Toxicity of fine and quasi-ultrafine particles: focus on the effects of extractable and non-extractable matter fractions

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Outline

1. Introduction
2. Objectives
3. Methodology of work
4. Results & Discussion
5. Conclusion

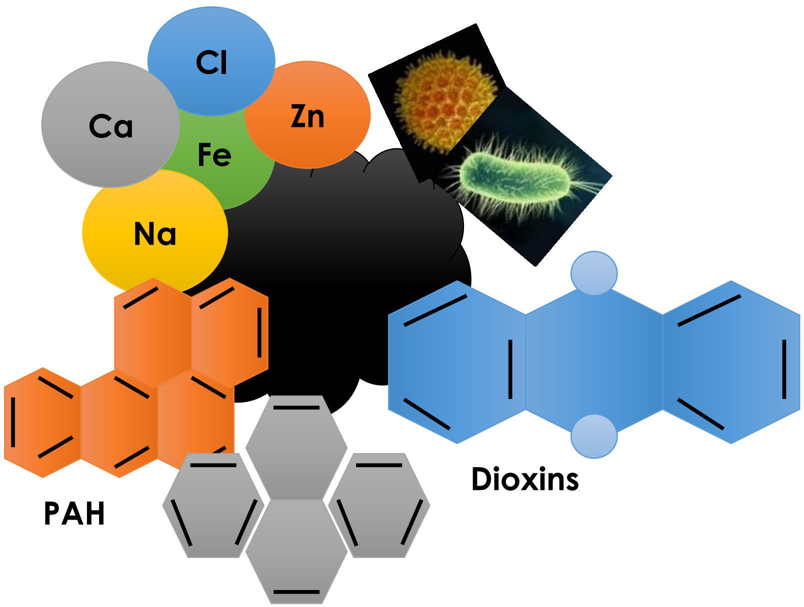
Fine atmospheric particles ($PM_{2.5}$)

Mixture of :

1. Inorganic compounds (e.g. metals, ions)

2. Organics : e.g. volatile organic compounds (VOCs), polycyclic aromatic hydrocarbons (PAHs)

3. Biological materials: (e.g. pollen, bacteria, fungi)



The **toxicity** of PM depends on their **composition**

Not constant → Varies according to the geographical location and several factors (climate…)

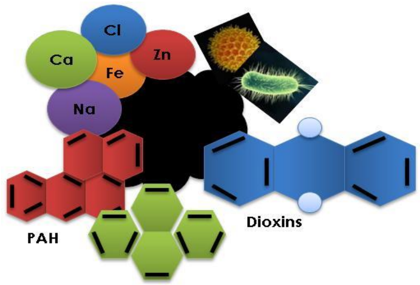
↓

This variation makes the determination of the toxic effects very <u>complex.</u>

- To date, a lot of research work has been done to identify the toxicological response of the **total PM**
- There is still a lack of knowledge about the specific **chemical components and/or fractions** within airborne PM, which could be mainly responsible for these effects

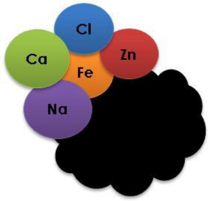
OBJECTIVES

1. Determine what is responsible in the PM for the observed toxic effects?



Total PM

Organic extractable matter fraction (OEM)



Non-extractable matter fraction (NEM)

2. Comparison of composition & toxicity between **fine** and **ultrafine** PM

Sampling of fine ($PM_{2.5\text{-}0.3}$) and quasi-ultrafine ($PM_{0.3}$) atmospheric particulate matter

Chemical characterization

Extraction of the PM organic extractable matter (OEM) and recovery of the PM non-extractable matter fraction (NEM)

In-vitro toxicological studies on Beas-2B cells

Sampling of fine ($PM_{2.5-0.3}$) and quasi-ultrafine ($PM_{0.3}$) atmospheric particulate matter

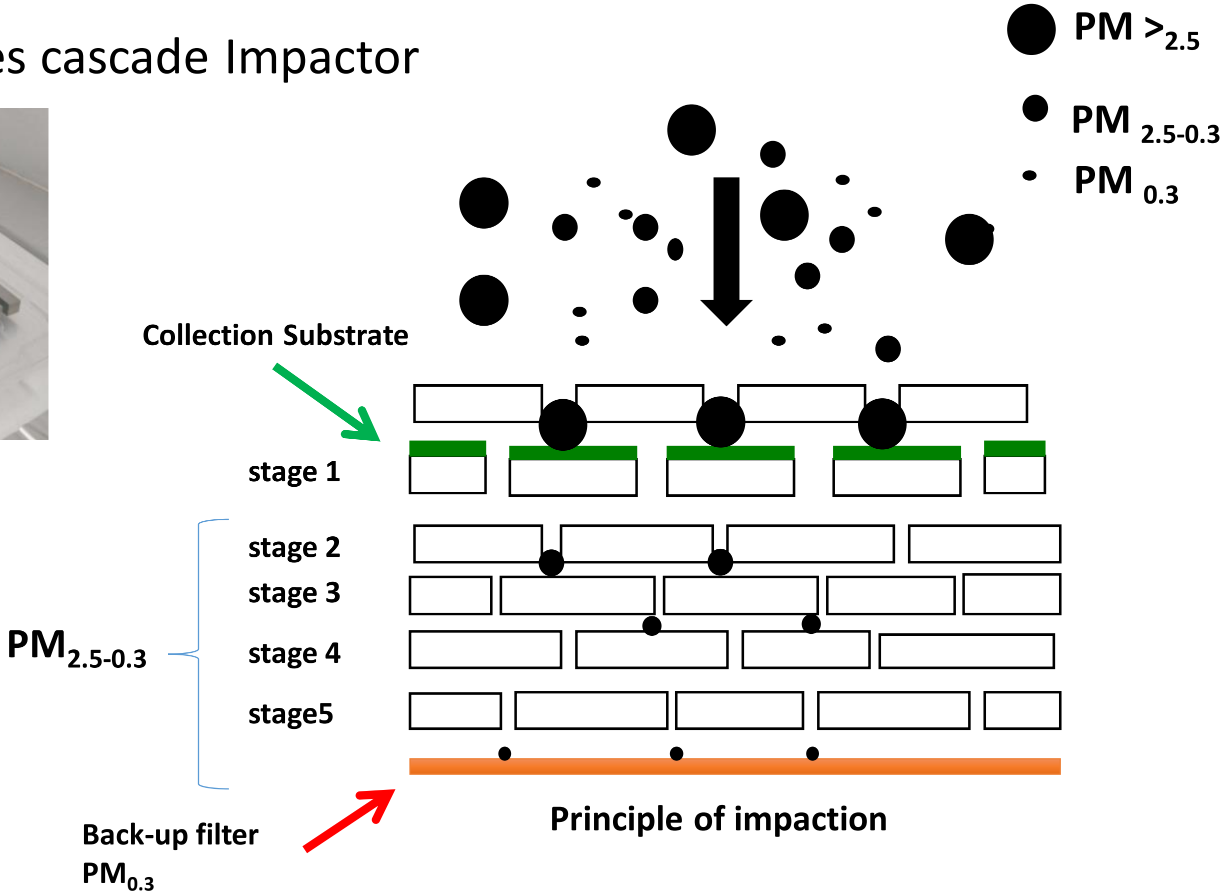
1. Typical road traffic site
2. Main axis between the capital and the airport
3. Surrounded by a strong residential area

Sampling of fine ($PM_{2.5-0.3}$) and quasi-ultrafine ($PM_{0.3}$) atmospheric particulate matter:

When? Sampling period 03/01/2017→ 08/03/2017

How? High volume 5 stages cascade Impactor

Cascade Impactor STAPLEX® 235



Principle of impaction

$PM_{2.5-0.3}$: impacted on plates from stage 2 to 5

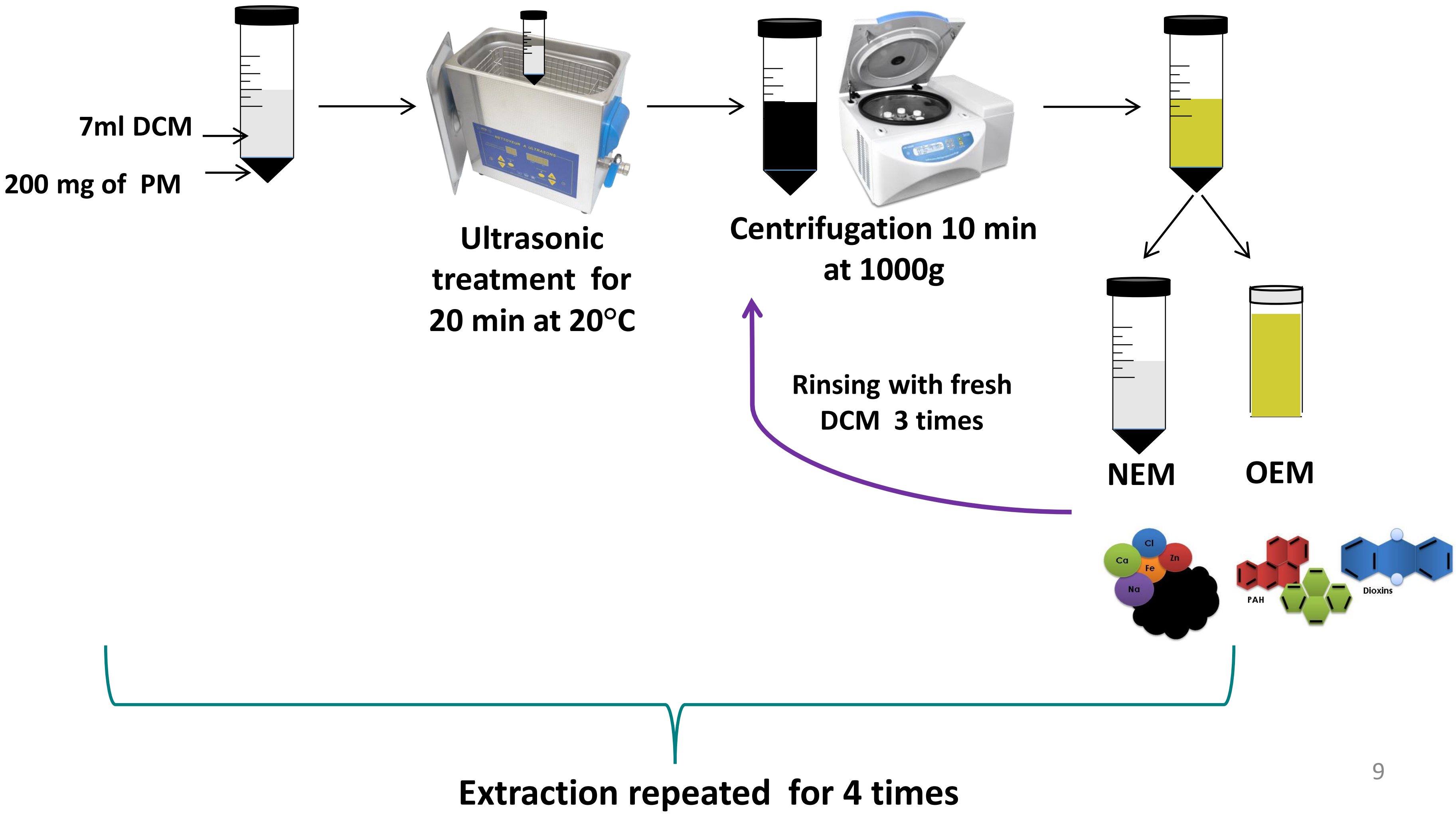
$PM_{0.3}$: trapped on a back-up filter

Chemical characterization

1. Determination and quantification of major and trace elements and water soluble ions (ICP-AES , IC)

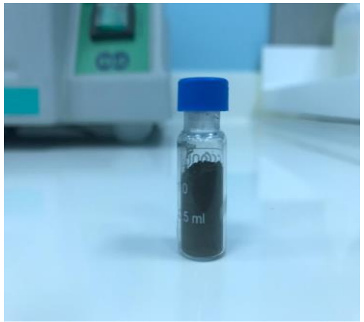
2. Quantification of PAHs (GC-MS)

Preparation of PM Organic Extractable Matter and Non-Extractable Matter

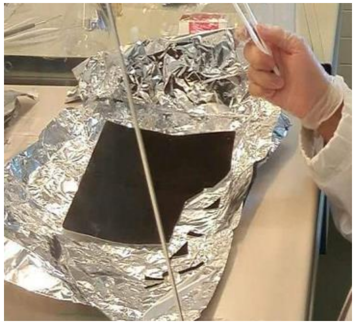


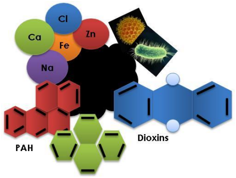
Samples for toxicological studies

$PM_{2.5\text{-}0.3}$ from stages 2 to 5



$PM_{0.3}$ from back-up filter

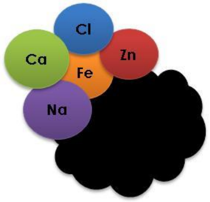




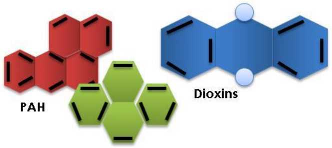
$PM_{2.5\text{-}0.3}$

DCM extraction

$NEM_{2.5\text{-}0.3}$



$OEM_{2.5\text{-}0.3}$

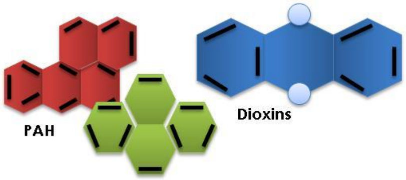


solvent change to DMSO

$OEM_{2.5\text{-}0.3}$

DCM extraction

$OEM_{0.3}$



solvent change to DMSO

$OEM_{0.3}$

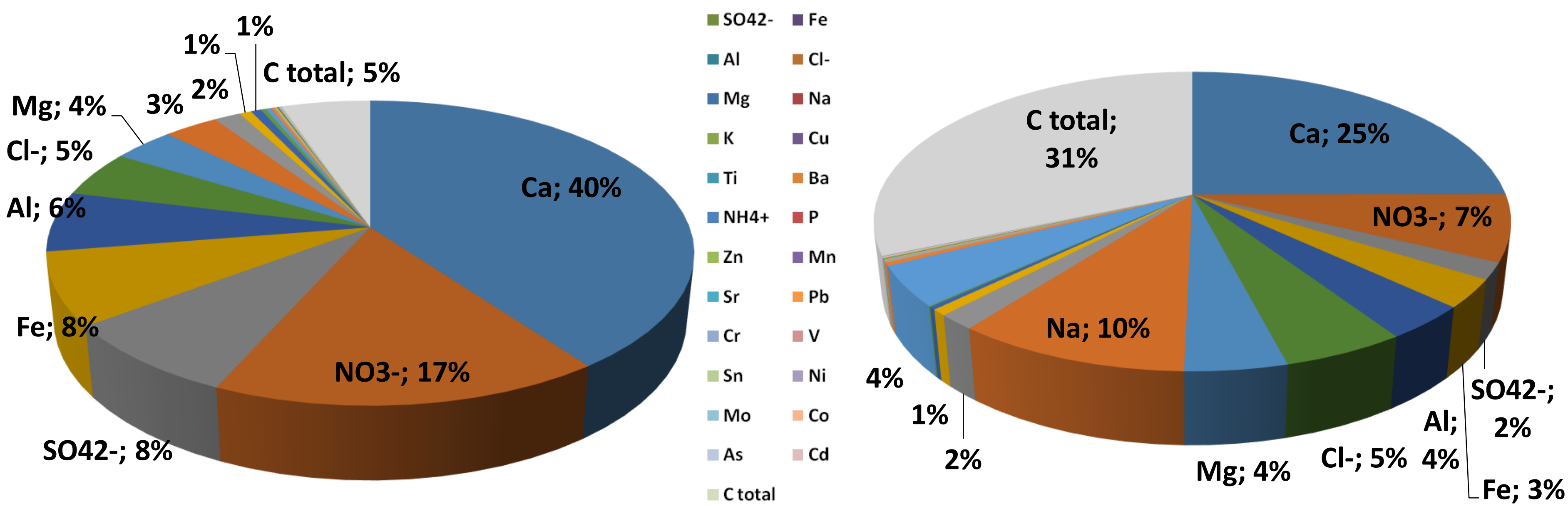
Evaluation of $PM_{2.5}$- induced oxidative stress and oxidative lesions

Results

Chemical characterization of PM

Major and trace elements and ions

$PM_{2.5-0.3}$ | $PM_{0.3}$



- <u>Predominant elements :</u>

Ca, Al, Fe, Mg → Resuspenion of soil dust **(Hans Wedepohl, 1995)**

- <u>Trace elements :</u>

Ba, Zn & Cu → Emission from engine's oil, tires and car brakes.

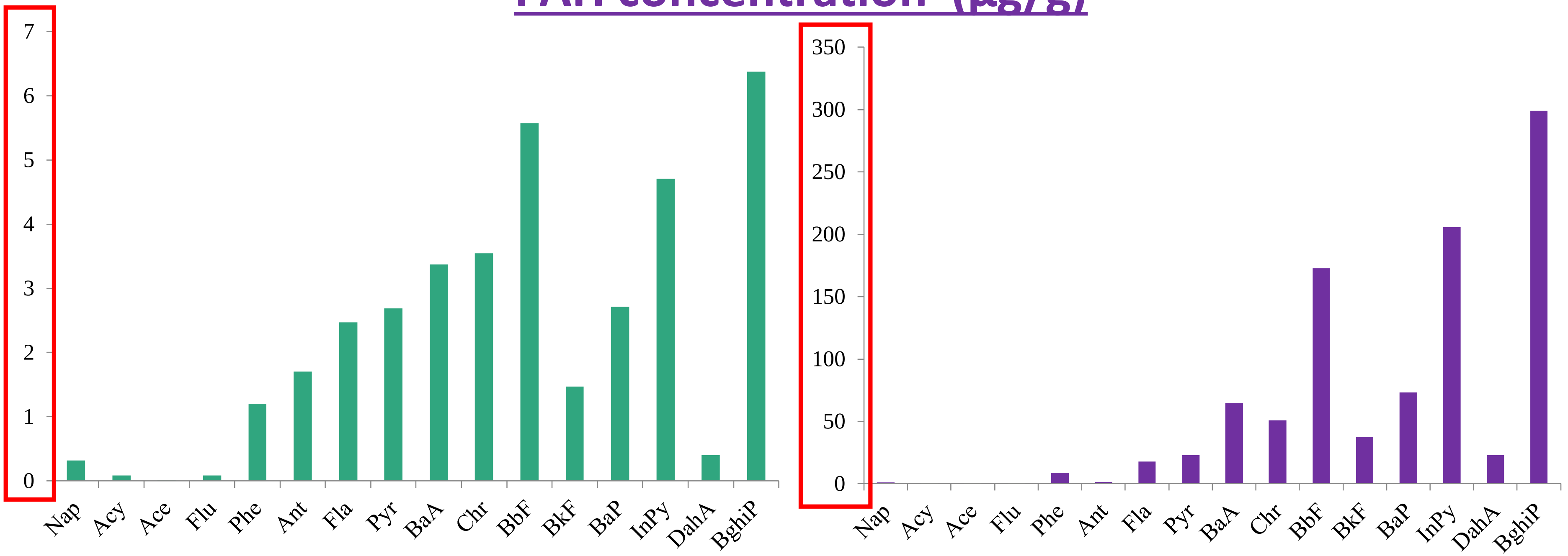
- <u>Predominant Ions:</u>

NO_3^- & SO_4^{2-} → Conversion of NO_2 and SO_2 precursor gases and long-range transport. **(Borgie et al., 2016; Luria et al., 1989)**

- <u>Total carbon :</u>

$PM_{0.3}$ >> $PM_{2.5-0.3}$: Combustion process and $PM_{0.3}$ emission

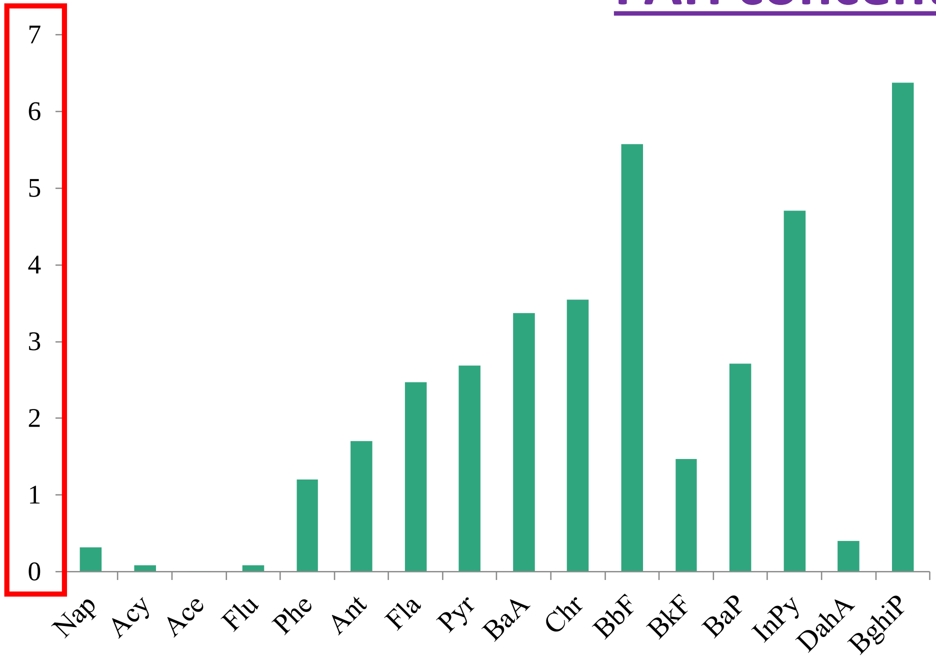
PAH concentration (µg/g)

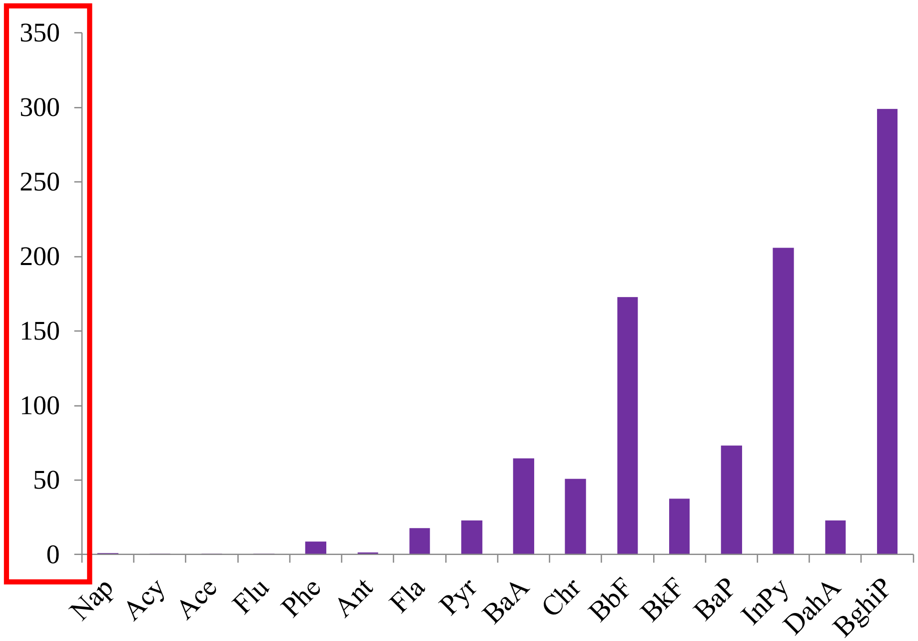


$OEM_{2.5\text{-}0.3}$ <<< $OEM_{0.3}$

→ Significant influence of ***anthropogenic activities*** and ***combustion sources*** (industries, road traffic and electric generators) on the emission of quasi-ultrafine particles and organic compounds.

PAH concentration (µg/g)





BghiP = *Benzo[g,h,i]perylene*

InPy = *Indeno[1,2,3-c,d]pyrene*

BkF = *Benzo[k]fluoranthene*

BaP = *Benzo[a]pyrene*

BbF = *Benzo[b]fluoranthene*

Usually emitted by ***combustion sources*** including gasoline motor vehicles, biomass burning, and industrial process (INERIS, 2005)

↓

Confirmed using characteristic **PAH ratios**

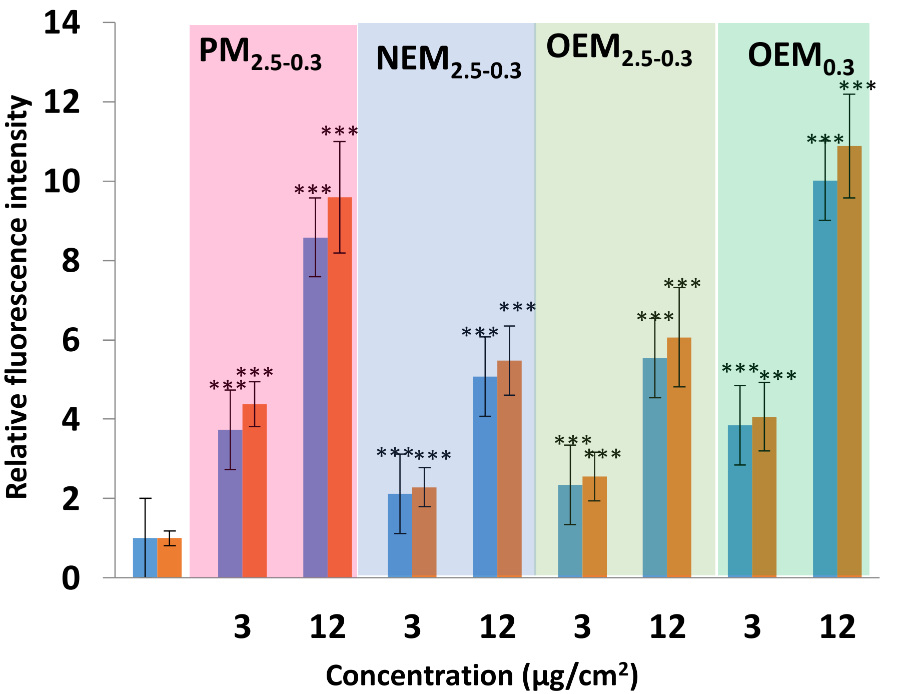
Results

Study of oxidative stress and oxidative lesions

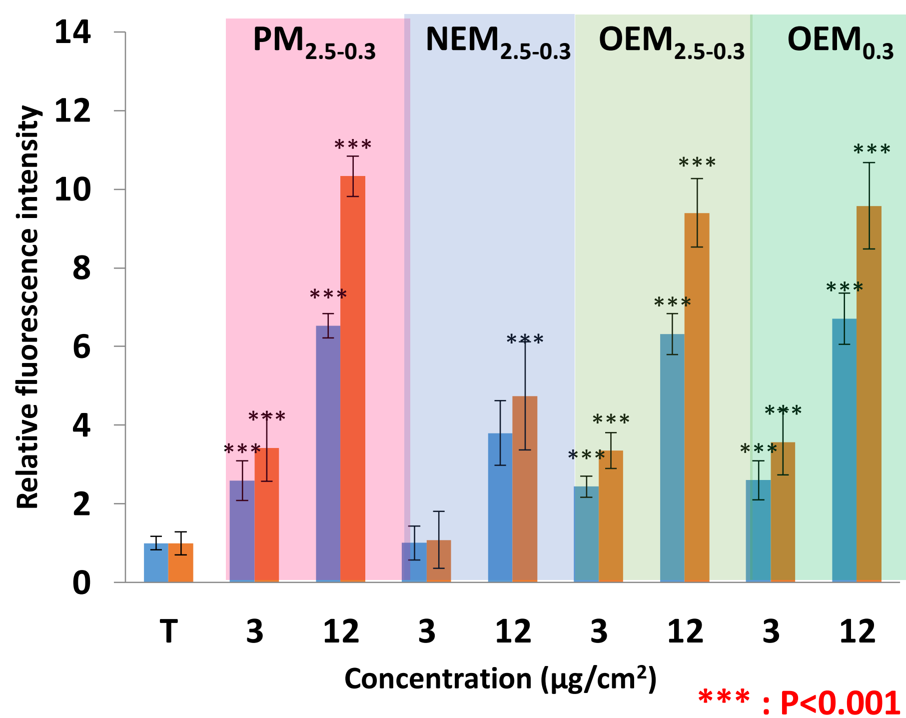
ROS production, antioxidant defense, stress damages

1- Ability to generate ROS (H_2O_2 & O_2^-)

1) Dihydroethidium (H_2O_2)



2) Carboxy-H2DCFDA (O_2^-)



ROS generation = dose and time dependent

ROS generation was more important after 24 H and C_2=12 µg/cm²

$PM_{2.5-0.3}$ > $OEM_{2.5-0.3}$ > $NEM_{2.5-0.3}$

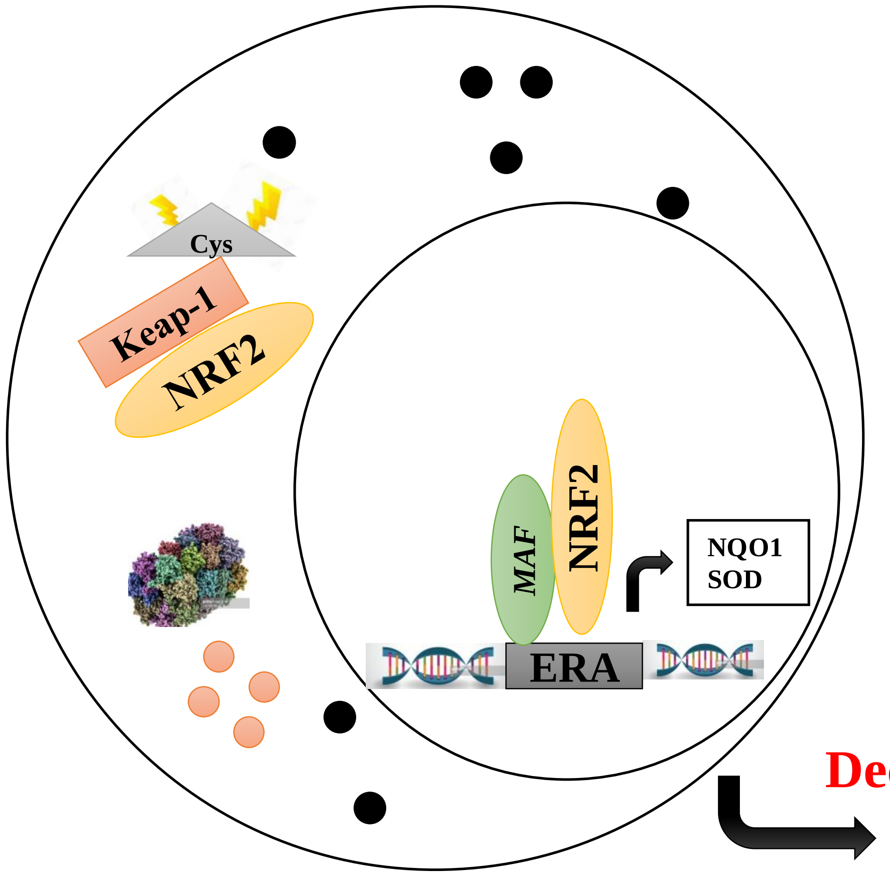
$OEM_{0.3}$ > $OEM_{2.5-0.3}$

- **Metal** and **water soluble ions** in NEM
- **Organic compouds (PAH)** in OEM

Can both contribute to **ROS overproduction.**

Defense against cellular stress: NRF2 antioxidant pathway

ROS = High level

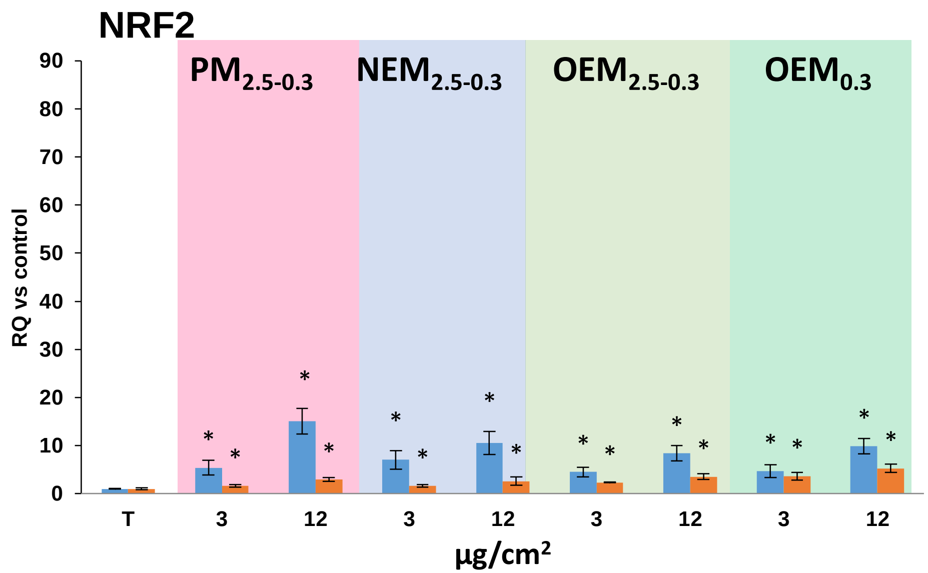


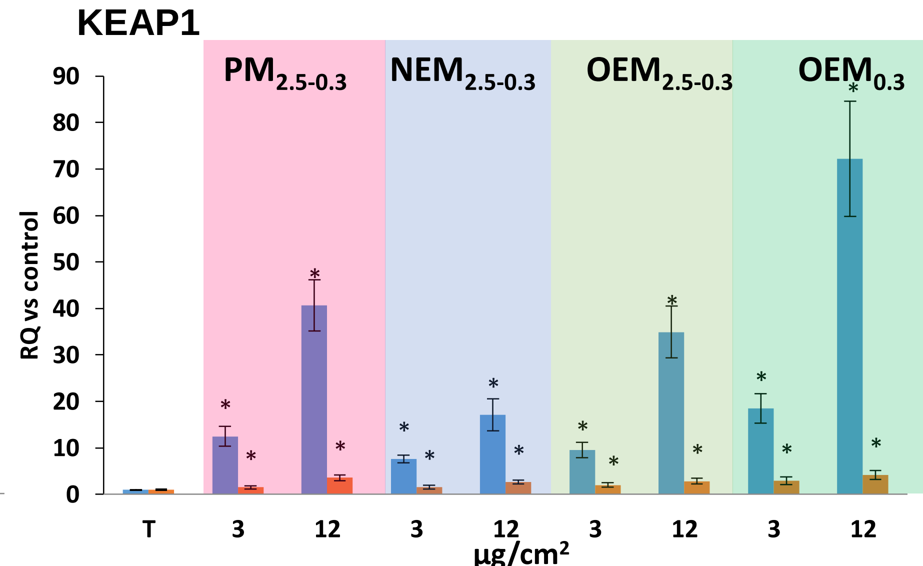
Decrease of oxidative stress

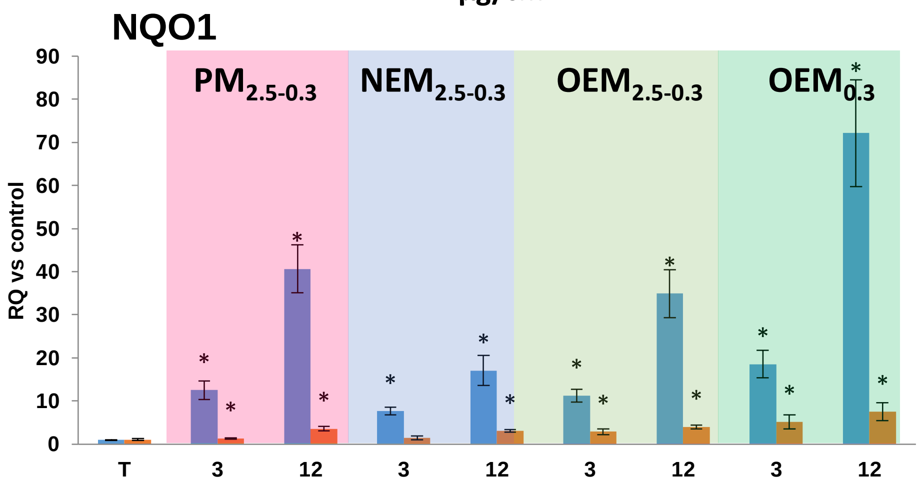
2. Evaluation of cellular defense against oxidative stress

a. NRF2, KEAP1and NQO1 gene expression

6h 24h







* : $P<0.05$

→ Significant increase after 6 and 24 H

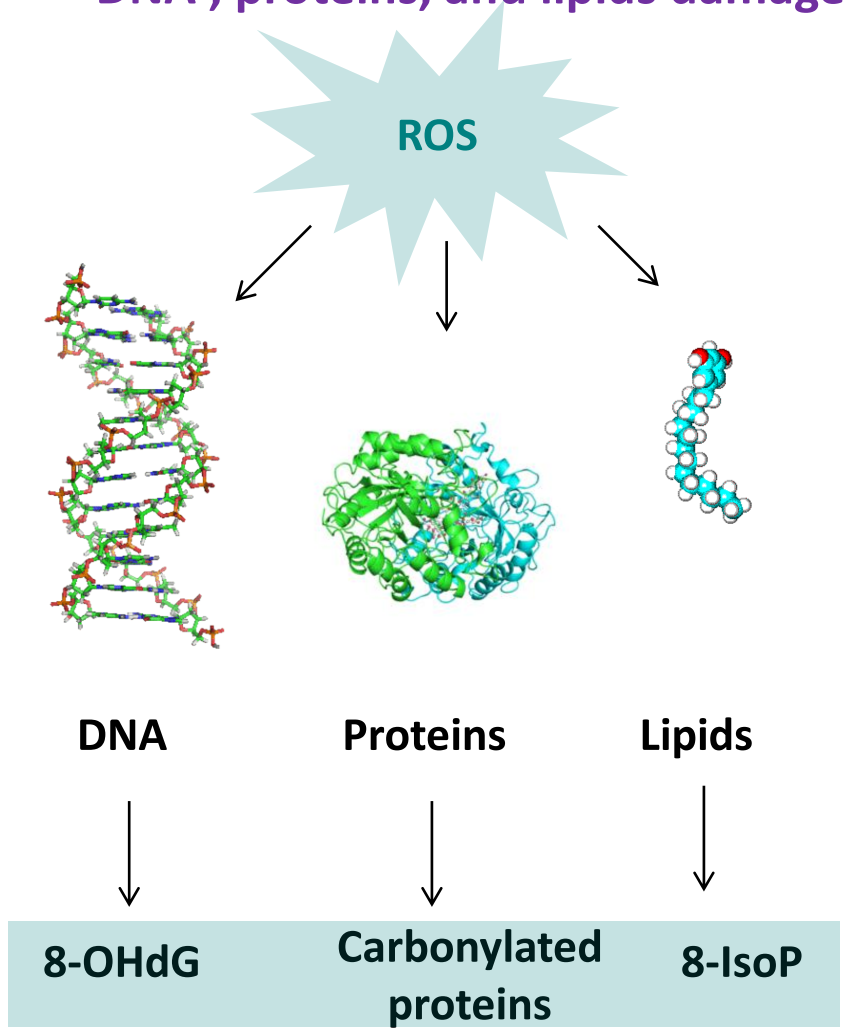
Increase was more important after 6H for C=12µg/cm²

$PM_{2.5\text{-}0.3}$ > $EOM_{2.5\text{-}0.3}$ > $NEM_{2.5\text{-}0.3}$

$EOM_{0.3}$ ≥ $EOM_{2.5\text{-}0.3}$

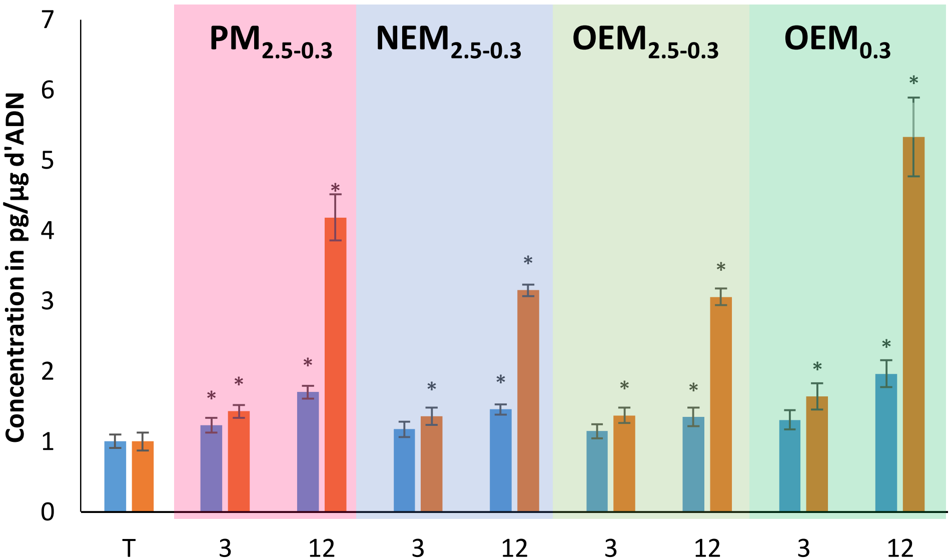
- After 6 hours of exposure, the cell produces few ROS with a strong cellular defense.
- After 24 hours, the production of ROS continues to increase, with less defense (as if the cell starts to give up).

DNA , proteins, and lipids damages

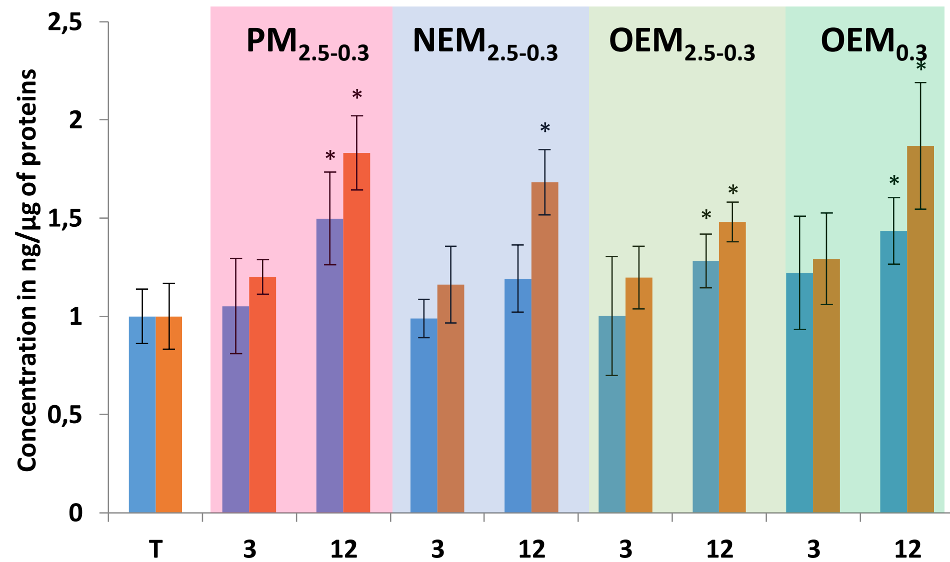


3. Evaluation of oxidative lesions: DNA , proteins, and lipids damages

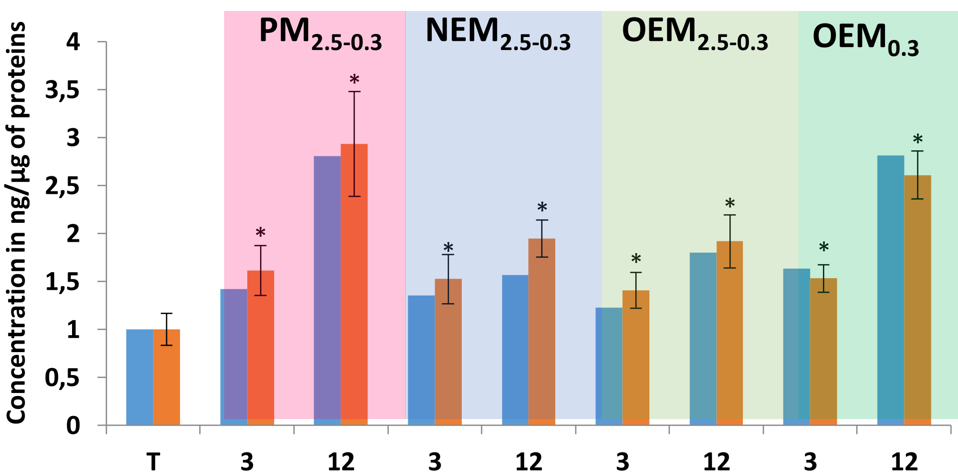
a. 8-OHdG



b. Carbonylated proteins



c.8-isoP



→ Increase was more important after 24 H to C=12µg/cm^2 when ROS are more produced with less antioxidant defense

$PM_{2.5-0.3}$ > $NEM_{2.5-0.3}$ = $OEM_{2.5-0.3}$

$OEM_{0.3}$ > $OEM_{2.5-0.3}$

- All PM fractions, NEM and OEM were able to induce oxidative stress and damages to the cellular macromolecules

- $OEM_{0.3}$ was able to induce toxicological effects more than the $OEM_{2.5\text{-}0.3}$, due to its higher concentration in PAH

Less ROS
More antioxidant defense
Less damages

More ROS
Less antioxidant defense
More damages

Toxicity of fine and quasi-ultrafine particles: focus on the effects of extractable and non-extractable matter fractions

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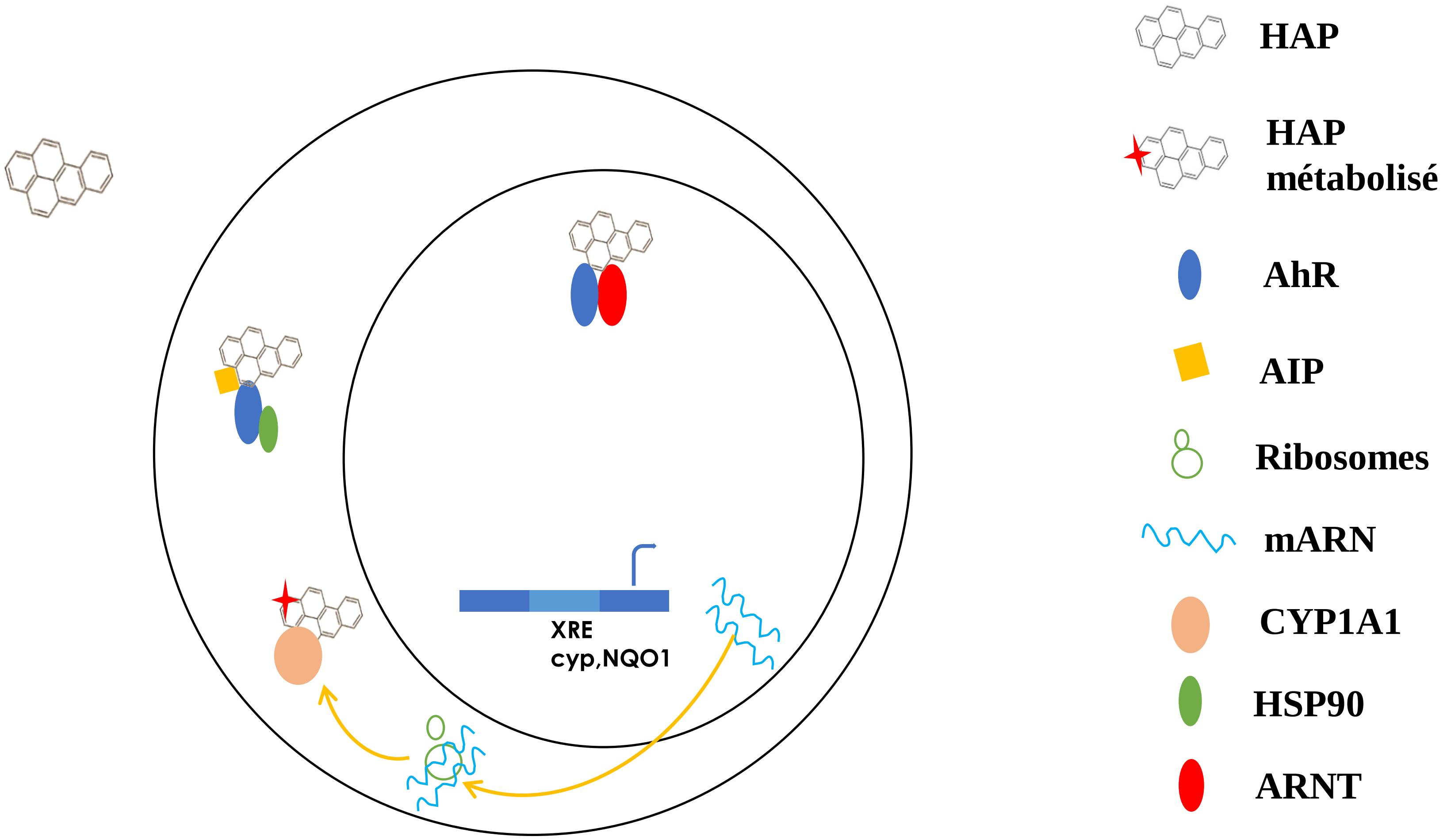
Study of the metabolic pathway of PAHs

Aryl hydrocarbon receptor pathway



Study of the metabolic pathway of PAHs

			$PM_{2.5-0.3}$		$NEM_{2.5-0.3}$		$-OEM_{2.5-0.3}$		$OEM_{0.3}$		BaP	NPyr	9-FluO
		Control	C1	C2	C1	C2	C1	C2	C1	C2	25 µM	25 µM	25 µM
6 h	AHR	1	+	+	–	–	–	–	–	+	–	+	+
	AhRR	1	+	+	–	–	–	–	–	+	–	+	+
	ARNT	1	+	+	–	–	–	–	–	+	–	+	+
	CYP1A1	1	+	+	–	–	–	+	+	–	–	+	+
	CYP1B1	1	+	+	–	–	–	+	+	+	–	+	+
	EPHX-1	1	–	–	–	–	–	–	+	+	–	+	+
	GSTA-4	1	–	+	–	–	–	–	–	+	–	+	+
24 h	AhR	1	–	+	–	–	–	–	–	+	+	+	+
	AhRR	1	+	+	–	–	–	–	–	+	+	+	+
	ARNT	1	+	+	–	–	–	–	–	+	+	+	+
	CYP1A1	1	+	–	–	–	–	+	+	+	+	+	+
	CYP1B1	1	+	+	–	–	–	–	–	+	+	+	+
	EPHX-1	1	+	+	–	–	–	–	+	+	+	+	+
	GSTA-4	1	–	–	–	–	–	–	+	+	–	+	+

$PM_{2.5-0.3}$: induction de la voie de metabolisation des HAPs après 6 et 24 heures d'exposition.

$EOM_{2.5-0.3}$ << $PM_{2.5-0.3}$

↓

Cinétique de métabolisation (< 6H ?) pour $OEM_{2.5-0.3}$

$NEM_{2.5-0.3}$: pas d'induction→ absence des composés organiques

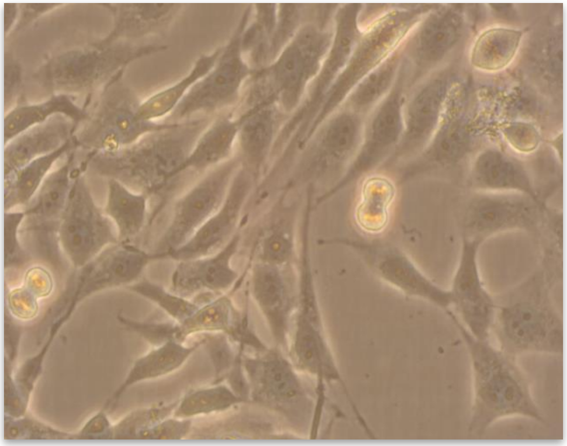
$EOM_{0.3}$>>> EOM2.5-0.3: ++ composés organiques

Gene expression of aryl hydrocarbon receptor (AHR), Aryl-Hydrocarbon Receptor Repressor (AHRR) and aryl hydrocarbon receptor nuclear translocator (ARNT), cytochrome P4501A1 (CYP1A1), cytochrome P4501B1(CYP1B1), epoxide hydrolase 1 (EPHX), glutathione S-transferase alpha 4 (GSTA4) enzymes in BEAS-2B cells exposed 6 and 24 h to $PM_{2.5-0.3}$,$OEM_{2.5-0.}$, $NEM_{2.5-0.3}$ and and positive controls (B[*a*]P, 1-NP and 9-FLO). These values are depicted as mean values and standard deviations of 3 replicates for controls and 3 replicates for exposed cells (Mann–Whitney U-test; vs. Controls, +: P<0.05).

Evaluation of $PM_{2.5}$ toxicity

Beas-2b Cells

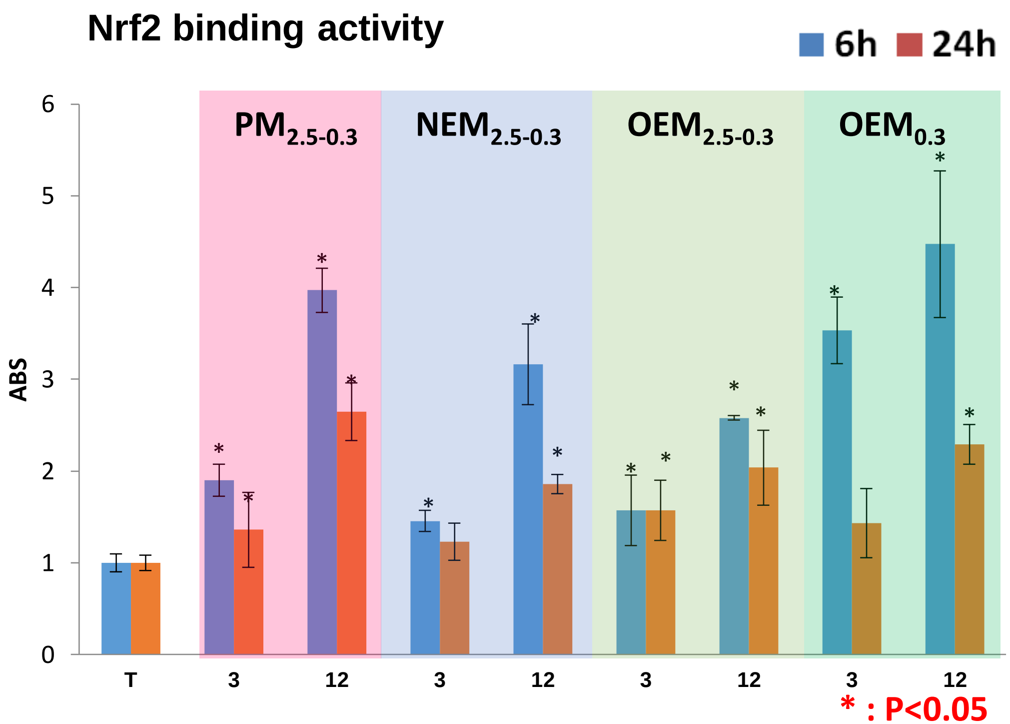
- **Human Bronchial epithelial cells**
- **Non-cancerous cells :** The cell line was originally isolated from the normal human bronchial epithelium of a cancer-free individual
- **Immortalized and transformed with Ad12-SV40 2B**



- **Beas-2B has been used extensively as an in vitro model of pulmonary epithelium in many experimental contexts, including toxicology testing, respiratory injury, wound healing, and neoplastic transformation (Zhao and Walter T. Klimecki ,2015).**
- **This is reflected in almost 1,200 publications referring to BEAS-2B in NCBI PubMed**

Evaluation of cellular defense against oxidative stress

b. NRF2 binding activity



Cellular surrender after 24 hours has been revealed at the protein level, with an important increase in the activity of Nrf2 at 6h

→ Significant increase after 6 and 24 H

Increase was more important after 6H to C=12µg/cm^2

$PM_{2.5\text{-}0.3}$ > $EOM_{2.5\text{-}0.3}$ > $NEM_{2.5\text{-}0.3}$

$EOM_{0.3}$ > $EOM_{2.5\text{-}0.3}$