21-25 febbraio 2018 wood energy days
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# Emission factors and UFP toxicity of firewood and pellets stoves from a real combustion cycle


**Senem OZGEN**
**senem.ozgen@polimi.it**

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LEAP
Laboratorio Energia e Ambiente Piacenza

LABORATORIO DI PROVA CALDAIE

# Frame and objectives of the project

## TOBICUP project

## (**TO**xicity of **BI**omass **C**ombustion generated **U**ltrafine **P**articles)

- deeper insight on possible negative health effects of ultrafine particle (UFP, Dp<100 nm) emissions from residential biomass combustion (RBC)
- evaluation of toxicological responses of UFP both from source samples and ambient samples impacted by RBC

### Objective 1

- **Physical-chemical** characterization of UFP (determination of water soluble ion content, carbonaceous compounds, elementary composition, polycyclic aromatic hydrocarbons (PAH), anhydrosugars).

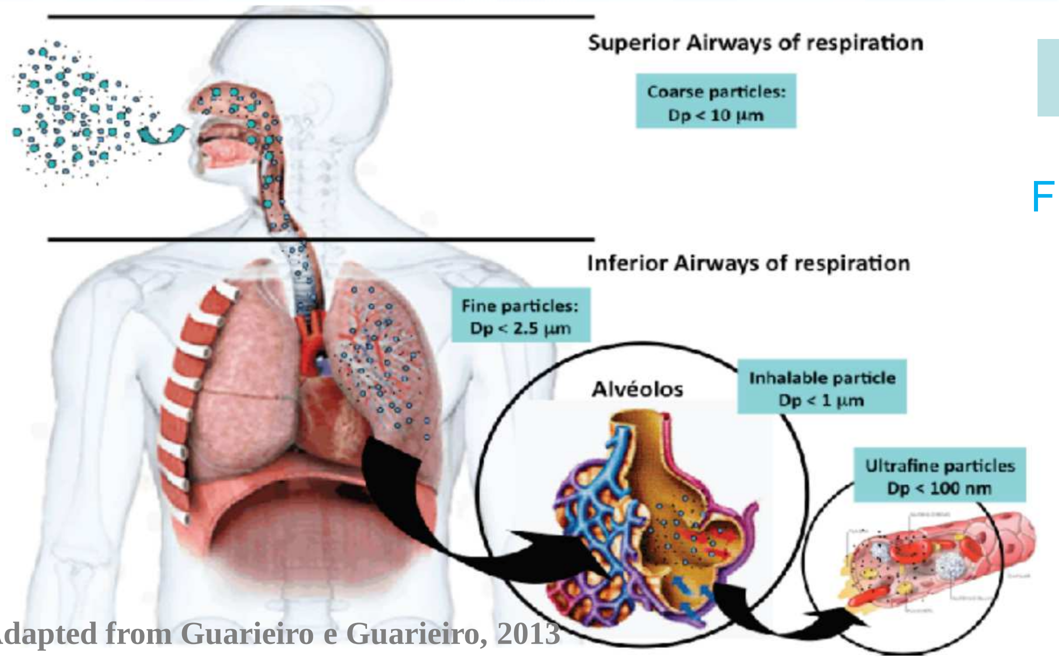
- Importance of objective 1:
  - PAH → potentially carcinogenic
  - levoglucosan and potassium → tracers for biomass combustion

### Objective 2

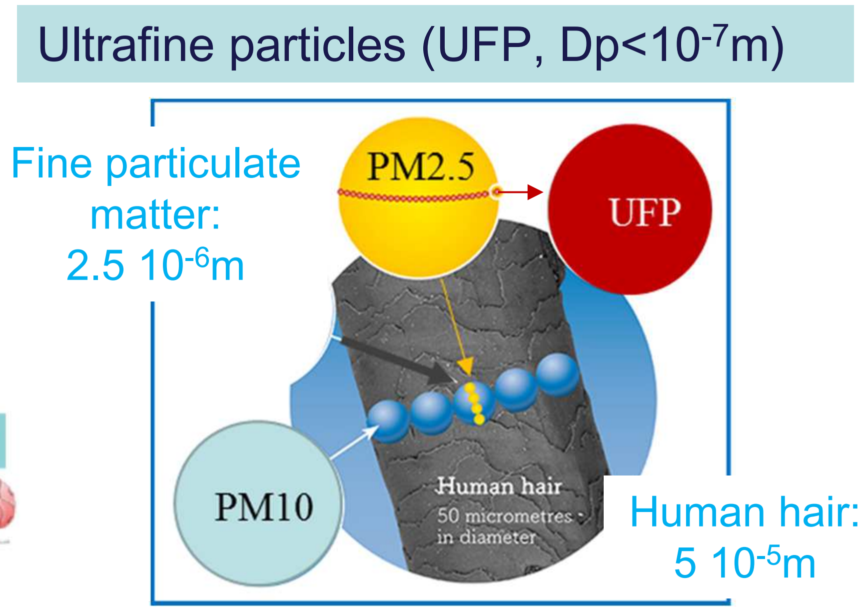
- Characterization of **biological activity** in the cells of the respiratory track (test in vitro on alveolar pulmonary cells) treated with UFP.

- Importance of objective 2:
  - Genotoxicity → predictive of situations which potentially can develop into a tumor
  - Oxidative stress → one of the causes of DNA damage
  - Inflammation → a local response to cellular injury, that serves as a mechanism initiating the elimination of noxious agents

# Why investigating ultrafine particles (UFP)?




Adapted from Guarieiro e Guarieiro, 2013

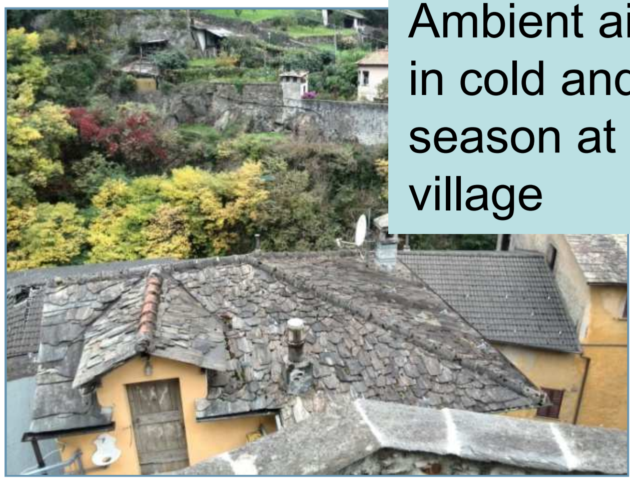



- UFP not a criteria pollutant, not routinely monitored at most air pollution monitoring stations, thus not regulated
- UFP are short-lived in the environment (e.g., some quickly accumulate into larger particles, others can evaporate)
- UFP <u>may be more toxic</u> than larger particulate matter for several reasons:

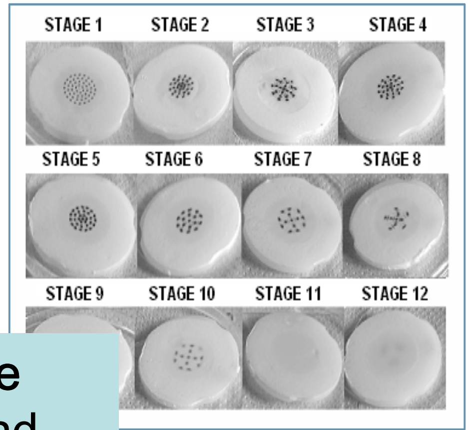
1) their small diameter enables UFPs to penetrate deep into the lungs more easily than larger particles;
2) UFPs are cleared less efficiently from the respiratory tract than larger particles and thus have more opportunity to translocate from the lung into the bloodstream and into other organ systems;
3) UFPs have a greater surface area to mass ratio compared to larger particles, providing a larger area to adsorb potentially toxic chemicals or metals and interface with pulmonary surfaces.

# Project activities

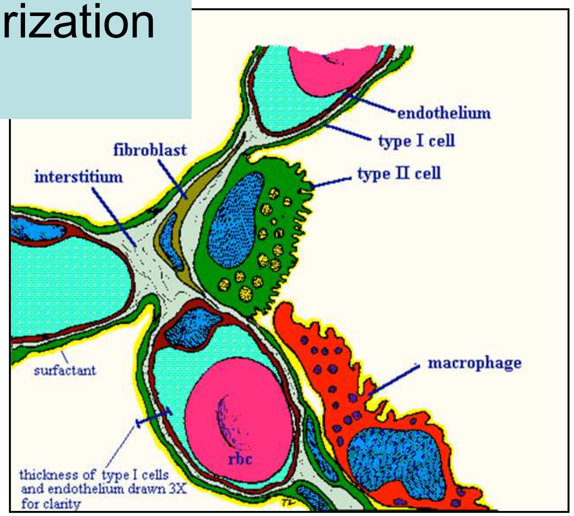
**Phase 1**
Emission testing. Tests on small scale domestic woody biomass automatic and manually fed appliances (i.e., pellet stove and wood stove



**Phase 2**
Ambient air sampling in cold and warm season at an Alpine village




**Outcome**
Chemical and toxicological characterization of UFP



# UFP sampling and characterization

*Corsini et al. (2017). Toxicology Letters 266: 74-84
**Corsini et al. (2017). Sci. Total Environ. 587-588: 223-231
#Marabini et al. (2017). Mutat Res Gen Tox En 820: 39-46

UFPs were collected by Multistage Cascade Impactors:
1 SDI (Small Deposit Impactor by Dekati)
2 MOUDIs (Micro-Orifice Uniform-Deposit Impactor by MSP Corp.)

Only the stages collecting particles with aerodynamic diameter $d_{ae} < 100$ nm were weighed and analysed (i.e. 2 stages + back-up filter for each impactor)

Aerosol particles were collected on different collection substrates (quartz-fiber filters, aluminum supports, polycarbonate and PTFE membranes) according to chemical analyses and toxicological tests.

Routine checks on filter blanks were carried out to avoid biases due to possible filter contamination.

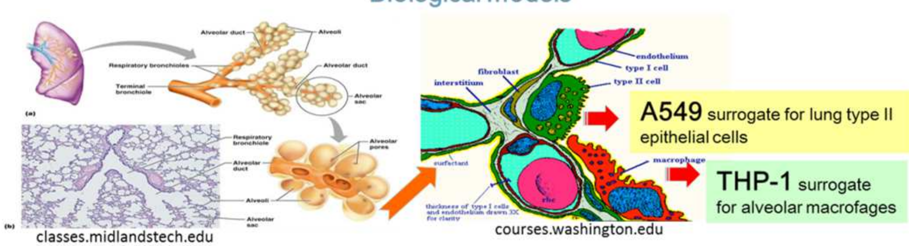
## Chemical characterization

| | |
|---|---|
| TOT on quartz fiber filters | total carbon (TC) |
| IC on quartz fiber filters | water soluble anions ($Cl^-$, $NO_2^-$, $NO_3^-$, $SO_4^{2-}$, formate, acetate, propionate, oxalate) and cations ($K^+$, $Ca^{2+}$, $Na^+$, $NH^{4+}$, $Mg^{2+}$) |
| HPAEC–PAD on quartz fiber filters | anhydrosugars (levoglucosan, mannosan and galactosan) |
| ICPAES on polycarbonate substrates | elements (Al ,As, Ba, Cd, Co, Cr, Cu, Fe, Mn, Mo, Ni, P, Pb, Sr, Ti, V, Zn) |
| GC-MS on quartz filters | PAHs (phenantherene, anthracene, fluoranthene, pyrene, benzo(a)anthracene, chrysene, benzo(b)fluoranthene, and benzo(a)pyrene) |

- TOT: Thermal optical transmittance
- IC: Ion chromatography (IC)
- HPAEC-PAD: high performance anion-exchange chromatography coupled with pulsed amperometric detection
- ICPAES (Varian 720-ES) methodology according to EN 14902:2005 ‘Standard method for measurement of Pb/Cd/As/Ni in the PM10 fraction of suspended particulate matter
- GC-MS: Gas chromatography – mass spectrometry

## Toxicologic characterization

Biological models




- Cytotoxicity[#]: MTT assay; Cell viability[*,**]: lactate dehydrogenase (LDH) leakage after 24-h;
- Cellular uptake[*,**,#]: by flow cytometer (FACScan analysis);
- ROS and RNS species production[#]
- Pro-inflammatory effects[*]: Interleukin-8 (IL-8) release
- Genotoxicity[**]: Comet assay (to evaluate single and double strand breaks), ɣ-H2AX test (biomarker of cellular response to double strand breaks)
- Other tests performed (results not shown in full in this talk):

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UNIVERSITÀ DEGLI STUDI DI MILANO
DIPARTIMENTO DI FISICA

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## Chemical characterization

## Toxicologic characterization

TOT on quar
fiber filters

IC on quartz
fiber filters

HPAEC–PAD
quartz fiber fil

ICPAES
polycarbo
substra

GC-MS on qu
filters

- Toxicological tests on two cell lines surrogate of human lung epithelial cells (they serve to enclose and protect the lungs, produce secretions) and alveolar macrofages (they destruct foreign material)
- Tests usually measure the release of biomarkers when the cells are exposed to UFP
- Concentrations used can be considered relevant for human exposure (the use of deposition models suggested that in vitro concentrations of 100ug/l may be representative of particles deposited in human lungs after 24h inhalation of ambient air ceoncentrations of 100-150 ug/m³)

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- GC-MS: Gas chromatography – mass spectrometry

• Other tests performed (results not shown in full in this talk):

# Phase 1 - Emission testing: investigated appliances

Tests performed on **small scale – commercially available - stoves** for residential heating, working under real-world operating conditions.
**Wood** types **largely used in the investigated alpine area** in Italy (**Fir** and **Beech**).

## Pellet stove

**Fir pellets** (soft-wood)

**Beech pellets** (hard-wood)

Nominal heat output: 11.1 kW
Nominal fuel consumption = 2.4 kg/h
Efficiency = 89.2%

## Wood stove

**Fir logs** (soft-wood)

**Beech logs** (hard-wood)

Nominal heat output: 8.2 kW
Nominal fuel consumption = 2 kg/h
Efficiency = 80.8%

Further details in Ozgen et al. (2017). Atmos Environ 150: 87-97

# Phase 1 - Emission testing - Real world combustion cycles



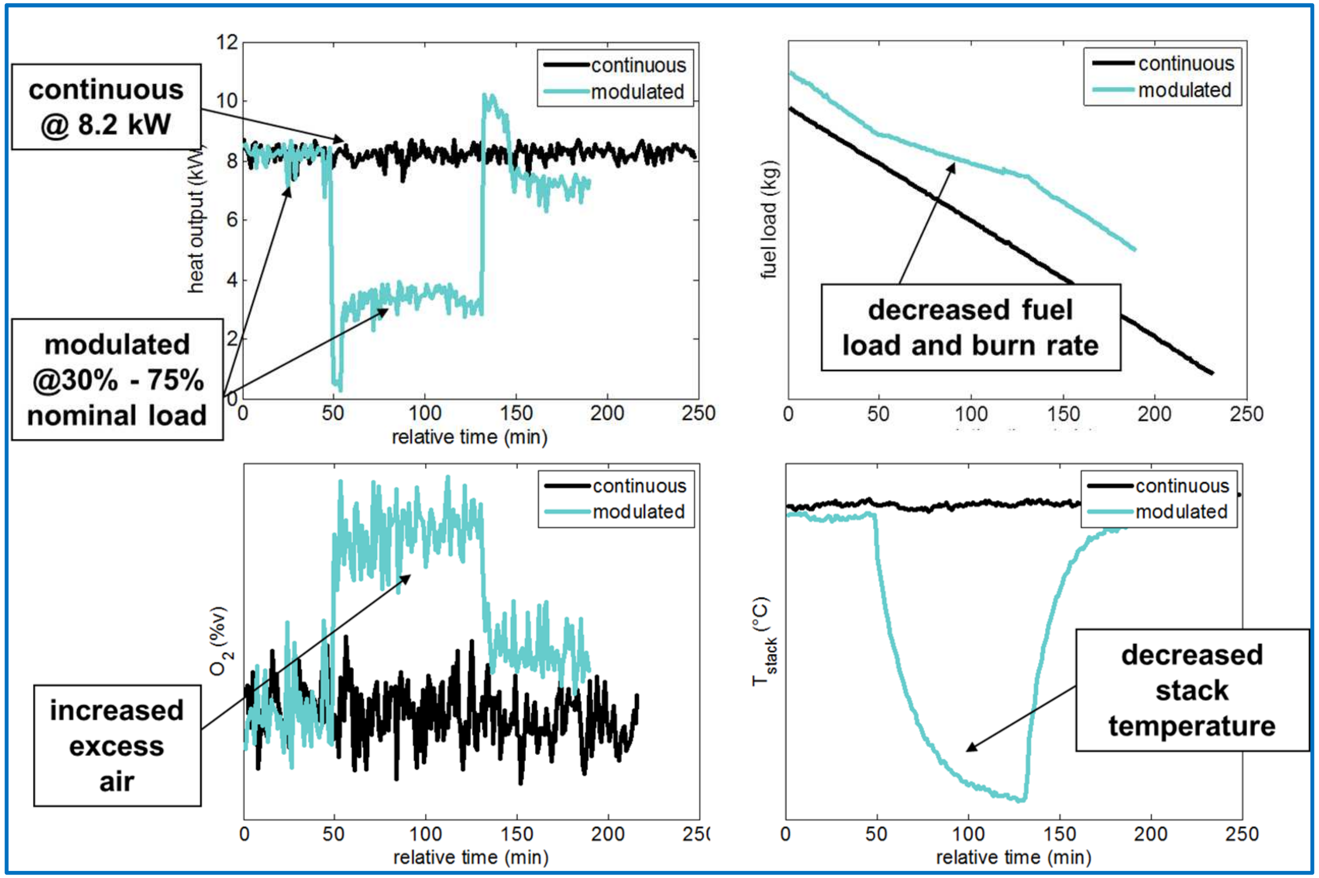


0 → Time

cold start | preheating | nominal load | overload and burn out (final weight 100-500 g)

- four to five consecutive batches comprising the cold start, eventual preheating period, two nominal loads and a final high load batch
- top ignition of the starting batch
- nominal load (about 2 kg/h, ~20 cm x ~11 cm x ~8 cm pieces)
- overload: final batch (about 1.3 times the nominal load) with slightly bigger logs.
- start-up and loading procedure followed the prescriptions of the manufacturer
- primary and secondary air supply manually controlled

# Phase 1 - Emission testing - Real world combustion cycles




modulated operation:

~ 55% of the runtime @75% nominal load

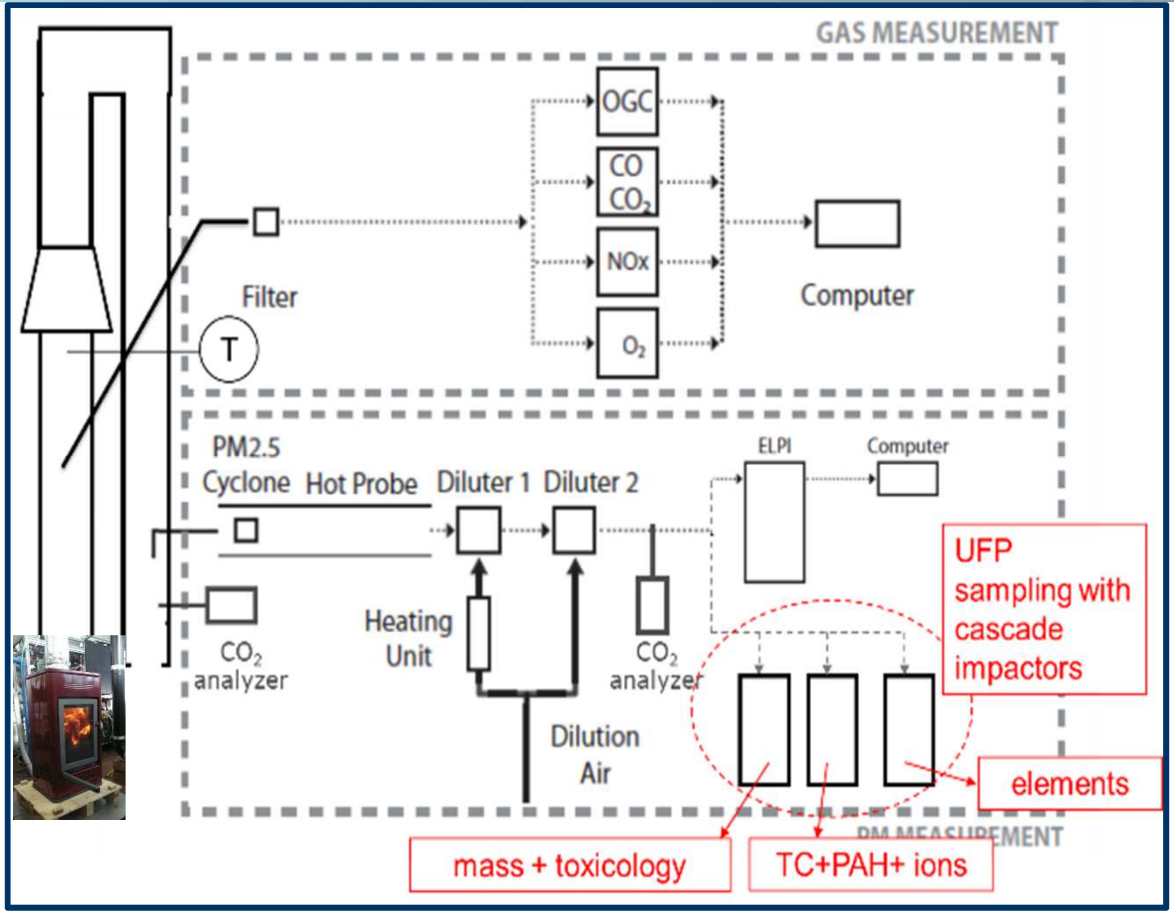
~ 45% of the runtime @minimum load (approx.30% nominal load)

(one test continuously @75%)

start up and shut down periods not included, only transitory for power modulation

- continuous operation at the nominal heat output much over the heating need hence the need to modulate the heat output
- sampling began when the stable operation conditions are reached

# Phase 1 - Emission testing – Experimental setup @ LEAP lab.




(test bench @ http://www.leap.polimi.it/leap/en/the-laboratory.html)

- combustion appliances set on a weigh-scale
- stove chimney under a laboratory extraction system (i.e., dilution tunnel with the hood)
- the tunnel allows the products of combustion to cool and mix with the indoor air upstream of the extractive sampling locations, allowing collection of samples in their ambient atmosphere form
- pellets stove experiments: flue gas extracted directly from the chimney

| Wood stove | Pellets stove |
|---|---|
| DR=400 -1000 | DR=90 -150 |
| Tsample=21-26°C | Tsample=28-32°C |

(DR: dilution ratio)

# Phase 2 - Ambient monitoring campaign - Measurement site


Site: Morbegno, Valtellina (alpine valley), Italy

- Elevation: 262 m
- Distance from Milan: ~100 km
- Population: ~12000 inhabitants

- Wood burning widely used for residential heating in winter
- Similar summer vs. winter source emissions apart from wood burning

## Winter campaign:

Courtyard of the town Council, in the city center (low traffic area)

- Period: 20 Jan 2015 – 27 Feb 2015
- Sampling integrated over three/four days

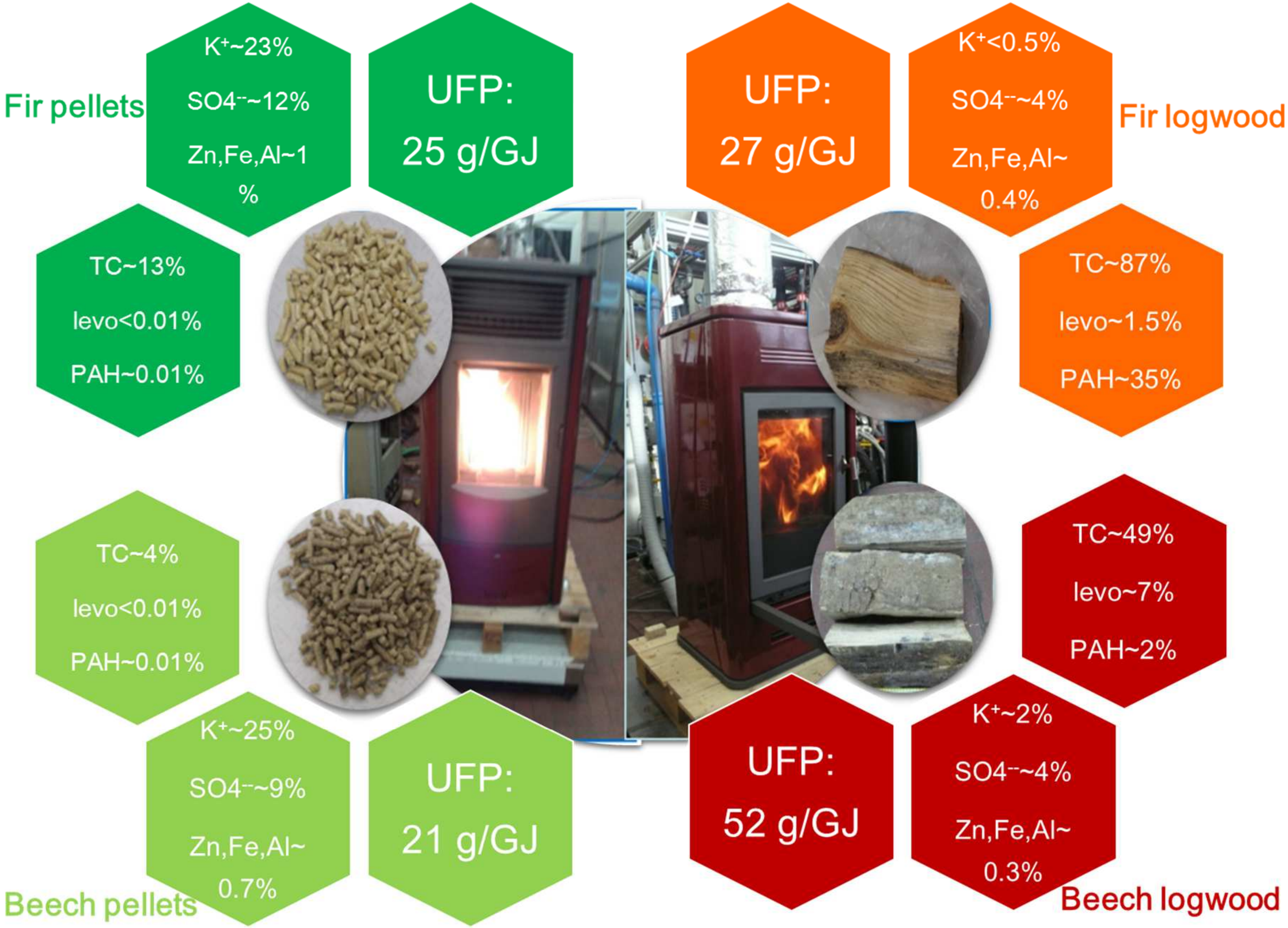
## Summer campaign:

Council open-air stock (low traffic area)

- Period: 8 Jun 2015 – 16 Jul 2015
- Sampling integrated over seven days

# Main findings – Phase 1: Emission testing

# UFP composition




Further details in Ozgen et al. (2017). Atmos Environ 150: 87-97

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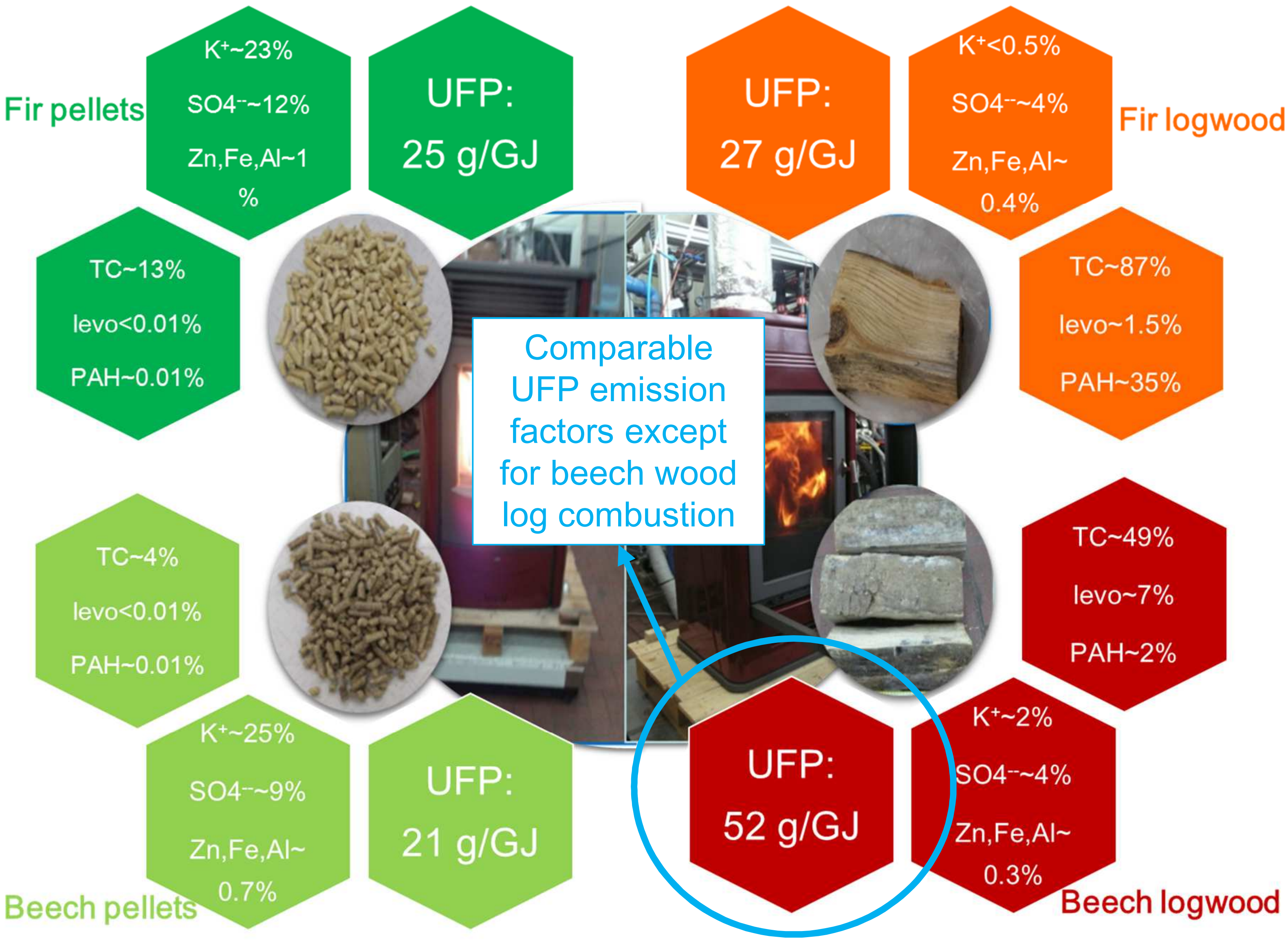


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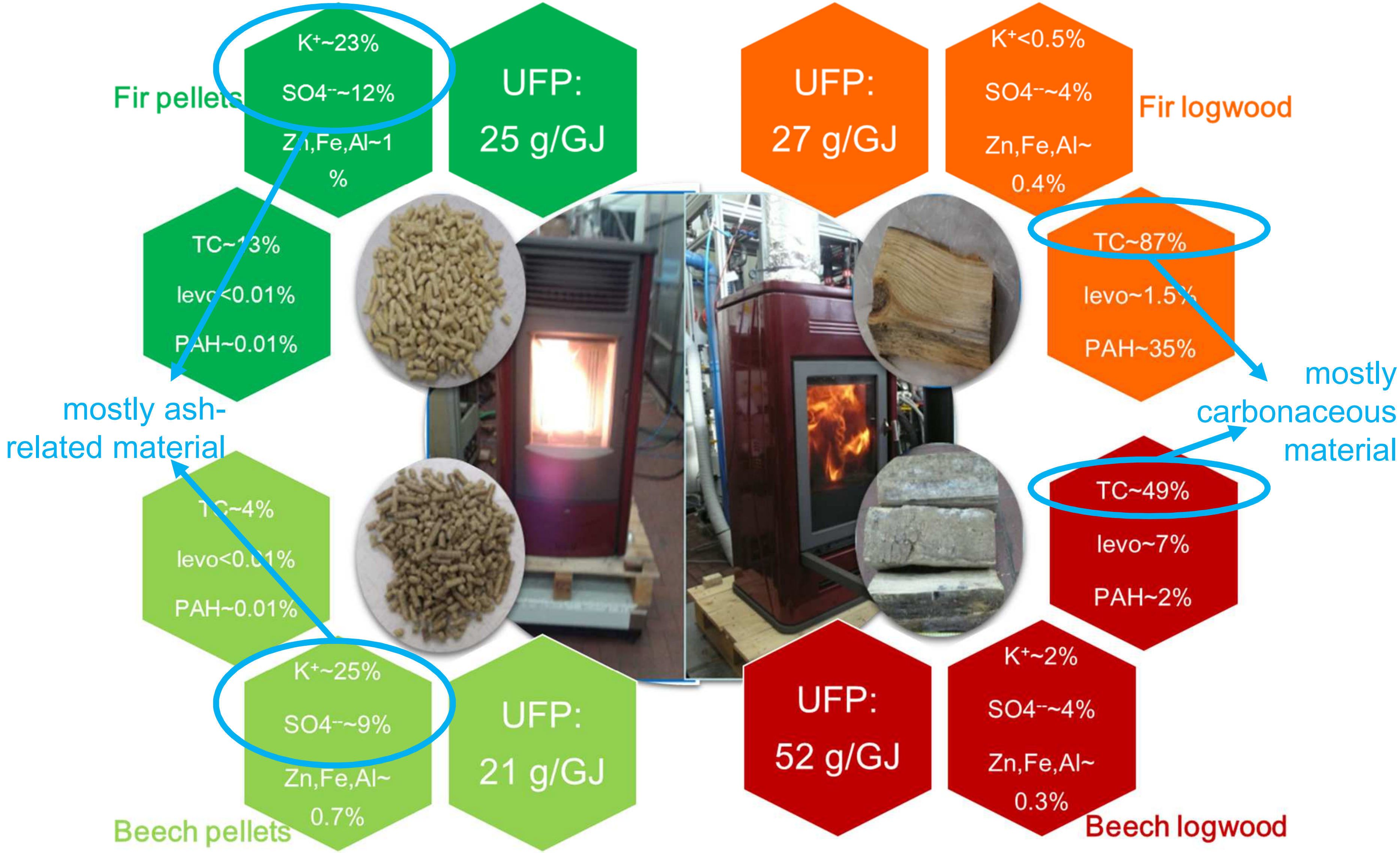


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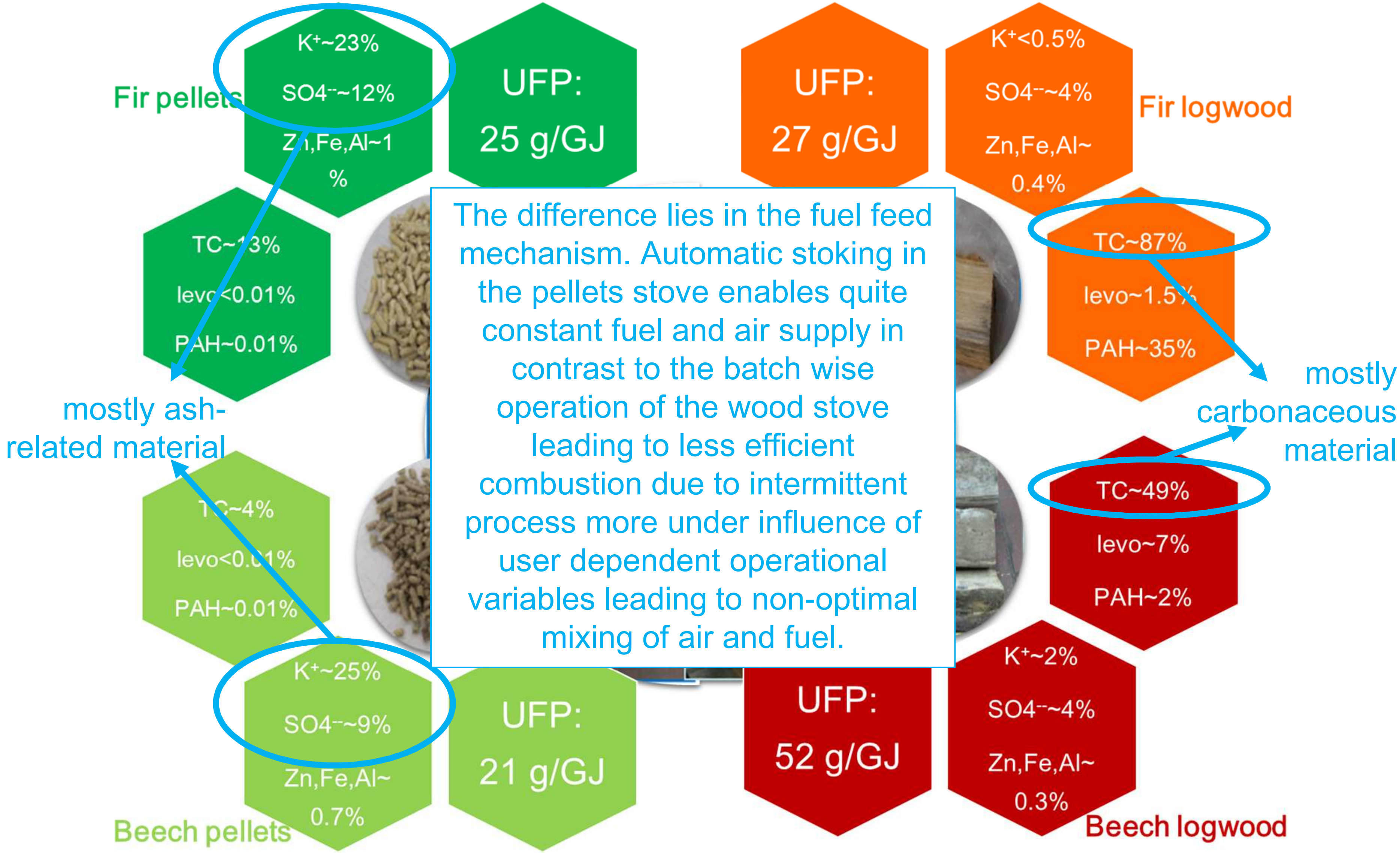
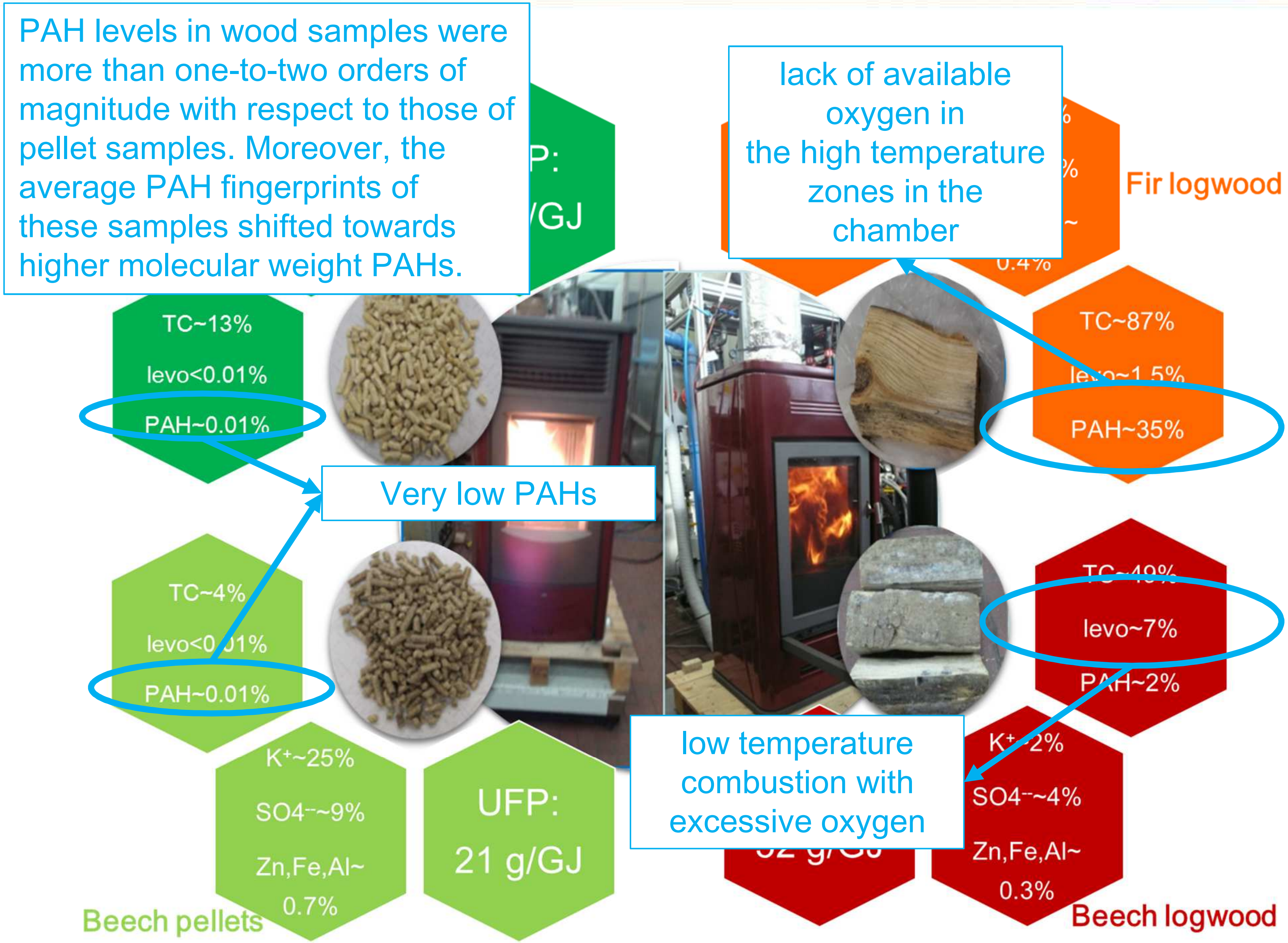



The difference lies in the fuel feed mechanism. Automatic stoking in the pellets stove enables quite constant fuel and air supply in contrast to the batch wise operation of the wood stove leading to less efficient combustion due to intermittent process more under influence of user dependent operational variables leading to non-optimal mixing of air and fuel.

Further details in Ozgen et al. (2017). Atmos Environ 150: 87-97

# UFP composition

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# UFP composition

**Fir pellets**

UFP: 25 g/GJ

- $K^+$~23%
- $SO4^{--}$~12%
- Zn,Fe,Al~1 %
- TC~13%
- levo<0.01%
- PAH~0.01%

**Fir logwood**

UFP: 27 g/GJ

- $K^+$<0.5%
- $SO4^{--}$~4%
- Zn,Fe,Al~ 0.4%
- TC~87%
- levo~1.5%
- PAH~35%

Almost negligible levoglucosan! Degradation process responsible for levoglucosan formation occurs at low temperatures not observed under normal operation of automatic appliances.

**Beech pellets**

UFP: 21 g/GJ

- TC~4%
- levo<0.01%
- PAH~0.01%
- $K^+$~25%
- $SO4^{--}$~9%
- Zn,Fe,Al~ 0.7%

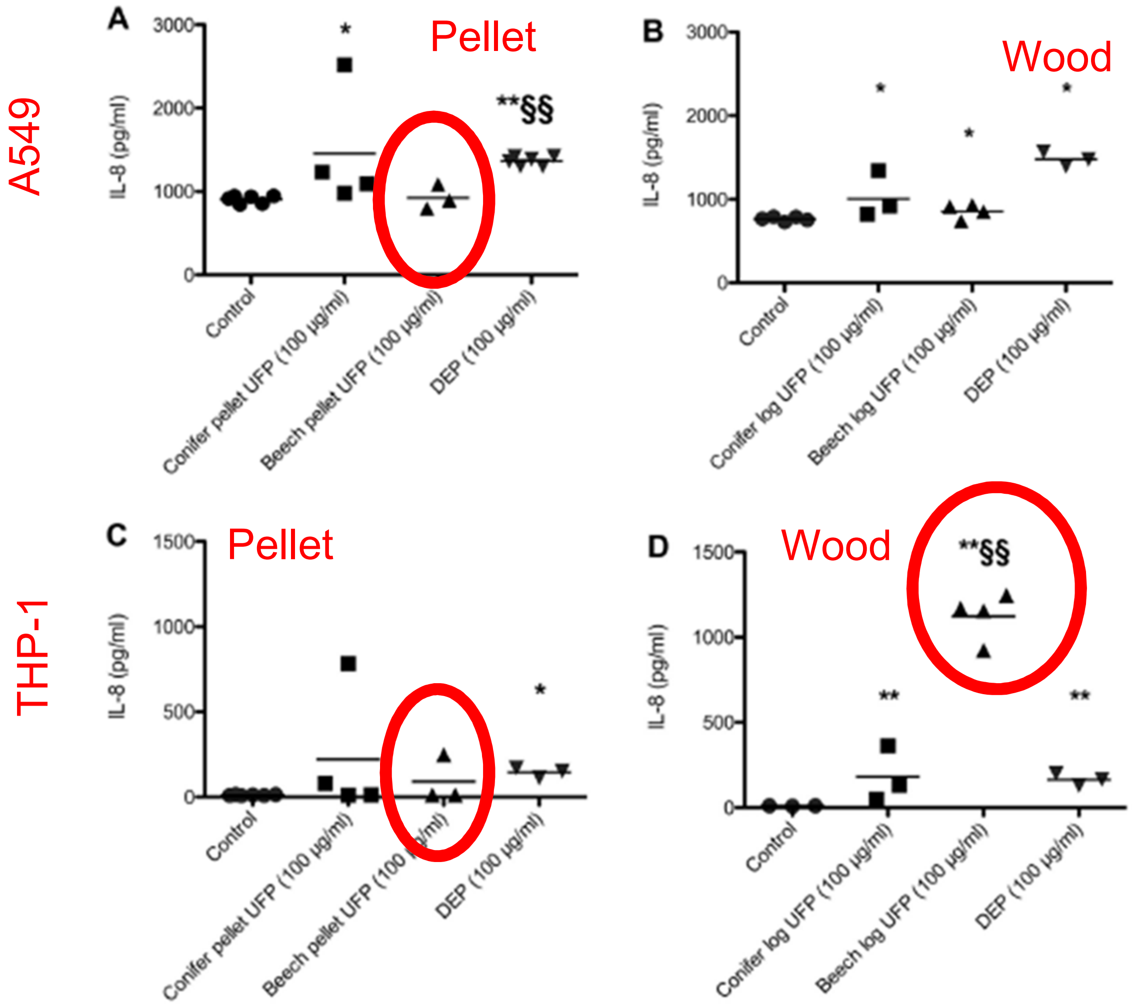
**Beech logwood**

UFP: 52 g/GJ

- TC~49%
- levo~7%
- PAH~2%
- $K^+$~2%
- $SO4^{--}$~4%
- Zn,Fe,Al~ 0.3%

# Pro-inflammatory effects from stove emissions

IL-8 secretion induced by UFP (dose based responses)




DEP=Diesel Exhaust Particles

Statistical analysis with *p < 0.05 and **p < 0.01 vs control cells (Cont), and §p < 0.05 and §§p < 0.01 vs UFP or DEP treated cells.

Further details in Corsini et al. (2017). Toxicology Letters 266: 74-84

- No effects observed in cell viability (assessed by LDH leakeage) → no cytotoxicological effects

- Pro-inflammatory effects are observed (assessed by IL-8 release):
  → UFPs from wood log combustion generally much more active than those from pellet
  → better combustion in pellet stove generated UFP with less inflammatory activity and negligible levoglucosan content

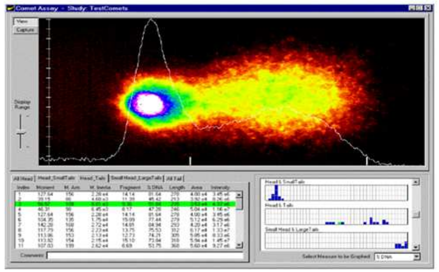
- High IL-8 release from beech wood log UFP

- THP-1 cells more sensitive to levoglucosan

# Oxidative stress and genotoxicological effects from stove emissions

- The alteration of the cellular redox status was the possible cause of DNA damage: ROS and RNS were probably generated directly by the particle surface in short times but also as secondary process due to inflammatory response
- ROS production may be associated both to K+ and levoglucosan
- In all the appliance and fuel type combinations investigated UFP samples induced significant genotoxic effect on human lung epithelial cells (A549), highest for fir wood
- Wood stove samples  the study of the UFP chemical composition suggested a combined effect of anhydrosugars (especially levoglucosan), elemental content (especially Fe, Al) and PAHs on the observed effects.

(dose based responses)



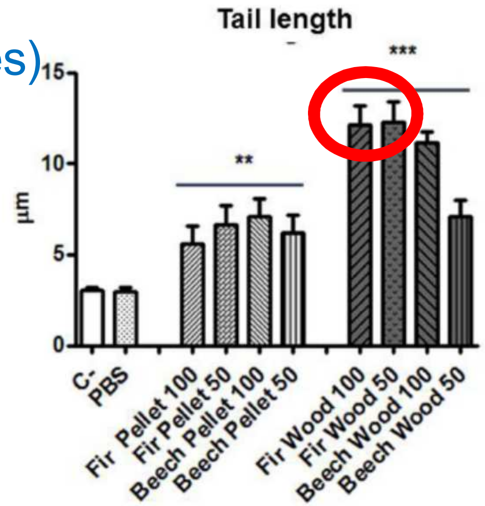



**Fig. 3.** Alkaline comet test evaluated as Tail lenght after treatments of A549 cells for 24 h with pellet and wood UFPs **p < 0.01 vs control ***p < 0.001 vs control.

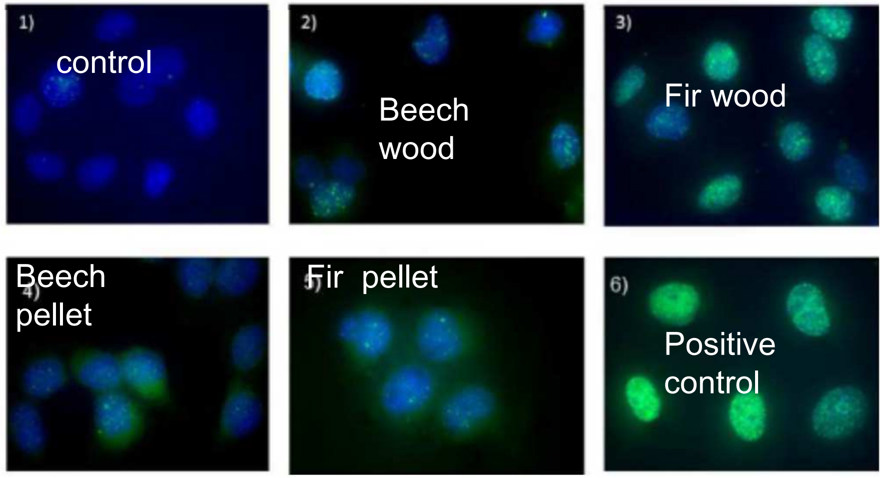
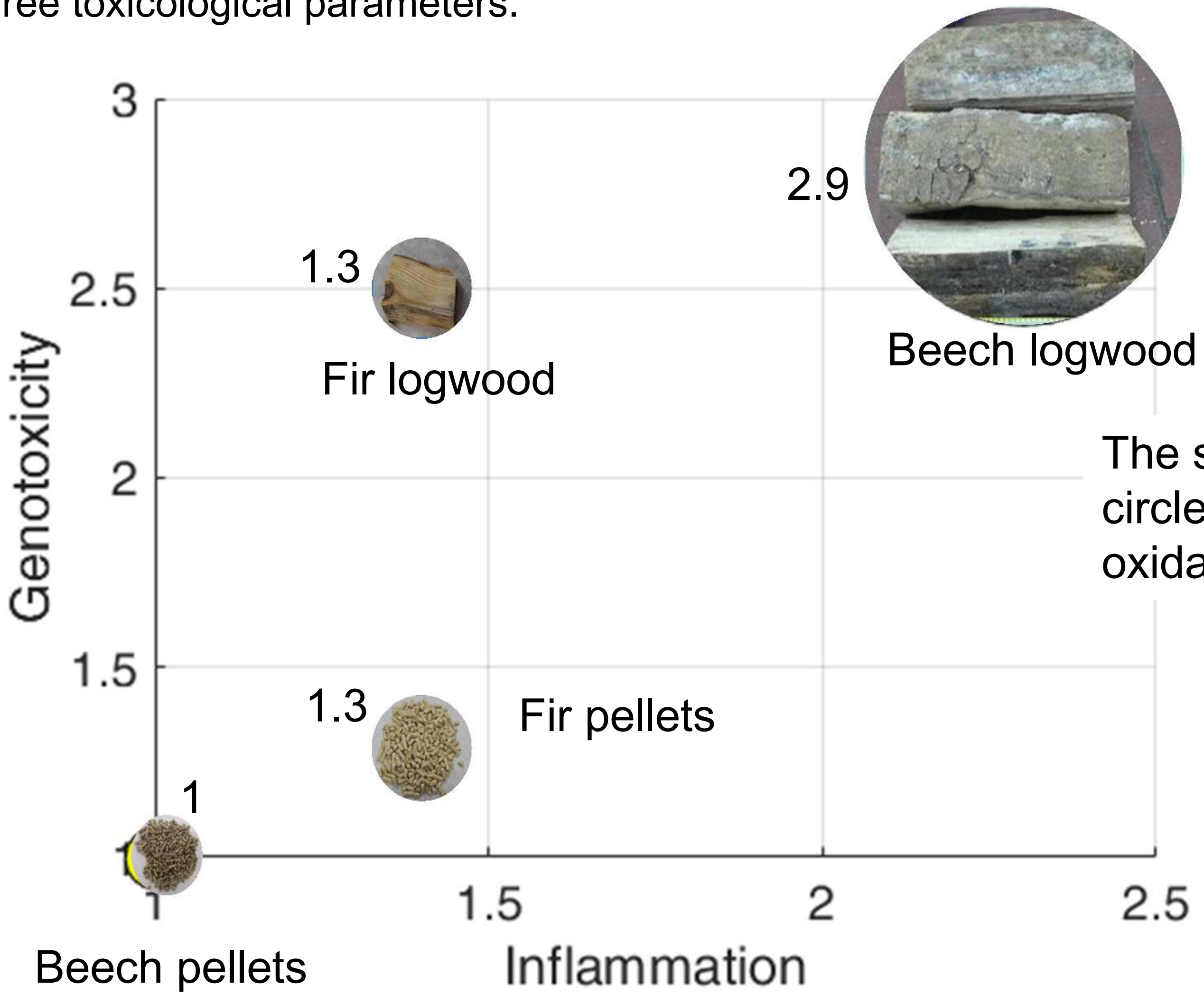



**Fig. 5.** DNA damage evaluated by γH2AX test after treatments of A549 cells for 24 h with pellet and wood UFPs (50 μg/mL) 1 Control; 2 Beech wood; 3 Fir wood; 4 Beech Pellet; 5 Fir Pellet; 6 Positive Control: Mitomycin c 0.5 μg/mL.

Further details in Marabini et al. (2017). Mutat Res Gen Tox En 820: 39-46.

# Relative toxicological responses

Energy-based toxicological response: Toxicological results are weighed according to the UFP emissions per unit energy input to the appliance, and expressed relative to the minimum response for each of the three toxicological parameters.




The size of the circles represent oxidative stress

The lowest response in each investigated parameter is indicated with 1.

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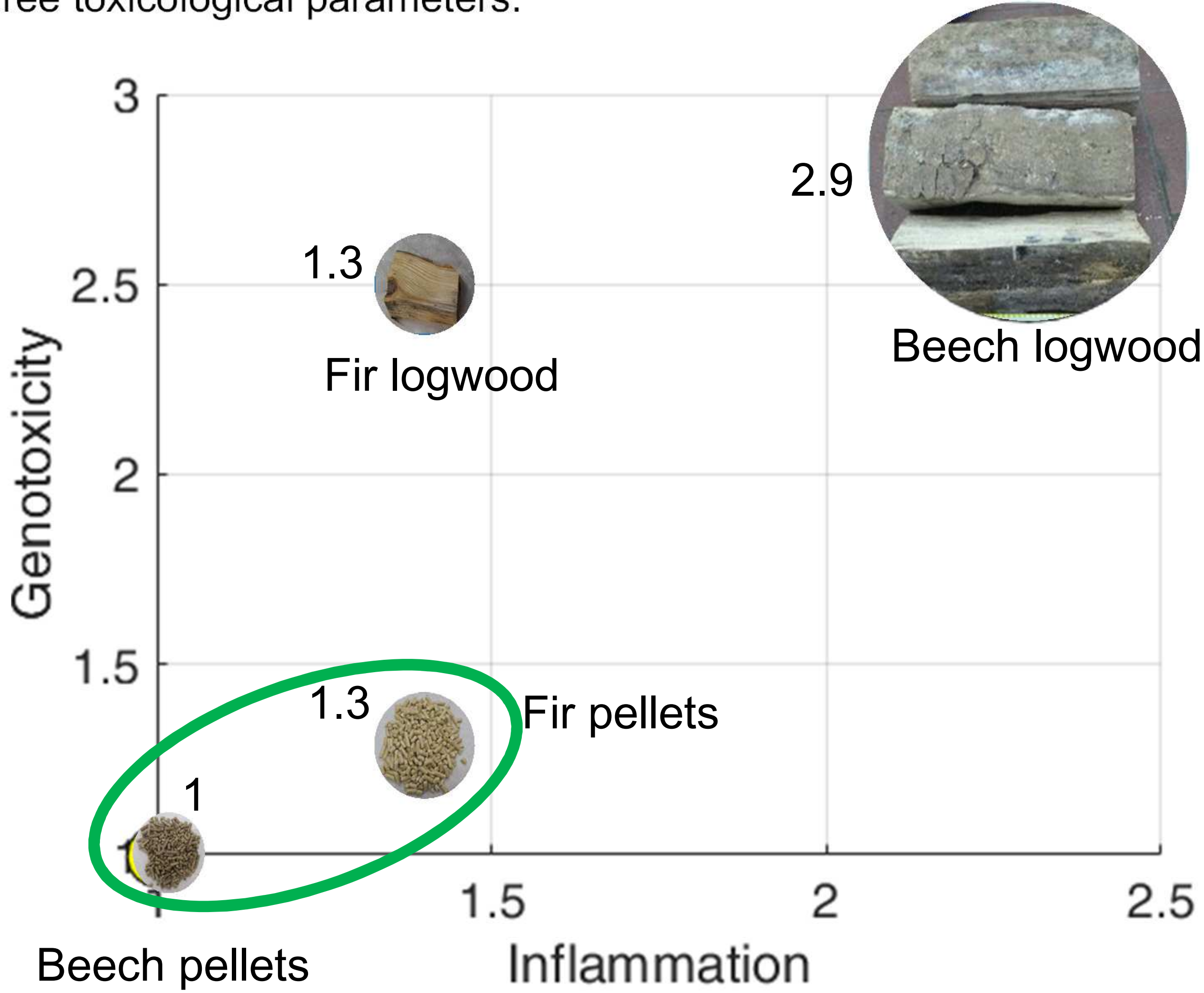


Less relative toxicological response associated with the automatic appliance with higher efficiency (pellet stove).

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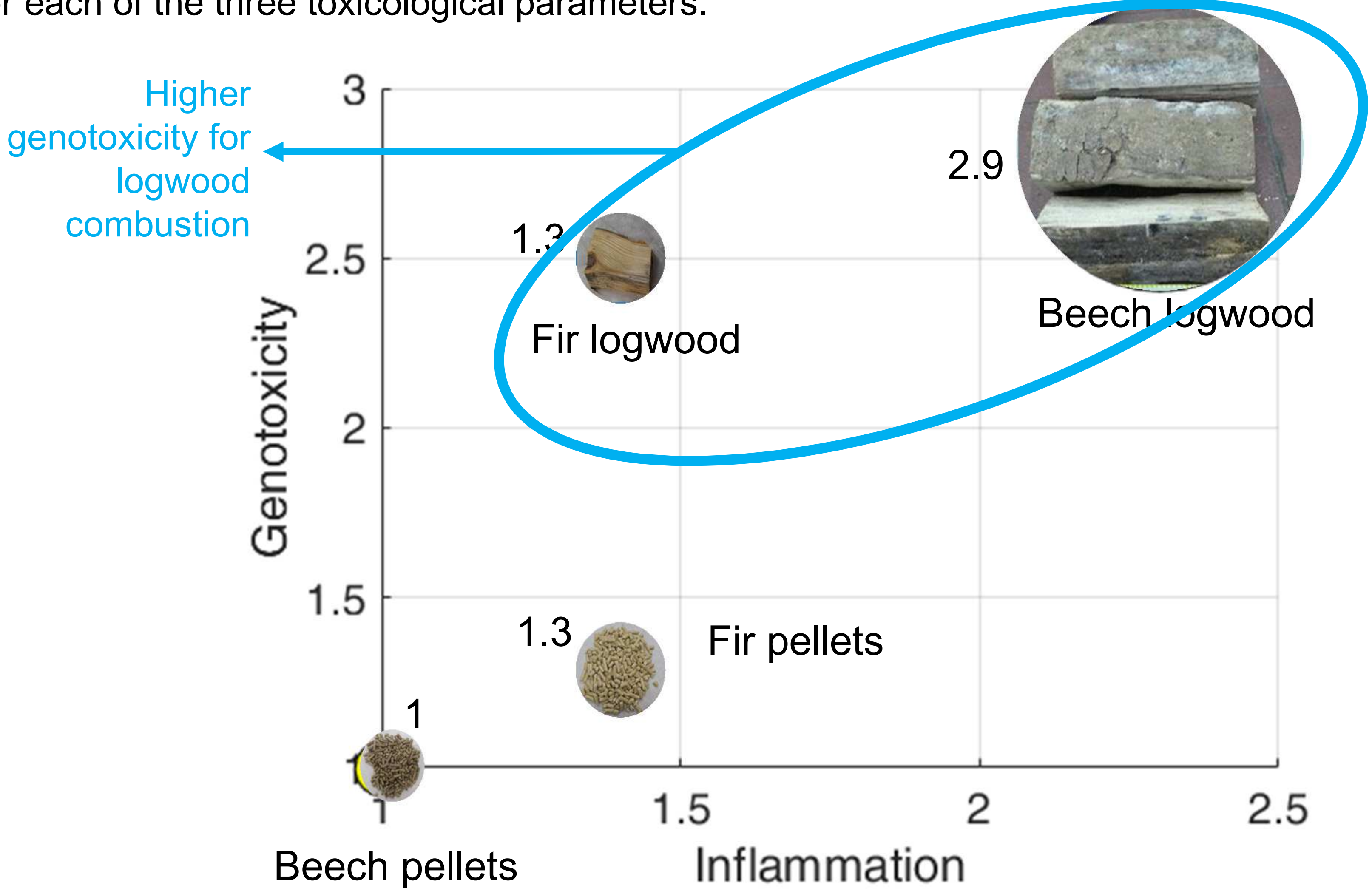

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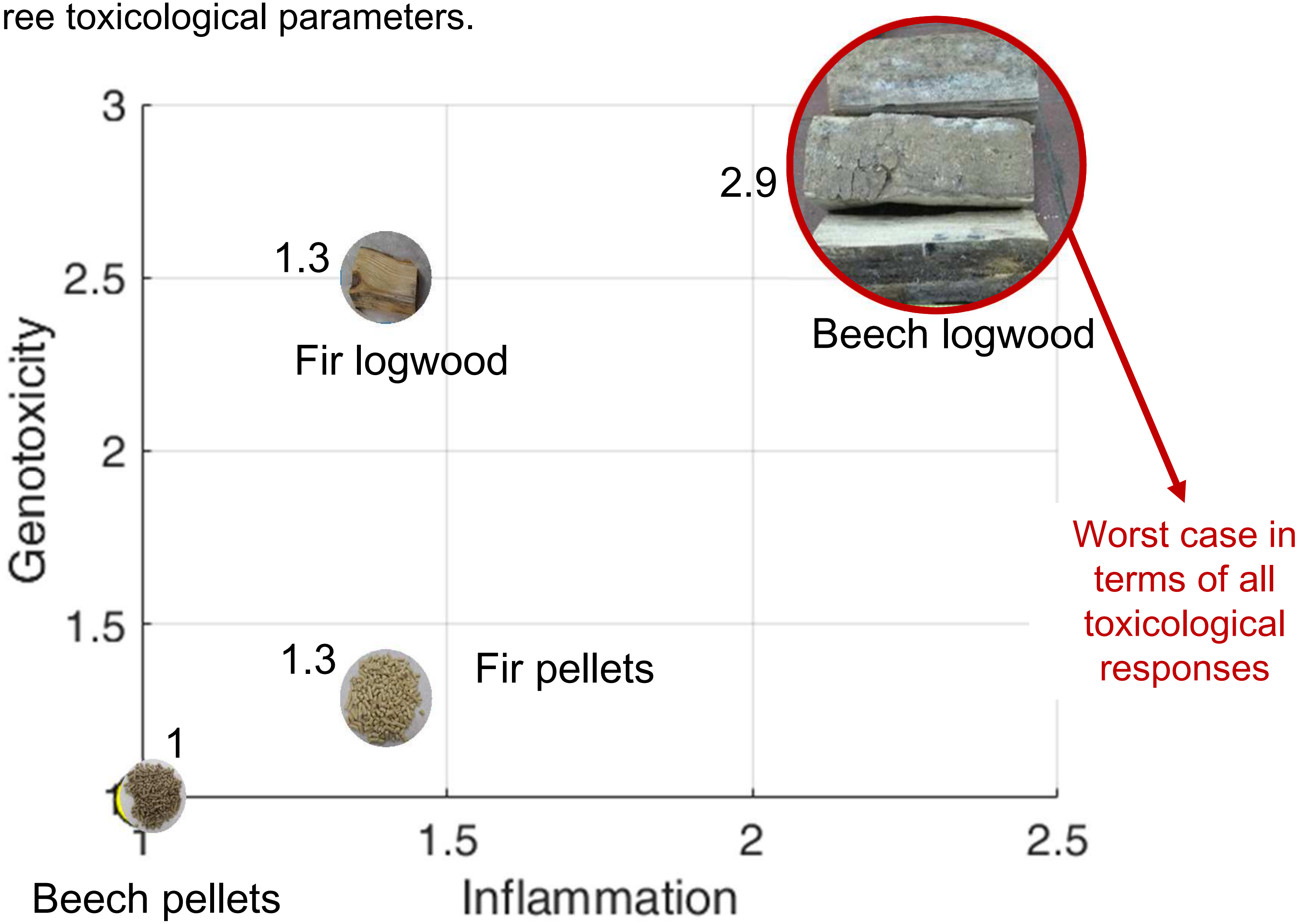


# Main findings – Phase 2: Ambient measurements

# UFP mass concentration and composition in ambient air

No seasonal differences in UFPs mass concentration
Winter:
2.2 (1.6–3.2) µg/m$^3$
Summer:
2.0 (1.0-3.1) µg/m$^3$

Levoglucosan and its isomers, $K_{+}$, and benzo(a)pyrene (i.e. wood burning tracers) with significant seasonal differences (high winter-to-summer ratios up to 8)

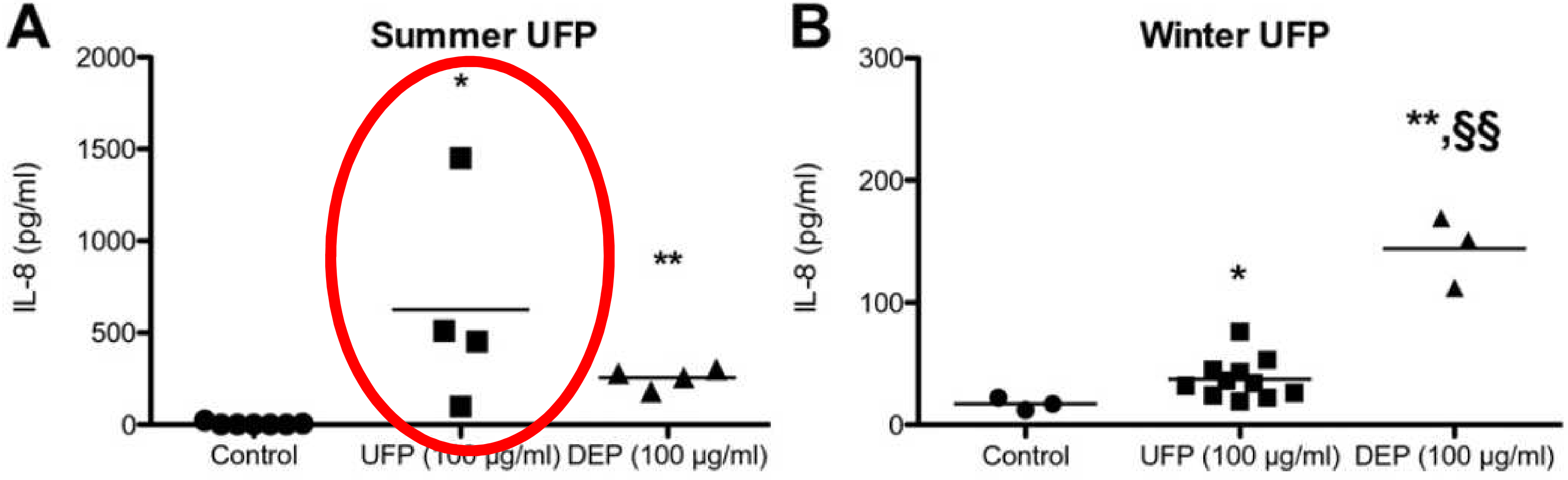
Wood Burning is an important source of UFP in Morbegno

Chemical composition showed seasonality

PAHs :
benzo(b)fluoranthene and benzo(a)pyrene gave much higher contributions in winter (i.e. consistent with LEAP emission data from small scale appliances where the same compounds were major contributors among PAHs).

# Pro-inflammatory effects of ambient UFPs

IL-8 secretion induced by UFP (dose based responses)




1. UFPs collected in summer more active in inducing IL-8 release than winter UFPs in both cells lines.
The release was overall similar to the one observed with DEP

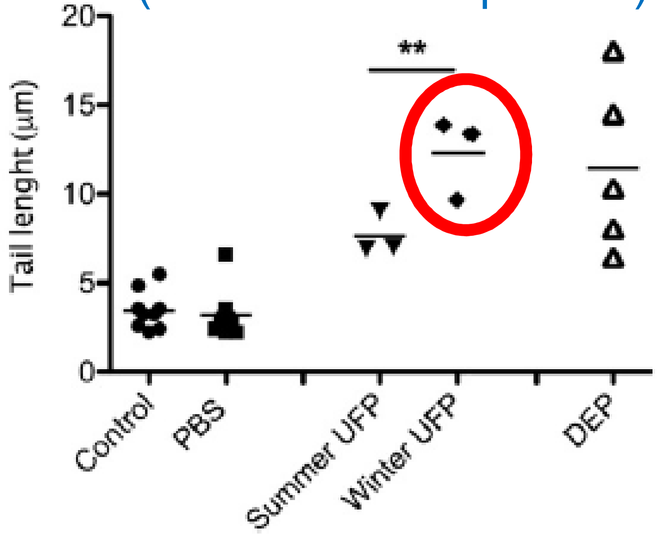
Role of photochemical processing with UFPs oxidation in typical summertime conditions.

2. Summer and winter UFPs (100 µg/ml) induced on average a 20% reduction in cell viability, with no statistically significant difference on a seasonal basis.

Further details in Corsini et al. (2017). Sci. Total Environ 587-588: 223-231

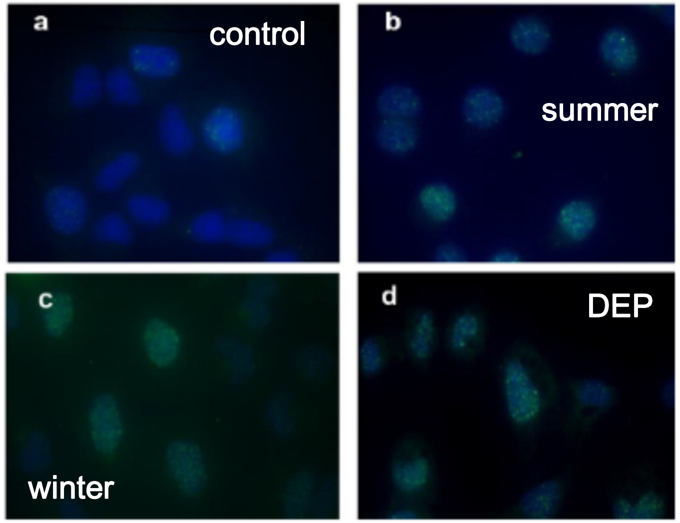
# Oxidative stress and genotoxic effects of ambient UFP

(dose based responses)




Higher effects with winter UFPs (opposite to inflammatory effects !)

seasonal differences in UFPs composition differently affected biological responses.




Genotoxicity observed in A549 cells → likely contribution of the presence of PAHs and metals in ambient air UFP.

In fact, PAHs are known to cause DNA damage and transition metals may cause DNA strand break by inducing ROS.

ROS increase at earlier timepoint which supports the DNA damage observed at 24h of exposure to UFP.

Further details in Corsini et al. (2017). Sci. Total Environ 587-588: 223-231

# Conclusive summary

**Phase 1 - Emission testing:**

- Critical situations (e.g., lack of available oxygen for the complete oxidation, too high or low burning temperatures, non-optimal mixing of air and fuel) will determine the composition of UFP and the expected health effects of the UFP;

- Type-testing may not be able to capture these critical situations

- The burning of the same wood types as logs in a manual appliance instead of as pellet (much drier and smaller) in an automatic roomheater may cause not only the increase of UFP emissions but also the alteration of the composition with potentially more toxic/carcinogenic substances.

**Phase 2 - Ambient Measurements:**

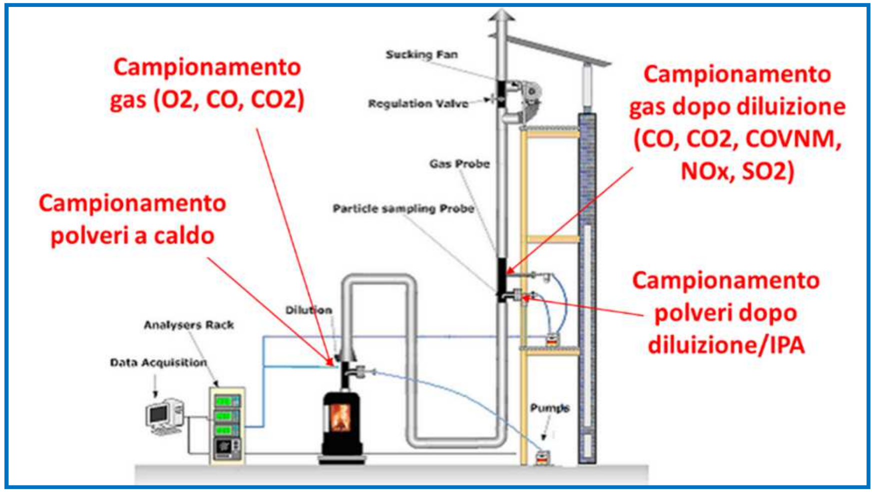
- Exposure to wintertime ambient UFPs more effective in inducing genotoxicity with limited pro-inflammatory responses compared to summer UFPs.

- Summer UFP (more oxidized by photochemical processes) causes more pro-inflammatory responses

# Other findings – Previous research project

## Emission factors from 6 residential heating appliances fed with various woody fuels

## real-world combustion cycles vs. type testing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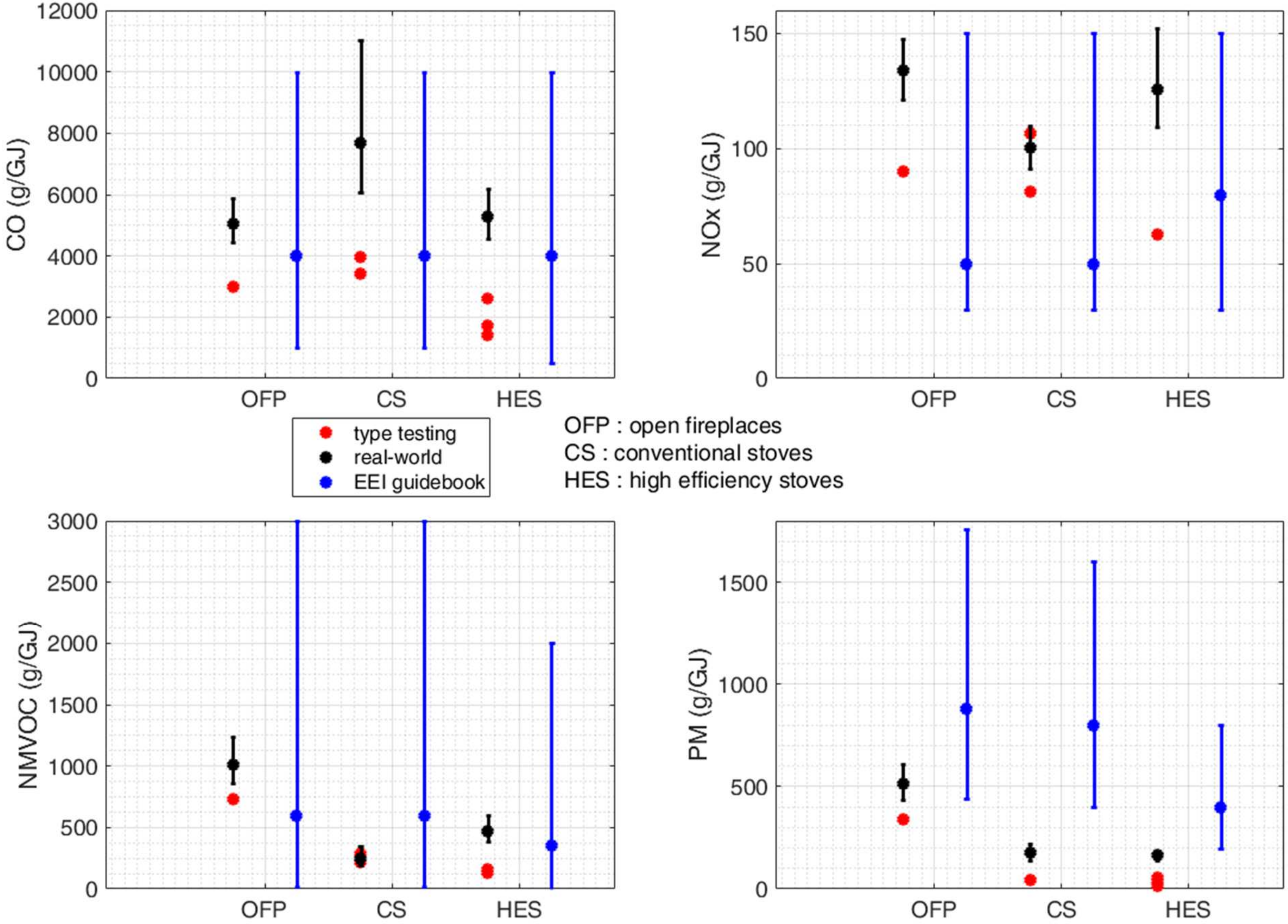
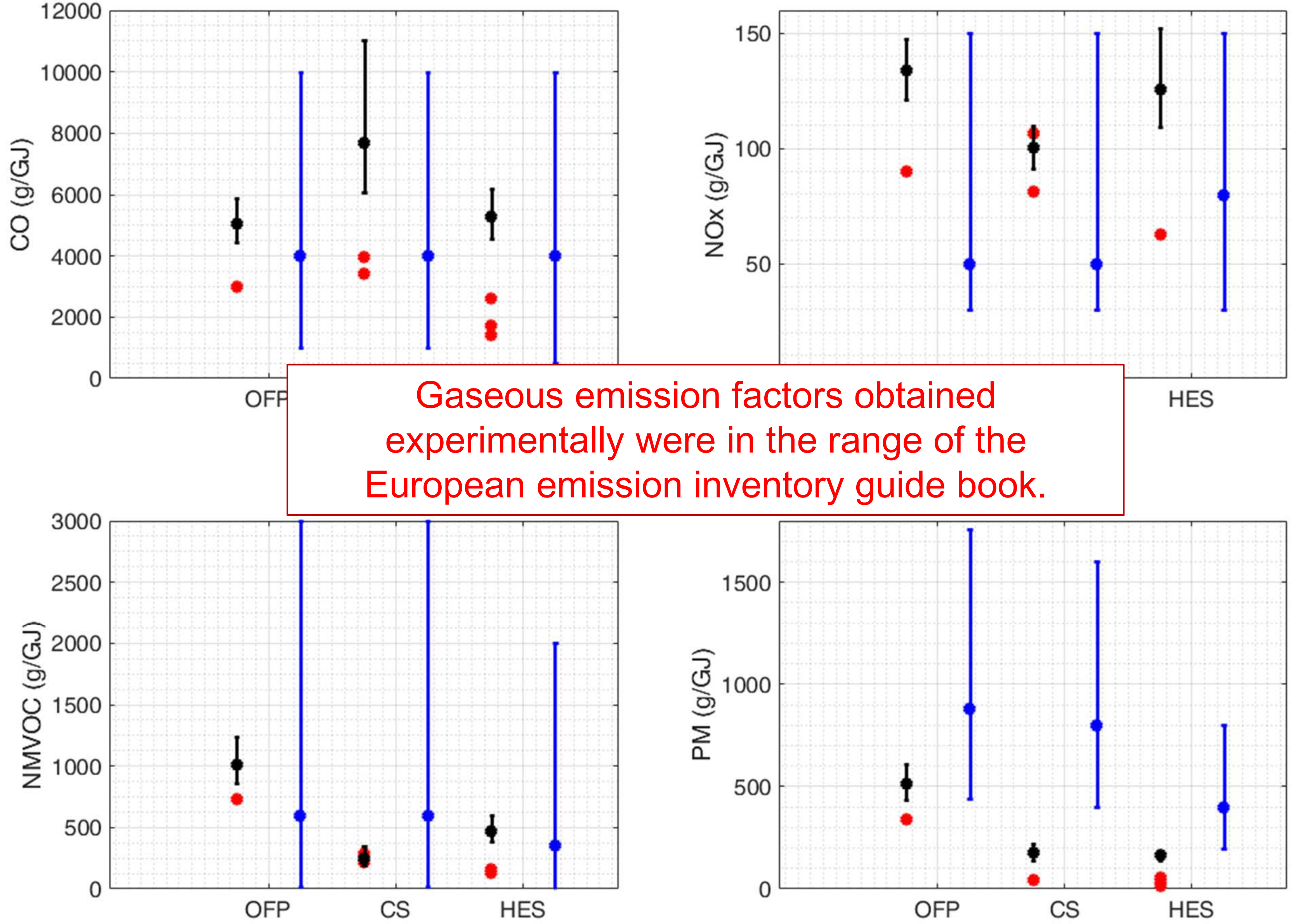
Further details in Ozgen et al. (2014). Atmos Environ 94: 144-153
Caserini et al. (2014). Ingegneria dell'Ambiente Vol. 1, n.1

# Summary of the experimental emission factors



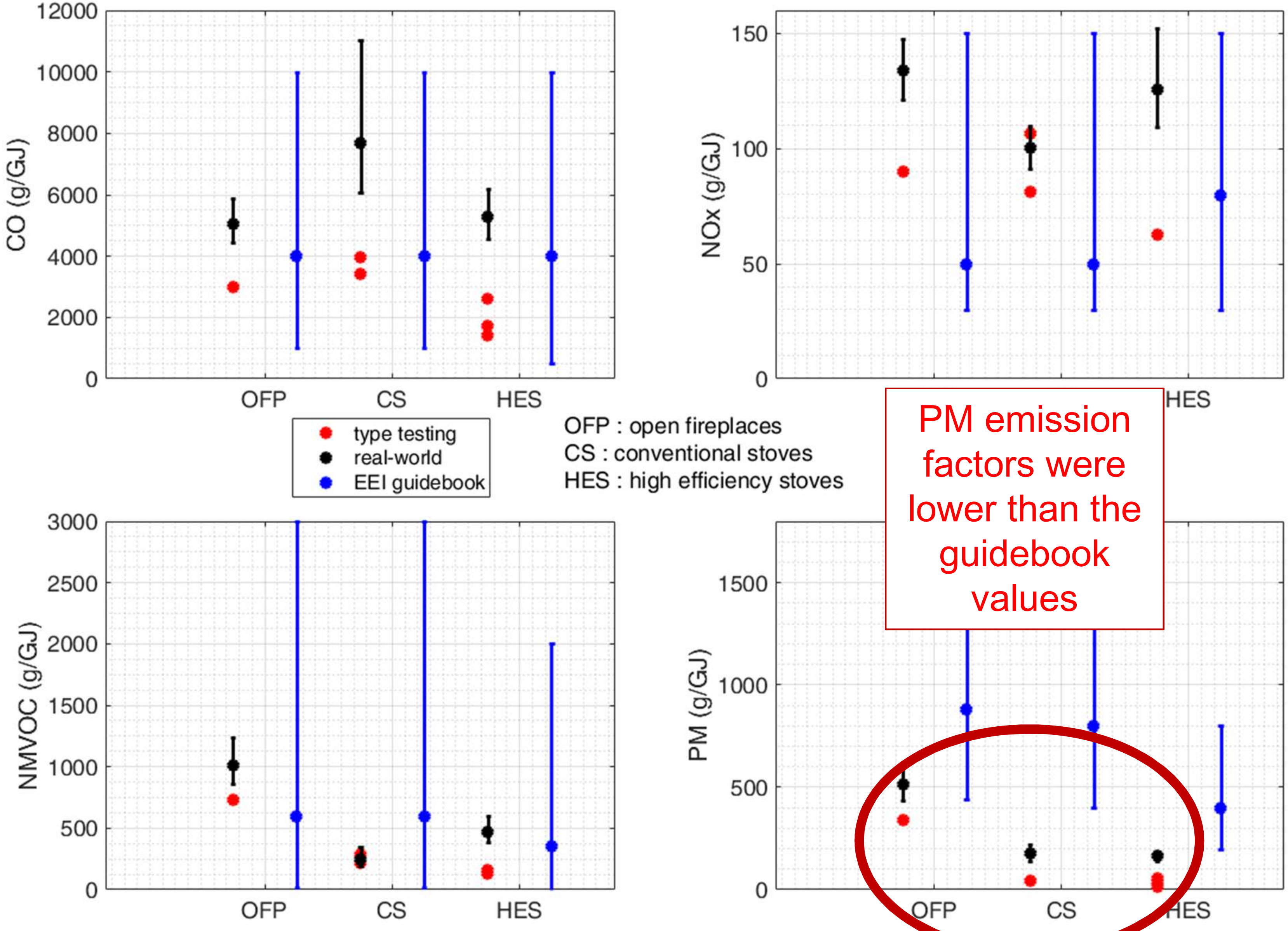

OFP : open fireplaces
CS : conventional stoves
HES : high efficiency stoves

# Summary of the experimental emission factors

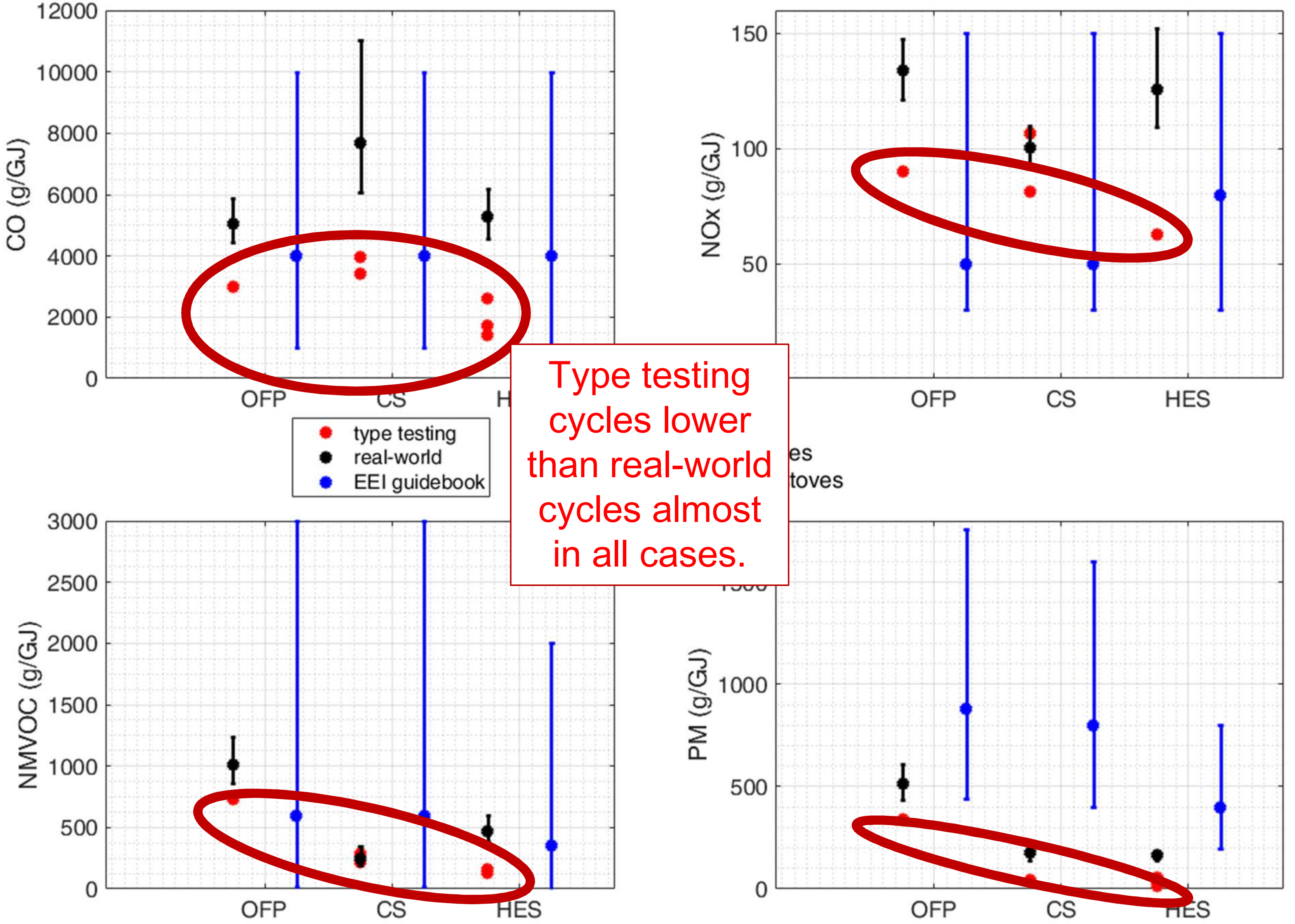



Gaseous emission factors obtained experimentally were in the range of the European emission inventory guide book.

# Summary of the experimental emission factors



# Summary of the experimental emission factors




Further details in Corsini et al. (2017). Sci. Total Environ 587-588: 223-231

# Tasks and working groups

WG1 - combustion tests, emission monitoring, UFP sampling, data analyses

POLITECNICO MILANO 1863
DIPARTIMENTO DI INGEGNERIA CIVILE E AMBIENTALE
LEAP
Laboratorio Energia e Ambiente Piacenza

WG2 - IC, HPAEC-PAD and TOT analyses

UNIVERSITÀ DEGLI STUDI DI MILANO

WG3 - ICPAES analyses, ambient monitoring, UFP sampling and data analyses

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DIPARTIMENTO DI FISICA

WG4 - Project leader, GC-MS analyses, toxicological analyses

UNIVERSITÀ DEGLI STUDI DI MILANO
DIPARTIMENTO DI SCIENZE FARMACOLOGICHE E BIOMOLECOLARI

# TOBICUP research project working groups

• WG1 - combustion tests, emission monitoring, UFP sampling, data analysis:

**S. Ozgen, G. Lonati, R. Tardivo, E. Tosi** – Department of Civil and Environmental Engineering - Environmental Engineering section, Politecnico di Milano

**S. Signorini** – Laboratory of Energy and Environment Piacenza (LEAP)

• WG2 - IC, HPAEC-PAD and TOT analyses:

**L. Corbella, P. Fermo** – Department of Chemistry, Università degli Studi di Milano

• WG3 – ICPAES analyses, ambient monitoring, UFP sampling and data analysis:

**V. Bernardoni, M. Dell'Acqua, G. Valli, R. Vecchi** – Department of Physics, Università degli Studi di Milano and INFN

**S. Becagli** – Department of Chemistry, Università degli Studi di Firenze

• WG4 – Project leader, GC-MS analyses, toxicologic analysis:

**C. Galli, D. Caruso, E. Corsini, L. Marabini, M. Marinovich** – Department of Pharmacological and Biomolecular Sciences, Università degli Studi di Milano

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# GRAZIE PER L'ATTENZIONE!

**Per informazioni:**

**senem.ozgen@polimi.it**

Politecnico di Milano
Dipartimento di Ingegneria Civile ed Ambientale (DICA) - Sezione Ambientale
Piazza L. da Vinci, 32 20133-Milano
Tel. +39-02-2399.6430. Cell: +39-328-1310609

Laboratorio Energia Ambiente Piacenza - Società Consortile a Responsabilità Limitata
Experimental Laboratories LEAP
http://www.leap.polimi.it/leap/en/the-laboratory.html