

On-site ALI versus Submerged Culture: Toxicological and Chemical Characterization of Brake Wear Nanoparticles

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27th ETH-Nanoparticles Conference (NPC-24)
and Aerosols-24, June 10-14, ETH Zurich,
Switzerland



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Outline

1. Introduction

- Framework & nPETS Project
- Aim of the work

2. Experimental

- Instrumentation
- Materials & Methods

3. Results

- Physico-chemical Characterization
- Toxicological Characterization

4. Conclusions

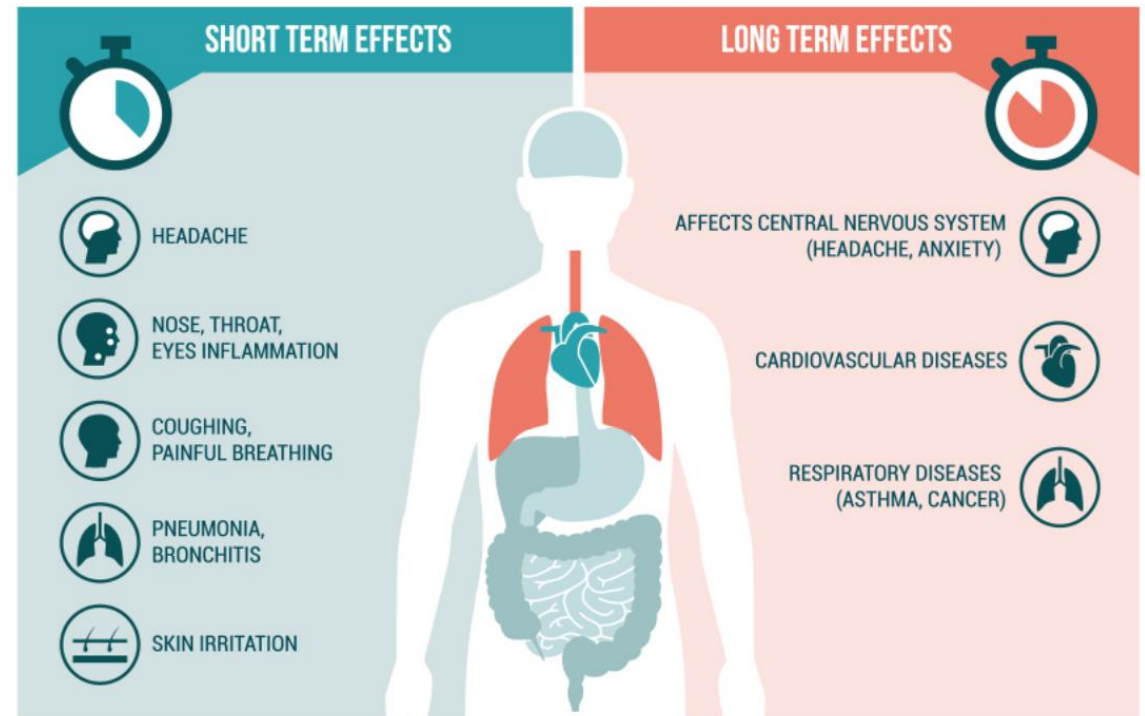
Framework

Air pollution is one of the major environmental risks to health
Almost every organ in the body can be affected by air pollution

- Air pollution is associated with generation of oxidative stress and inflammation in human cells, which may lead to chronic diseases
- Respiratory tract is the main system affected by air pollution

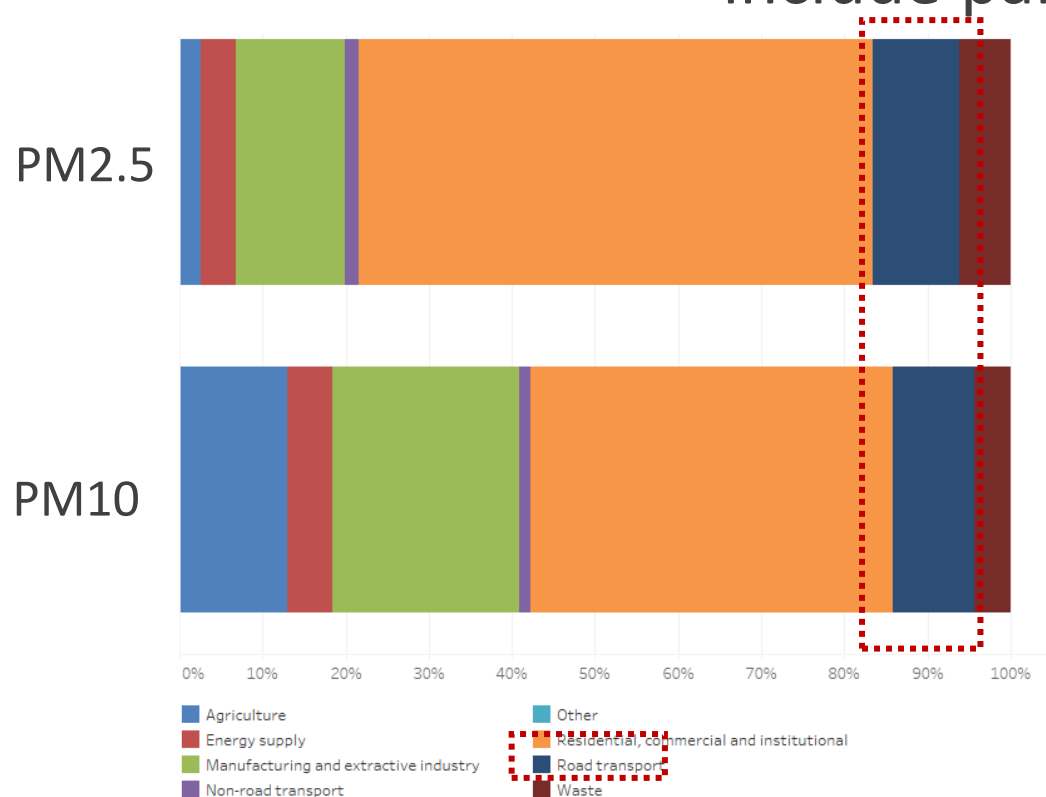
World Health Organization (2021). WHO global air quality guidelines: particulate matter (PM2.5 and PM10), ozone, nitrogen dioxide, sulfur dioxide and carbon monoxide.

12/06/2024



Framework

Air pollutants with the strongest evidence for public health include particulate matters (PMs)



- Respectively 10.4 % and 9.7 % of total PM_{2.5} and PM₁₀ are related to the road transport
- PMs from the road transport are divided in Exhaust and Non-Exhaust emissions

Air pollution in Europe: 2023 reporting status under the National Emission reduction Commitments Directive. doi: 10.2800/149724



nPETS Project

- **Aim** - Characterization of nano-emissions generated by the transport sector
- **WP3:** collection of nano-particulates generated by brakes during laboratory tests
- **WP6:** physico-chemical and toxicological characterization of nano-emissions from different traffic sources (inorganic fraction)




**Nanoparticle Emissions
From The Transport Sector:
Health And Policy Impacts**



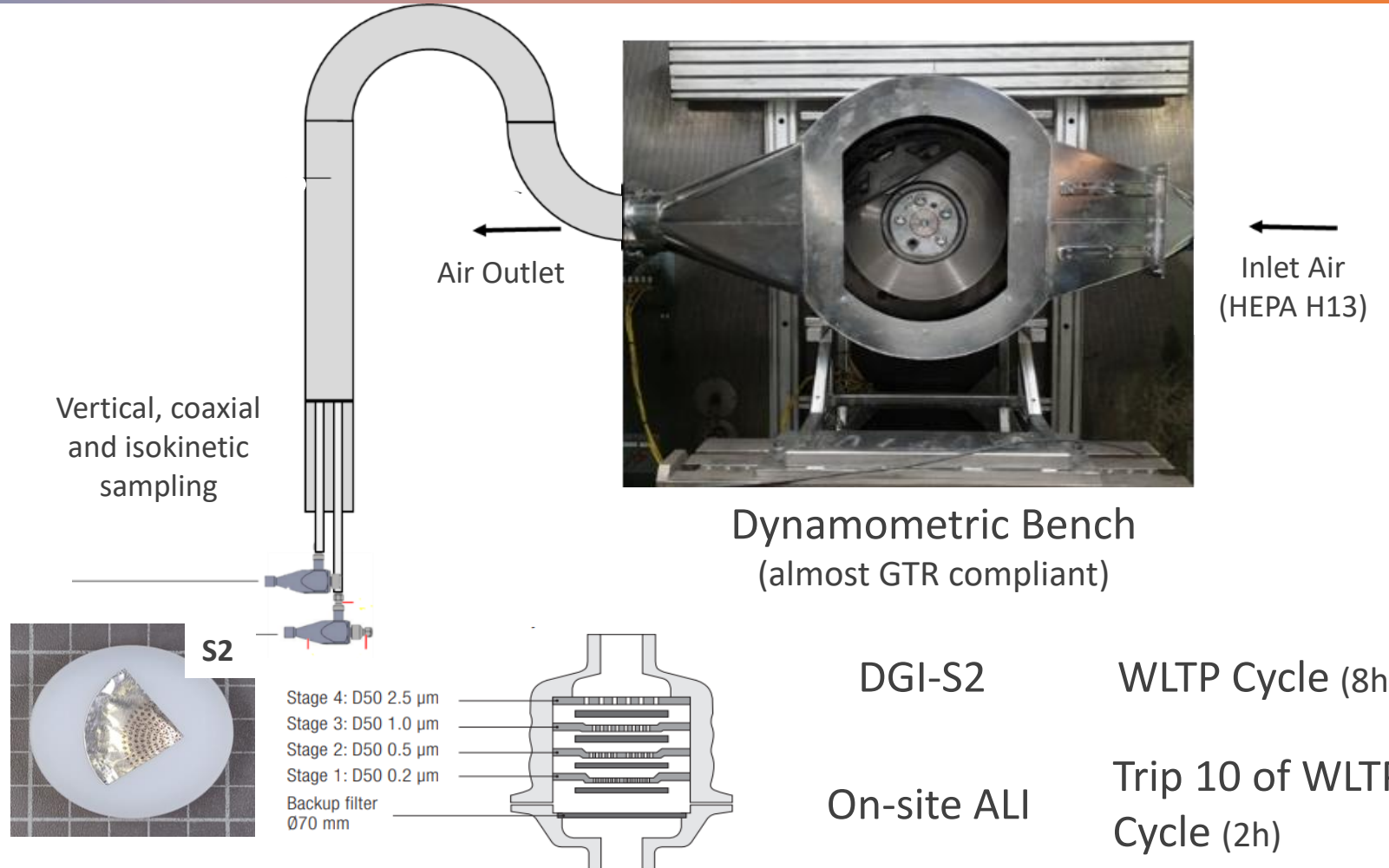
Horizon 2020 – Grant n°954377



Aim of the Work

- Perform reliable physico-chemical characterization of non-exhaust nano-emissions produced by brakes
 - Compare the toxicity response, of A549 cells exposed to nano-emissions, in submerged and on-site ALI conditions
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Instrumentation



Materials & Methods

Materials

Grey Cast-Iron
Braking Disc

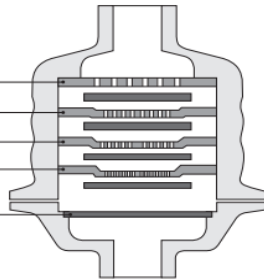


+

M1a & M1b
(Low Steel)



DGI	
Stage	d50 / μm
S4	2.10-2.20
S3	0.80-0.90
S2	0.40-0.45
S1	0.13-0.15
Back-up	-

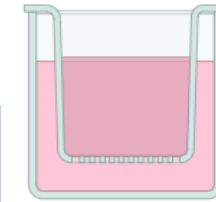
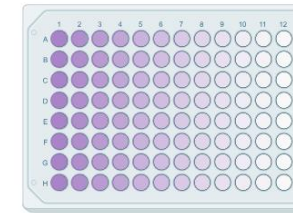


Chemical Characterization

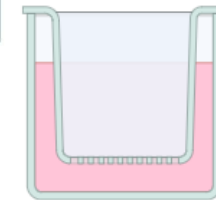


Toxicological Characterization

Alamar Blue Assay



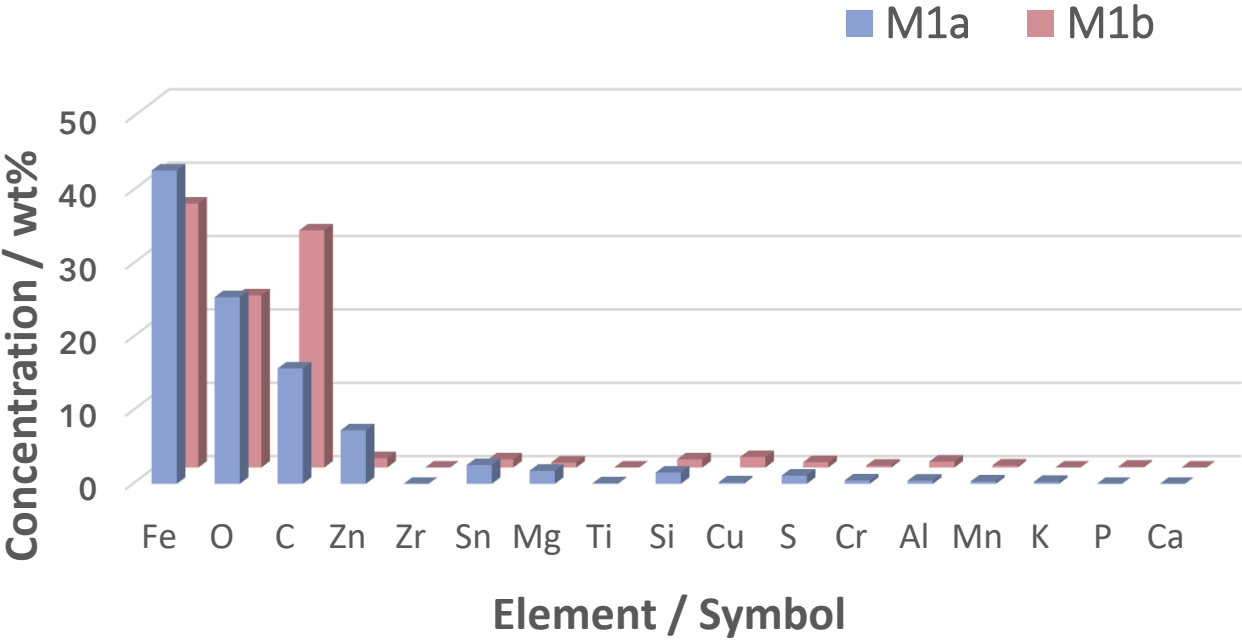
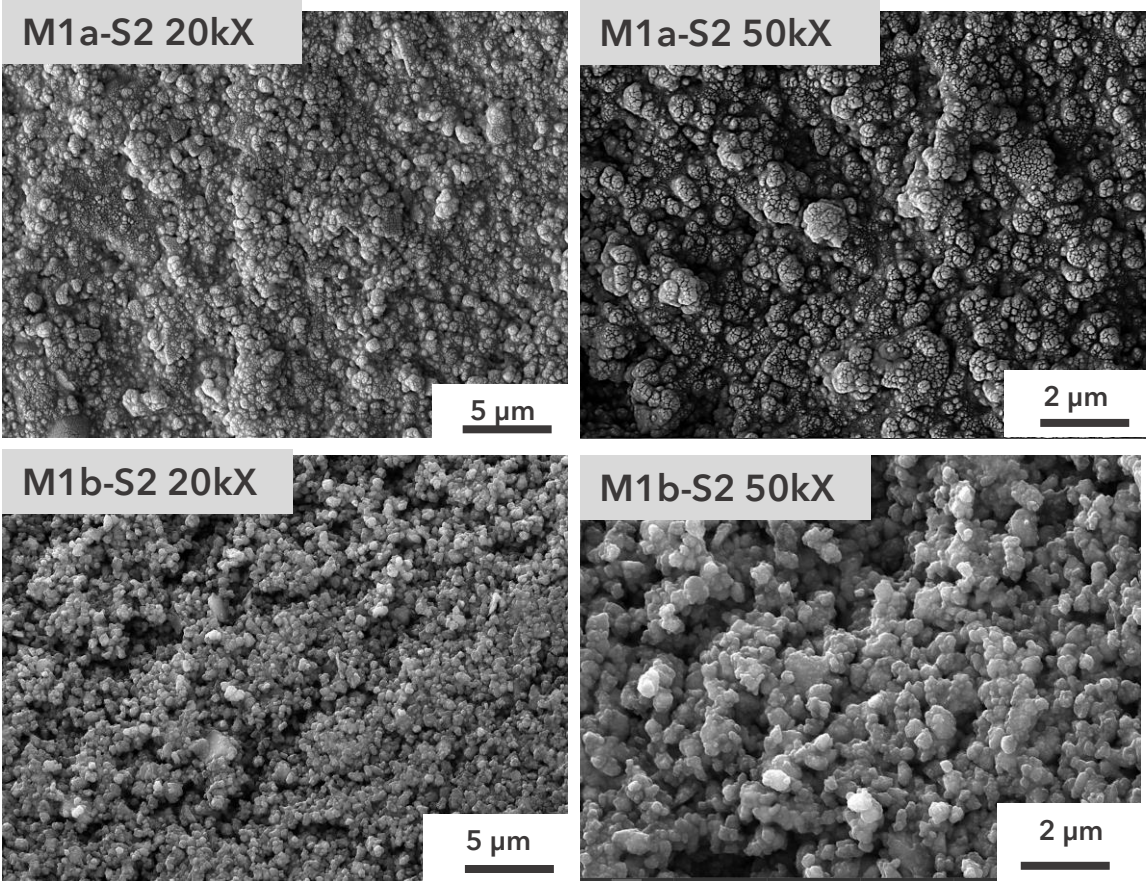
Submerged
Conditions (A549 &
dTHP-1)



On-site ALI
Conditions (A549)

ALI PMs exposure	2 hours
Incubation	24 hours
Alamar Incubation	3 hours
Replicates	3 test for each friction material

SEM/EDS Characterization



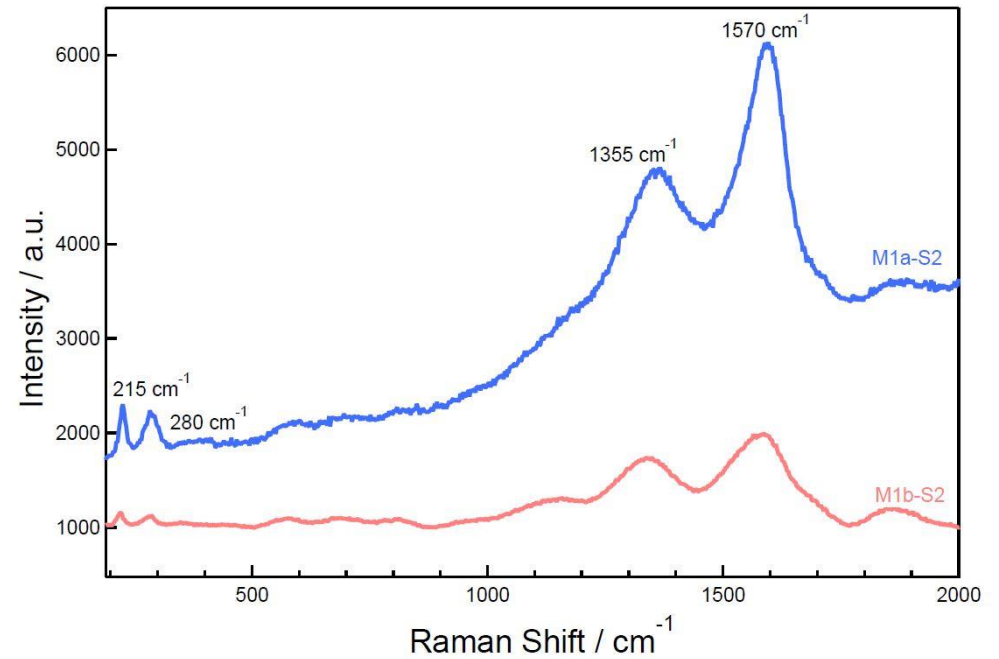
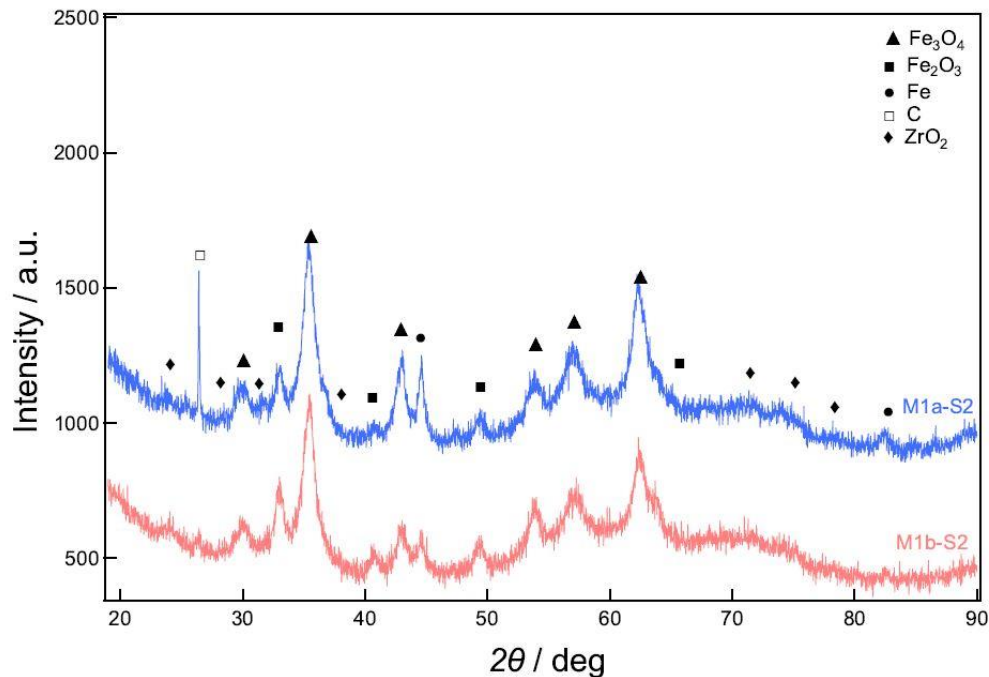
Distribution of elements as measured for the **M1a** and **M1b** samples of DGI S2.

The elemental composition of NPs emission is dominated by three main elements, **Iron**, **Carbon** and **Oxygen**.

XRD & Raman Characterization

X-Ray Patterns

- Fe_3O_4 Magnetite M1b > M1a
- Fe_2O_3 Hematite M1a > M1b
- Fe Iron M1b > M1a
- C Graphite M1a > M1b

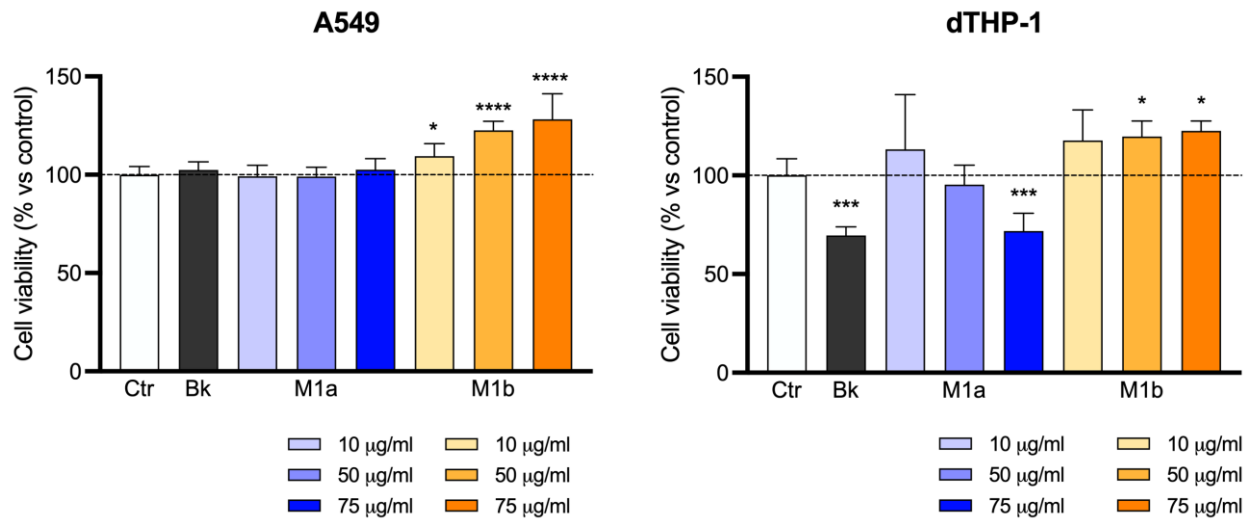


Raman Spectra (confirms XRD findings)

- 50 to 700 cm^{-1} signals related to Iron oxides
- 1200 to 2000 cm^{-1} signals related to elemental Carbon

Toxicological Characterization

Submerged Conditions

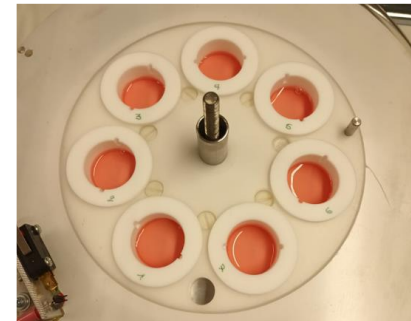
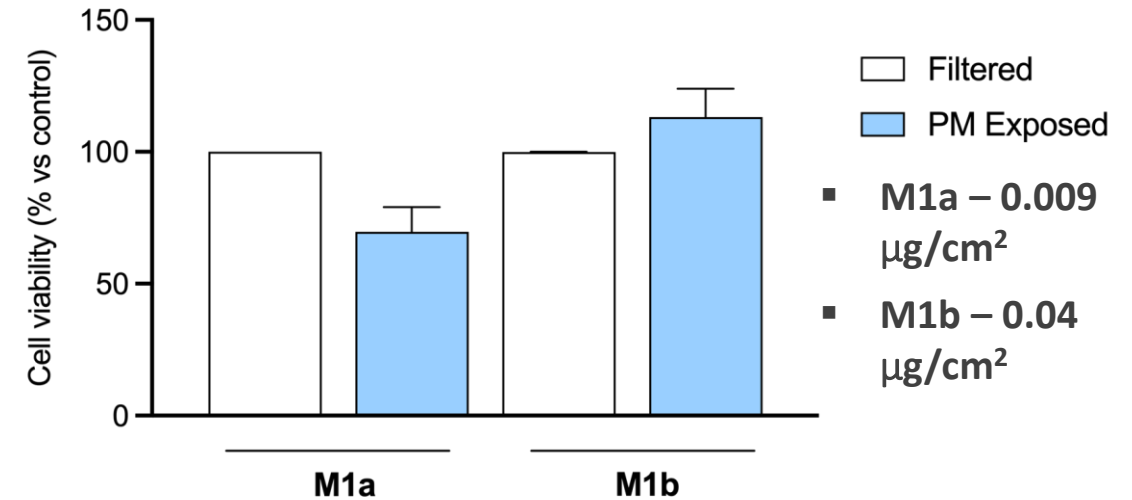


- **A549** cell viability is not affected by M1a and M1b emissions
- For **dTHP-1** a dose dependent behaviour was observed for M1a emission

Statistical analysis: differences were established using a two-way ANOVA and considering significant at * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, **** $p \leq 0.0001$

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On-site ALI



There are no statistical differences in the **A549** cell viability of M1a and M1b emissions



Conclusions

- M1a and M1b non-exhaust nano-emissions (<450 nm) were collected and analysed
- In spite of the observed differences in chemical composition between M1a and M1b, the cell viability of A549 was not affected for both in submerged and on-site ALI conditions
- M1a emission exhibit a dose-dependent effect in cell viability in dTHP-1. This might be related to the higher amount of metals compared to the M1b emission

Acknowledgments

This research was supported by the European Commission's Horizon 2020 research and innovation programme: nPETS (grant agreement No 954377, <https://www.npets-project.eu/>)

Thank You!

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