Toxicity of real-world subway emissions in an ALI exposure model

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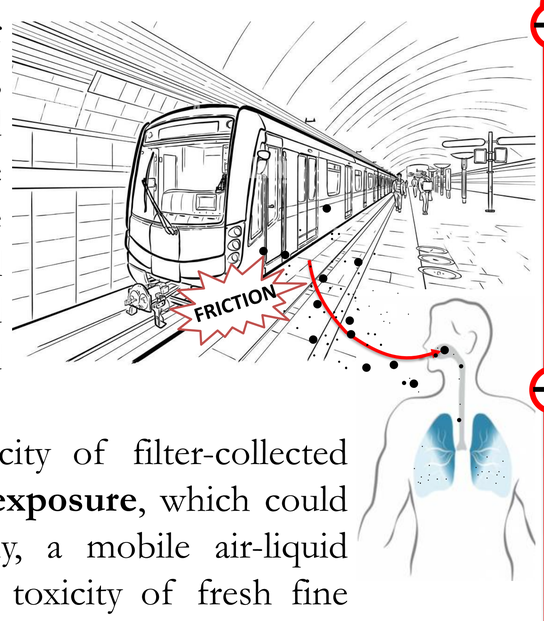
BACKGROUND AND RATIONAL

In subways, $PM_{2.5}$ can reach 5-10 times higher levels compared to a busy street. Thus, particles are produced in an isolated environment with limited access to the outside (1,2). $PM_{2.5}$ generated by the **friction of the train wheels, the brakes**, are rich of metal content and become harmful when inhaled because they are small enough to reach and deposit in the deepest region of the lung.



Different studies have investigated the toxicity of filter-collected particles, but not yet direct **on-site *in vitro* exposure**, which could provide more realistic results. In this study, a mobile air-liquid interface (ALI) system was used to test the toxicity of fresh fine particles emitted in real time from subway trains on a platform in Stockholm metro station.

MATERIAL AND METHODS

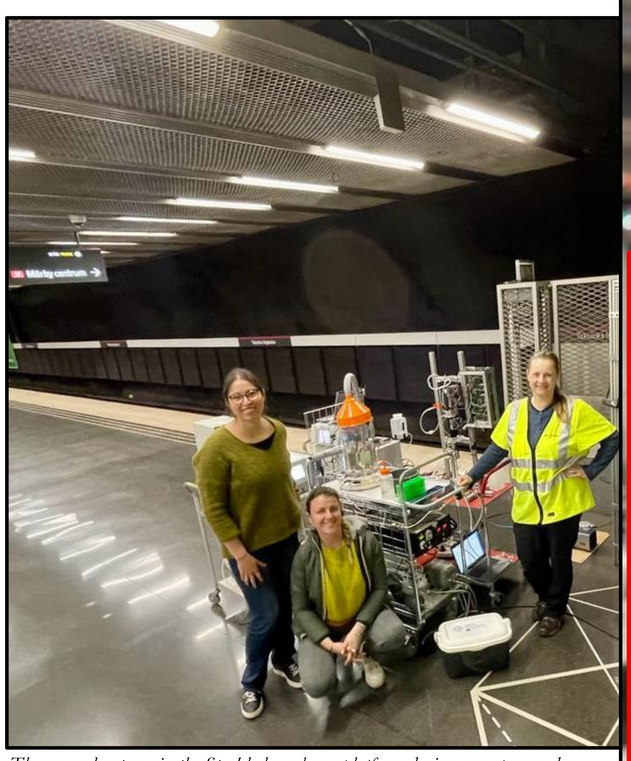
Human cell culture: A549 human cells cultured alone or co-cultured with THP-1 for higher sensitivity. The alveolar model was chosen to mimic the toxicity of $PM_{2.5}$ in the deepest regions of the lungs where less clearance occurs, and particles accumulate.

Exposure and incubation: Cells were exposed for 2 h during weekdays to trains emissions and incubated for 24 h. A shorter incubation period (3 h) was tested but did not provide enough time for cells to produce and release cytokines.

Particles <2.5 μm: Directly sampled from the subway platform. Particle concentration was increased five times (using a concentrator, 3), before administered to the cells. Particle size selection was performed using a cyclone and the concentration was measured with an optical particle counter before and after the ALI system.

Toxicity endpoints: a) Cytotoxicity (Alamar blue);
b) Inflammation:
- Cytokine release (Multiplex assay)

SUBWAY PLATFORM:
in situ exposure



The researcher team in the Stockholm subway platform during an exposure day.

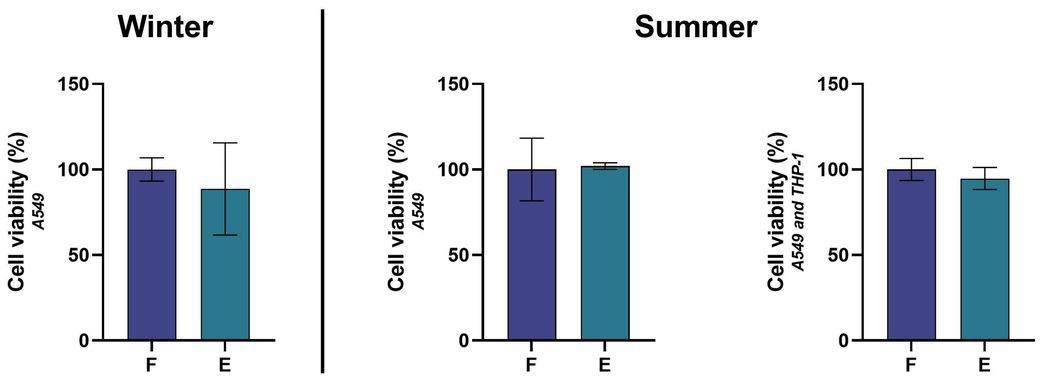
In situ exposure: the ALI system with alveolar cells sampling when a train was passing.

WINTER *vs* SUMMER

In subways, the quality of air and its circulation can be affected by various factors such as the frequency of trains and the number and location of platforms and exits. During the **summer** months, the **ventilation system is activated** to let fresh air in, which also helps to lower the temperature. As a result, the concentration of particles emitted by the trains decreases.

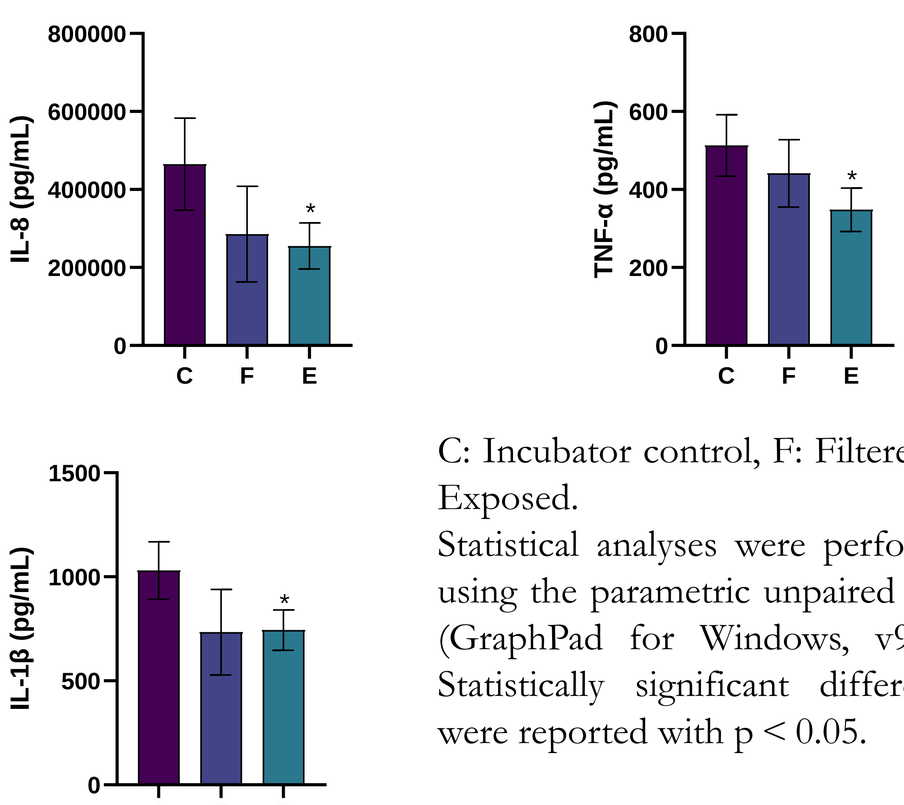
RESULTS

- **Cell viability (%)**



Results are expressed as mean ± SD of at least 4 independent experiments. Exposed groups (E) are normalized on filtered control groups (F).

- **Cytokine release in A549 in winter**



C: Incubator control, F: Filtered, E: Exposed.
Statistical analyses were performed using the parametric unpaired t-test (GraphPad for Windows, v9.4.0). Statistically significant differences were reported with $p < 0.05$.

- **Estimated exposure dose**: 1,39 μg/cm².

CONCLUSIONS

$PM_{2.5}$ particles sampled in the subway platform did not affect cell viability at the studied conditions. Despite that, during winter, there is higher variability in cytotoxicity results between filtered and exposed groups than in summer. This comparison confirmed that ventilation has to be considered when testing particle toxicity in the subway.
In **winter**, when the ventilation was off, it was observed a **statistical decrease in inflammatory cytokines** (IL-8, TNF-α, IL-1β) release in A549. The exposure dose was variable between days and relatively low compared to lab conditions. Further analyses are required to confirm the decreased inflammatory trend e.g., measure of cytokines gene expression in mono (A549) and co-culture (A549 and THP-1).

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