

P15. Cytotoxicity and mutagenicity of particulate matter from the open burning of tree pruning wastes

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Open burning of agricultural wastes is a worldwide long-standing practice due to its economic and practical benefits. This study aimed to examine the cytotoxicity and mutagenicity of particulate matter below 10 μm (PM10) released from the burning of pruning residues common in Portugal and other countries of the Mediterranean region.

Field measurements were conducted to collect PM10 samples from open burning of vines, olive, willow and acacia pruning branches. To assess the cytotoxicity of the PM10 total organic extract, the A549 cell line, representative of the alveolar type II pneumocytes of the human lung, was used. The potential cytotoxicity was checked using the WST-8 and the LDH assays. The mutagenicity of the PM10-bound polycyclic aromatic hydrocarbons was screened through the Ames test, using *S. typhimurium* TA98 and TA100 strains with and without metabolic activation by the S9 fraction (rat liver microsomal fractions).

PM10 organic extracts induced LDH release in a dose-dependent manner. Regarding the cellular metabolic activity, dose-dependency was lacking for most of the samples. Combined WST-8 and LDH data indicate that PM10 exposure induce the necrotic cell death pathway in which the cell membrane integrity is lost. Significant correlations were found between the cytotoxic response and the PM10 organic component.

Three samples presented mutagenic effects towards TA98 strain without metabolic activation. However, when the S9 fraction was introduced into this strain, the mutagenic effect disappeared, suggesting that these samples lost their mutagenicity after being metabolised by the enzymes from the S9 liver fraction.



A queima de resíduos agrícolas é uma prática mundial comum devido ao seu benefício económico e prático. Este estudo teve como objetivo analisar a citotoxicidade e mutagenidade de matéria particulada inferior a 10 μm (PM10) libertada pela queima de resíduos de poda comuns em Portugal e outros países da região mediterrânea.

No campo, foram realizadas amostragens de PM10 durante a queima de ramos de poda de videiras, oliveiras, salgueiros e acácias. Para avaliar a citotoxicidade do extrato orgânico total do PM10 foi utilizada a linha celular A549, representativa de pneumócitos alveolares tipo II do pulmão humano. A potencial citotoxicidade foi analisada através dos ensaios WST-8 e LDH. A mutagenidade dos extratos de hidrocarbonetos policíclicos aromáticos foi avaliada através do teste Ames, usando as estirpes *S. typhimurium* TA98 e TA100 com e sem ativação metabólica pela fração S9 (frações microssomais de fígado de rato).

Os extratos orgânicos de PM10 induziram uma libertação dose-dependente de LDH. Em relação à atividade metabólica celular, não se verificou uma libertação dependente da dose na maioria das amostras. Dados combinados de WST-8 e LDH indicam que a exposição a PM10 induz morte celular necrótica, na qual a integridade da membrana celular é perdida. Foram observadas correlações significativas entre a resposta citotóxica e a componente orgânica do PM10.

Três amostras apresentaram efeitos mutagénicos para a estirpe TA98 sem ativação metabólica. No entanto, quando a fração S9 foi introduzida nesta estirpe, o efeito mutagénico desapareceu, sugerindo que estas amostras perderam a sua mutagenicidade após serem metabolizadas pelas enzimas da fração S9 do fígado.

Cytotoxicity and mutagenicity of particulate matter from the open burning of tree pruning wastes

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BACKGROUND:

Open burning of agricultural wastes is a worldwide long-standing practice due to its economic and practical benefits (Estrellan and Iino, 2010). Biomass open burning releases a range of air pollutants that contribute to the deterioration of air quality and are harmful to human health. The characterization of particle and gaseous emissions, from the open burning of vine, olive, willow and acacia branches was previously described (Alves et al., 2019), however the toxicity of emissions from burning agricultural waste has been largely neglected (Andreae and Merlet, 2001; Lemieux et al., 2004; Naeher et al., 2007; Chuang et al., 2019).

OBJECTIVES:

This study aimed to examine the cytotoxicity and mutagenicity of particulate matter below 10 µm (PM₁₀) released from the burning of pruning residues common in Portugal and other countries of the Mediterranean region.

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METHODS AND RESULTS:

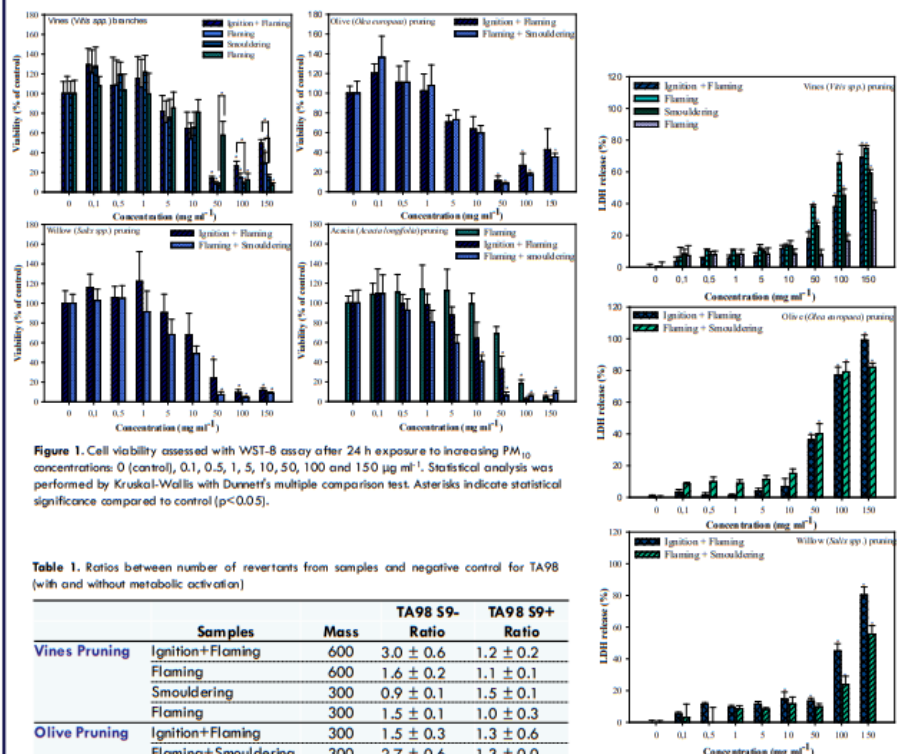
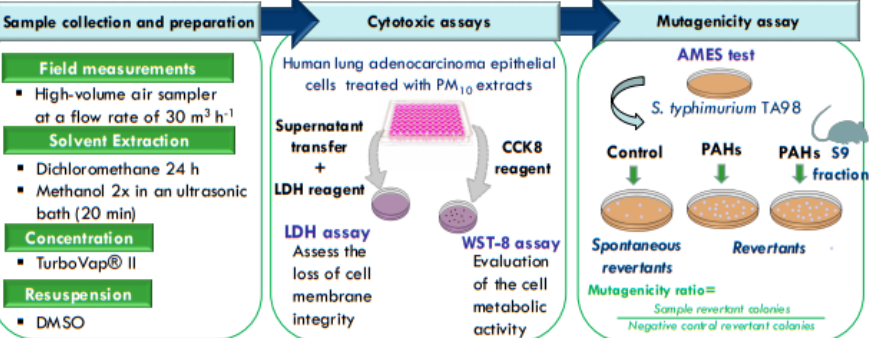


Figure 1. Cell viability assessed with WST-8 assay after 24 h exposure to increasing PM₁₀ concentrations: 0 (control), 0.1, 0.5, 1, 5, 10, 50, 100 and 150 µg ml⁻¹. Statistical analysis was performed by Kruskal-Wallis with Dunnett's multiple comparison test. Asterisks indicate statistical significance compared to control ($p < 0.05$).

Table 1. Ratios between number of revertants from samples and negative control for TA98 (with and without metabolic activation)

Samples	Mass	TA98 S9-		TA98 S9+	
		Ratio	Ratio	Ratio	Ratio
Vines Pruning	Ignition+Flaming	600	3.0 ± 0.6	1.2 ± 0.2	
	Flaming	600	1.6 ± 0.2	1.1 ± 0.1	
	Smouldering	300	0.9 ± 0.1	1.5 ± 0.1	
Olive Pruning	Ignition+Flaming	300	1.5 ± 0.1	1.0 ± 0.3	
	Flaming+Smouldering	300	1.5 ± 0.3	1.3 ± 0.6	
Willow pruning	Ignition+Flaming	150	2.5 ± 0.6	1.9 ± 0.2	
	Flaming+Smouldering	150	1.1 ± 0.4	1.8 ± 0.4	
Acacia pruning	Flaming	40	1.1 ± 0.3	1.0 ± 0.1	
	Ignition+Flaming	25	1.7 ± 0.2	1.2 ± 0.2	
	Flaming+Smouldering	50	0.9 ± 0.2	1.1 ± 0.3	
Blank		4.5	0.8 ± 0.3	1.1 ± 0.2	

CONCLUSIONS:

- PM₁₀ organic extracts induced LDH release in a dose-dependent manner
- Regarding the cellular metabolic activity, dose-dependency was lacking for most of the samples
- Significant correlations were found between the cytotoxic response and the PM₁₀ organic component
- Three samples presented mutagenic effects towards TA98 strain without metabolic activation
- When the S9 fraction was introduced the mutagenic effect disappeared, suggesting that these samples lost their mutagenicity after being metabolised by the enzymes from the S9 liver fraction

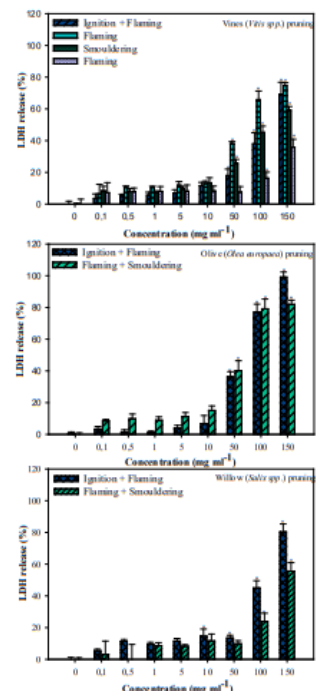


Figure 2. LDH release after 24 h exposure to increasing PM₁₀ concentrations: 0 (control), 0.1, 0.5, 1, 5, 10, 50, 100 and 150 µg ml⁻¹. Statistical analysis was performed by Kruskal-Wallis with Dunnett's multiple comparison test. Asterisks indicate statistical significance compared to control ($p < 0.05$).