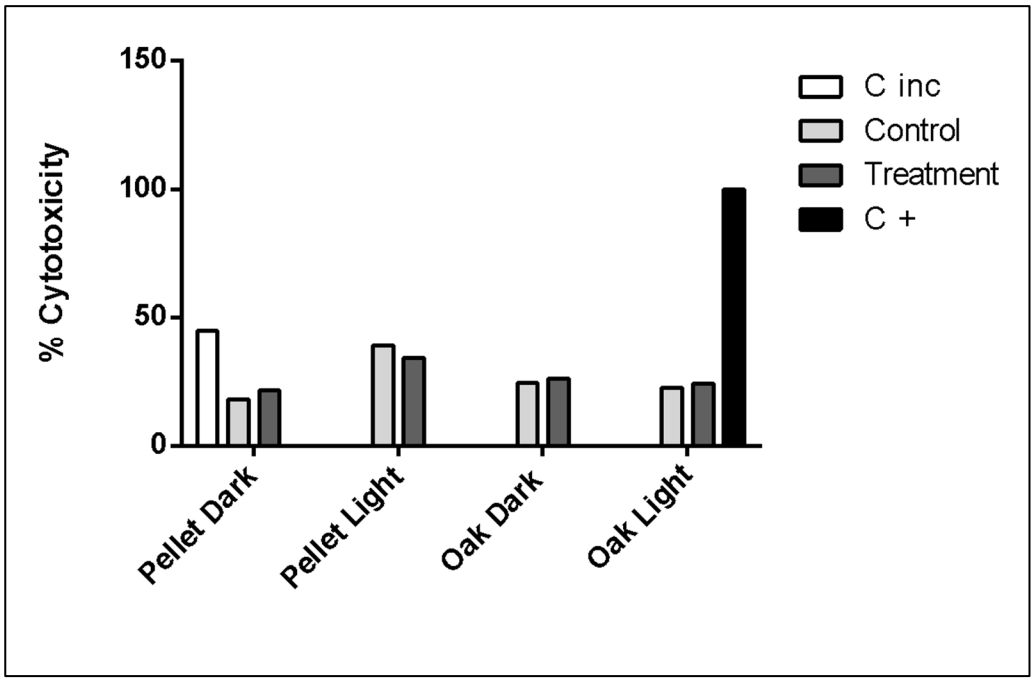
the results of the cells in the CO_2 incubator, which should be representative of the optimal cellular conditions, showed an unexpected increase cellular death as measured by LDH (Figure 18).

This result is confirmed by the slightly higher release of IL-8 (Figure 19) from the incubator control cell, suggesting a pre-existing cell inflammatory condition which undermines our possibility to clearly analyze the exposure outcomes.

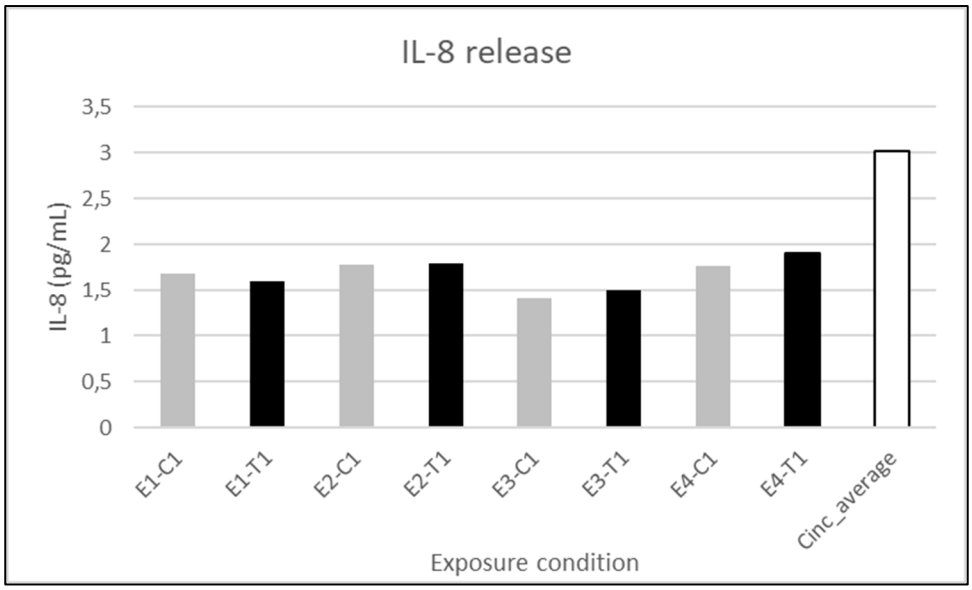
As an example, we report here the expression of the gene CYP1B1 measured in ALI control and exposed cells (Figure 20) normalized versus the expression of the same gene in the cell maintained at the ALI in the CO_2 incubator. The results suggest a significant reduction of the gene expression in both control and exposed cells. This increased suppression of the gene is likely related to a pre-existing over-expression in the incubator cells.

Normalizing CYP1B1 gene expression of the exposed cells versus the relative ALI control cells, a not significant modification is observed (Figure 21). The variation among the different exposure condition is low, undermining the possibility to define specific relation between the exposures and the biological outcomes.

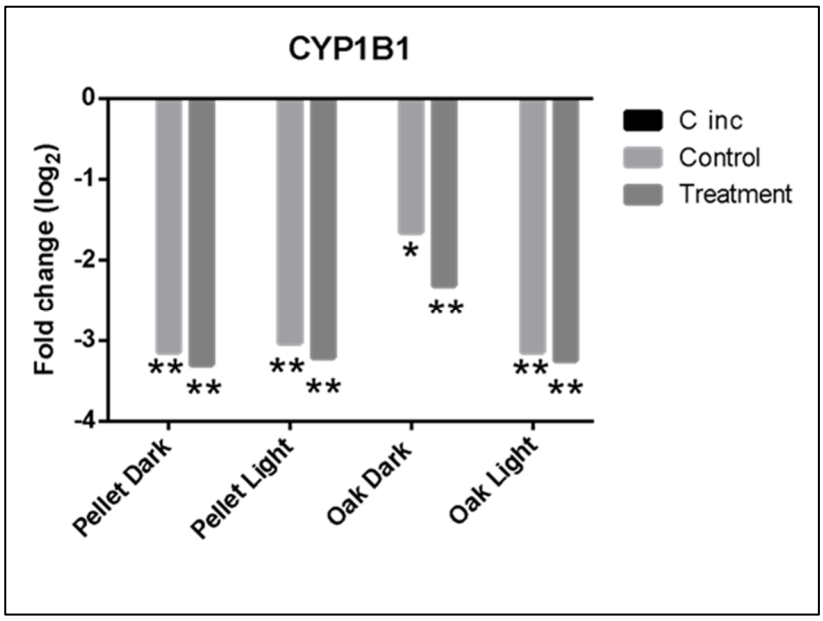
Similar outcomes are obtained for the other genes analyzed and are not reported nor discussed in detail.



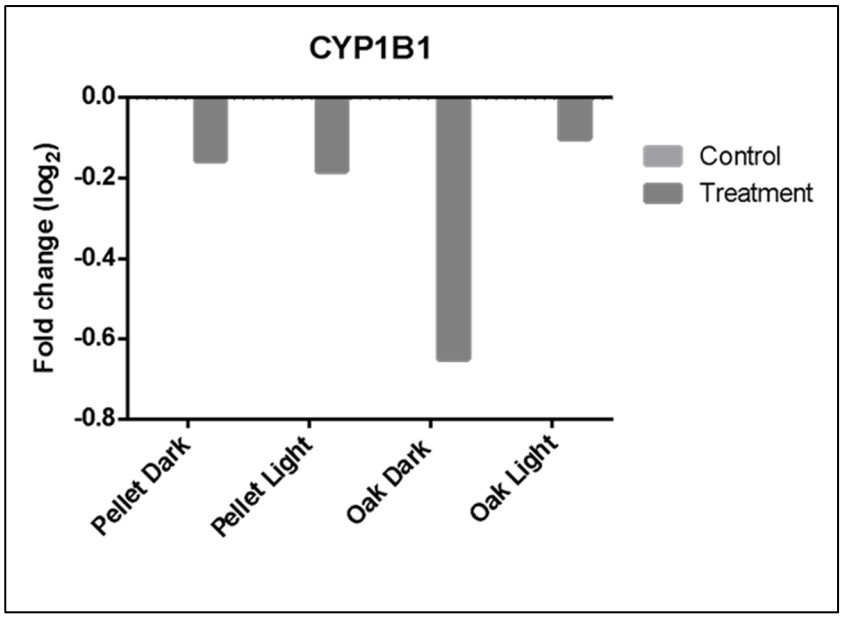
Figures 18- Cytoxicity in exposed and control cells under the different conditions and of the positive control (C+, cellular lysate with lysing solution 100% cell death) and of the incubator control (C inc).



Figures 19 - Inflammatory responses, measured by IL-8 release, in exposed and control cells under the different conditions and of the incubator control cells (Cinc). Higher release of IL-8 is related to the incubator control cells, possibly in relation to the higher cytotoxicity measured by LDH release.



Figures 20 - CYP1B1 gene expression measured by RT-qPCR in exposed and ALI control cells normalized versus the incubator control cells. A significant reduction of the gene expression is reported (one way ANOVA, *$p < 0.05$, **$p < 0.01$). Gene expression of the incubator control cells is normalized and corresponds to the x-axis (value equal to zero)



Figures 21 - CYP1B1 gene expression measured by RT-qPCR in exposed and normalized versus the ALI control cells. Lack of significant modulation of the gene expression is reported. Gene expression of control cells is normalized and corresponds to the x-axis (value equal to zero)