P17. Assessment of in vitro toxicity of particles from residential biomass combustion

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 Because it represents an important source of ambient particulate matter, residential biomass burning has a significant impact on indoor air quality. Nevertheless, indoor particles arising from the use of biomass combustion appliances has been scarcely characterised in relation to their chemical composition and toxicity. In the present study, samples of particulate matter with aerodynamic diameter lower than 10 µm (PM_{10}) were collected in two households equipped with distinct combustion appliances (open fireplace and woodstove) and in the absence of other indoor sources. Simultaneously, sampling was carried out outdoors to investigate the toxicity of particles in a rural area strongly impacted by biomass burning emissions. The overall toxicity of the particles was assessed with the Vibrio fischeri bioluminescence inhibition assay. The cytotoxicity was evaluated in human lung epithelial cells (A549) by two distinct assays: the mitochondrial reduction of WST-8 and the release of the enzyme lactate dehydrogenase (LDH). Indoor particles generated during the operation of the fireplace were the most toxic to Vibrio Fischeri, while indoor particles from the woodstove operation and outdoor air had low toxicity or were non-toxic. Samples collected during the fireplace operation induced the highest decrease in the cellular metabolic activity of A549 cells but did not compromise the integrity of the cytoplasmatic membrane. Significant correlations were recorded between the metabolic activity reduction of Vibrio Fischeri, as well as between A549 cells and several PM_{10} chemical components (e.g. polycyclic aromatic hydrocarbons).

 Ao representar uma fonte importante de material particulado na atmosfera, a queima doméstica de biomassa tem um impacto significativo na qualidade do ar interior. No entanto, as partículas libertadas para o interior das habitações através da utilização de equipamentos de combustão de biomassa têm sido pouco caracterizadas do ponto de vista químico e toxicológico. Neste trabalho, realizou-se a amostragem de partículas com diâmetro inferior a 10 µm (PM_{10}) em duas habitações durante a utilização de equipamentos de combustão distintos (lareira aberta e recuperador de calor) e na ausência de outras fontes interiores. Simultaneamente, foi feita a colheita de PM_{10} no exterior para avaliar a toxicidade das partículas numa zona rural fortemente impactada pelas emissões de queima de biomassa. A toxicidade global das partículas foi examinada com o teste de inibição da bioluminescência da Vibrio Fischeri. A citotoxicidade foi avaliada em células do epitélio pulmonar humano (A549) utilizando dois ensaios distintos: redução mitocondrial do WST-8 e libertação da enzima lactato desidrogenase (LDH). As partículas amostradas durante a operação da lareira aberta apresentaram toxicidade elevada para a Vibrio Fischeri, enquanto que partículas colhidas durante a operação do recuperador de calor e no exterior apresentaram valores de toxidade reduzida ou nula. As amostras obtidas durante a operação da lareira aberta provocaram uma redução mais acentuada na atividade metabólica das células A549 não comprometendo, contudo, a integridade da membrana citoplasmática. Foram observadas correlações significativas entre a redução da atividade metabólica das células A459 e vários compostos químicos determinados nas PM_{10} (e.g. hidrocarbonetos aromáticos policíclicos).

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Background:

In addition of being a recognised important source of ambient particulate matter (PM) (Vicente and Alves, 2018, and references therein), residential biomass burning has also a noticeable impact on indoor air quality (e.g. Castro et al., 2018; de Gennaro et al., 2015; Guo et al., 2008; Salthammer et al., 2014; Vicente et al., 2020). Furthermore, people spend most of their time in indoor environments (e.g. Schweizer et al., 2007), meaning that it is where most of human exposure occurs (Morawska et al., 2013). Nevertheless, indoor particles arising from the use of biomass combustion appliances has been scarcely characterised in relation to their chemical composition and toxicity. The toxicological profile of a relevant indoor pollution source is of utmost importance to better understand the potential health risk posed by the source and to develop appropriate control strategies.

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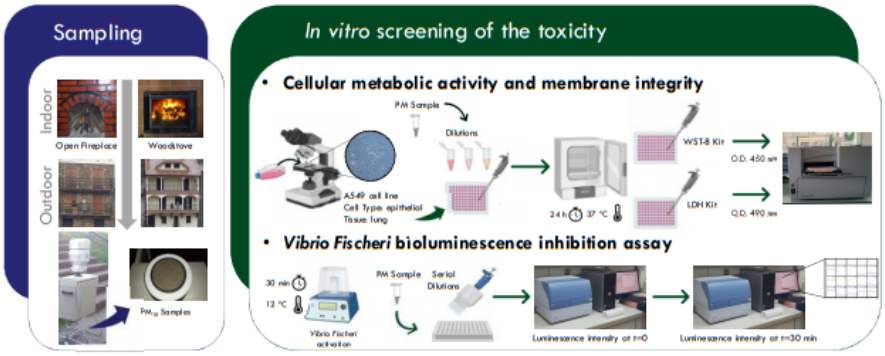
Objectives:

- Evaluate the toxicity of indoor PM generated from Portuguese combustion appliances (open fireplace and woodstove), which are common in the Mediterranean region, using different *in vitro* tests.
- Compare the toxicity results of indoor generated particles with those from outdoor particles, collected in a rural area strongly impacted by biomass burning emissions.

Methods and Results:



A549 Mitochondrial Metabolic Activity

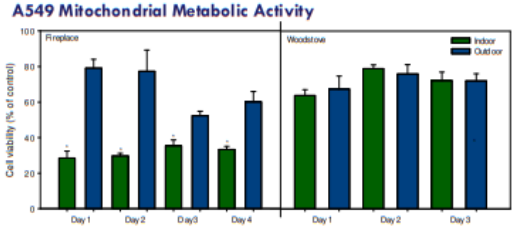
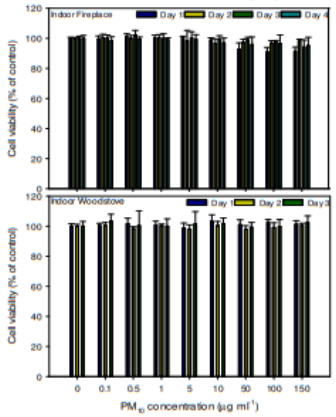
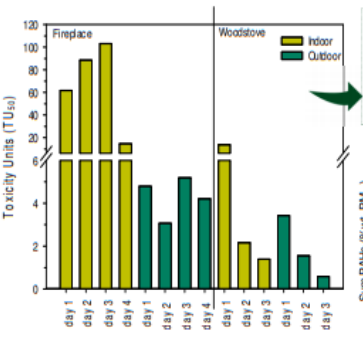


Figure 1. Cell viability assessed with WST-8 assay after 24 h exposure to 150 μg m^{-3} PM_{10}. Asterisks indicate statistical significance compared to outdoor PM_{10} samples ($p<0.05$) assessed by Kruskal Wallis with Dunnett's multiple comparison test.

A549 Cellular Membrane Integrity



Vibrio Fischeri Bioluminescence Inhibition Assay



$TU_{50} \leq 1$ non-toxic
$1 < TU_{50} \leq 10$ toxic
$10 < TU_{50} \leq 100$ very toxic
$TU_{50} > 100$ extremely toxic

Figure 3. Toxicity Units (TU_{50}) calculated from EC_{50} values obtained with Vibrio Fischeri bioassay

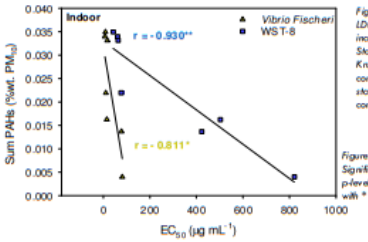


*Figure 4. Pearson correlation coefficients. Significant correlation coefficients at the p-level < 0.05 and 0.01, are marked with * and **, respectively.*

Figure 2. Cell viability assessed with LDH assay after 24 h exposure to increasing PM_{10} concentrations. Statistical analysis was performed by Kruskal-Wallis with Dunnett's multiple comparison test. Asterisks indicate statistical significance compared to control ($p<0.05$).

Conclusions:

- PM_{10} samples did not compromise the integrity of the cytoplasmatic membrane of A549 cells.
- Outdoor samples affected the A549 cells metabolic activity significantly less than their corresponding indoor air counterparts during the operation of the fireplace, whereas no such effect was observed with the woodstove samples.
- The bioluminescent inhibition assay revealed that indoor particles generated during the operation of the fireplace were the most toxic.
- For indoor-generated PM_{10}, organic carbon and PAH were significantly correlated with cellular metabolic activity and bioluminescence reduction, suggesting a role of organic compounds in toxicity.