Methodology development for the characterization of toxicological risks related to particulate pollution in underground stations

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Introduction

Context: During a day, a traveler will pass through one or many microenvironments (Duan, 1982) (e.g., parking, wagon, cabin) and may be exposed to spatially and temporally variable (e.g., in concentration and in chemical composition) particle pollution. The cumulative effects of Particulate Matter (PM) on the health of travelers are poorly studied, due to the lack of PM sampling methods for *in vitro* studies. For example, filter sampling can be used to determine the PM composition by directly analyzing the filter, but it is necessary to desorb the PM from the filter to expose cells to PM *in vitro studies*. However, during this desorption step, PM are only partially desorbed and could be modified (composition, diameter), which would bias the interpretation of the results (Roper et al, 2015). It is thus required to find a **sampling method suitable both for *in vitro* tests** and **physicochemical characterizations**. This method must also **collect sufficient PM mass** for *in vitro* test (Kumar et al, 2021), **be movable in microenvironments** and **collect representative PM** (i.e., without under- or overestimating the true concentration value of the considered environment).

Study: Within the framework of the Aerorep thesis and the TOXinTRANSPORT project, 2 cyclones (Bertin Instruments) were tested in real conditions in underground stations: (1) the Coriolis Micro directly samples in a liquid, while (2) the Coriolis Compact uses dry sampling in a cone. The 2 cyclones were tested under different conditions (sampling liquid, sampling duration) and the samples were exposed on human cells (see coculture model on the right). The poster presents the methodologies used, from the deployment of the cyclones in underground stations to *in vitro* tests of the samples. Preliminary results on the sampling efficiency of the Coriolis are also presented.

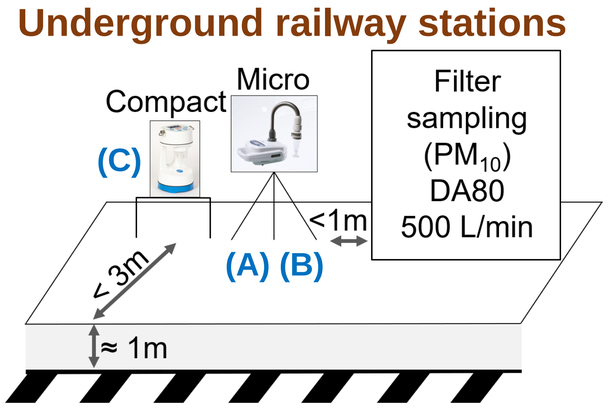
Coculture model:

Human monocyte-derived macrophages **THP-1**

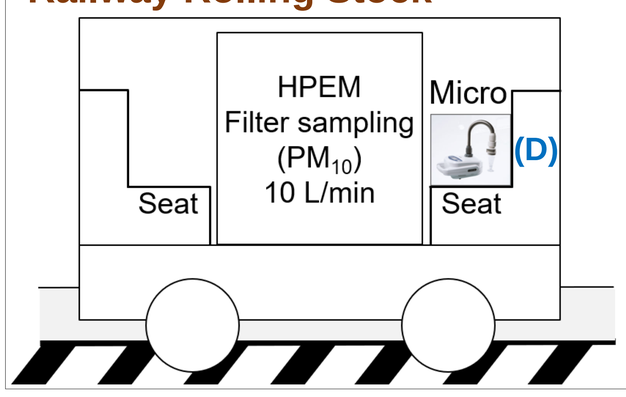
Human pulmonary epithelial cells **A549**

1. Particles sampling: 2 campaigns, 4 scenarios

Underground railway stations



Railway Rolling Stock



Coriolis Scenario	Sampling duration	Collection medium
Micro (A)	2h (n=13)	**Culture medium RPMI 1x + 1% Fetal Bovine Serum** (FBS) filled with 9/10 water/RPMI every minute
Micro (B)	4h (n=3)	**Milli Q water**, filled with Milli Q water every minute
Compact (C)	2h (n=6) 4h (n=3)	**Dry sampling** in a cone
Micro (D)	10 min (n=2)* 2h (n=1) 4h (n=2) 8-9h (n=4) 14h (n=1)	**Milli Q water** with 0,001% **surfactant** (Tween®20), filled with Milli Q water every minute

Compact: 50 L/min
Micro: 100 L/min except for * at 300 L/min

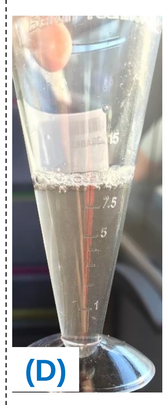
2. Particles extraction for *in vitro* tests

(A)

Samples are exposed directly on the cells.

Few preparation steps. Some contamination risks.

Cell culture medium (RPMI) concentration is modified during sampling (evaporation), which can have an impact on cells.



(D)

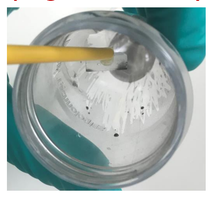
Dilution 9/10 in RPMI **10x** + 10% FBS, before exposure on cells.

PM are not adsorded on walls due to surfactant. Samples can not be tested directly on cells. Use of 10X medium limits dilution but is only available in RPMI.

(B)

Not exposed on cells.

PM are adsorbed on walls. Need agressive methods for desorption (e.g. wall scraping).



(C)

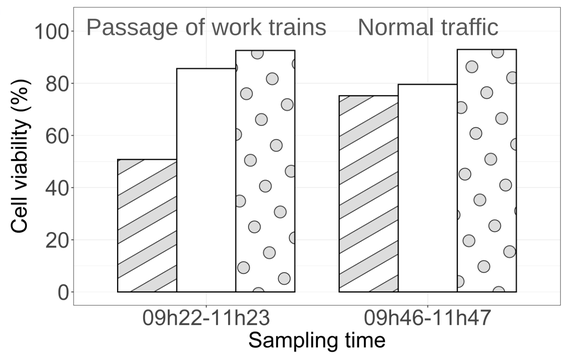
Resuspended in RPMI 1x + 10% FBS.

PM are not adsorded on walls due to surfactant. Use of « fresh » culture medium that limits contamination risks. Control of the medium volume.

3. Samples exploitation: *in vitro* test examples

Cell viability: Measure with Prestoblue test after 24h sample exposure on cells.

(C)



	Work trains	Normal traffic
Sum of Al, Ba, Cr, Cu, Fe, Si (Compact)	20 mg/L	35 mg/L
Organic carbon (DA80)	44 µg/m³	17 µg/m³
PAH (DA80)	11 ng/m³	4 ng/m³

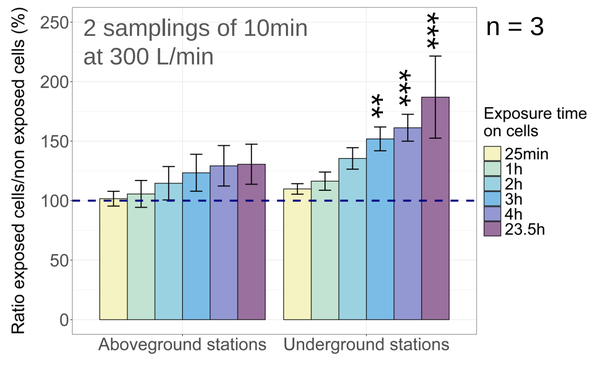
Sample volume : 8 mL

(D)

Considering the 1:2 dilution, the lowest cell viabilities were obtained for the 8h test sampled during a heat wave and the 14h test , with 85% and 86% respectively.

Reactive Oxygen Species (ROS) production: Measure by DCFH-DA test.

(D)



** p<0.01, *** p<0.001 compared to unexposed cells (Anova, Dunnett test)

	Aboveground stations	Underground stations
Sum of Ba, Cr, Cu, Fe, Si, Ti (Micro)	2 mg/L	21 mg/L

Sample volume : 6 mL

(C)

All 9 samples significantly induced ROS on cells after 2 or 3h of exposure compared to unexposed cells.

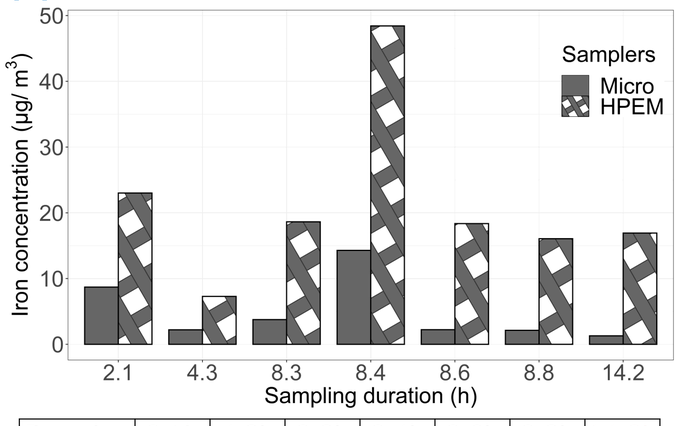
4. Representativeness of collected samples

(C)

Ratio between Fe concentration in Compact samples and in DA80 filters sampled in parallel one after the other

Fe (µg/m³)	Duration	2h	2h	4h	4h	4h
	Compact	16.6	35.2	30.3	10.6	40.5
	DA80 mean	14.6	79.2	64.5	69.4	81.9
	Ratio samplers	114%	44%	47%	15%	49%

(D)



Duration	2.1h	4.3h	8.3h	8.4h	8.6h	8.8h	14.2h
Ratio samplers	38%	30%	20%	29%	12%	13%	7%

Conclusions and outlook

Compatibility between the scenarios and the different analyses:

Coriolis	PM recovery	*In vitro* tests	Metals analyses	PAH analyses
Micro (A)	✓	x	x	✓
Micro (B)	x	Not tested	Not tested	Not tested
Compact (C)	✓	✓	✓	✓
Micro (D)	✓	✓	✓	✓

- Both Coriolis **collect enough PM** mass for *in vitro* studies with **2h samplings** and **50 µg/m³** of PM_{10} in the sampled air.
- The **effects induced** on the cells are **different** according to the **physicochemical characteristics** of the test samples.
- **Underestimation** of Fe **sampling** compared to filter-based ones.
- The **sampling efficiencies** of both 2 Coriolis should be further investigated.
- Need to **standardize** the sampling **parameters** (e.g., collection time) in future campaigns to compare assays with each other.

References

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