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Universidade de Aveiro Departamento de Ambiente e Ordenamento

BINH NGAN VU

MUTAGENICITY ASSESSMENT OF AEROSOLS IN EMISSIONS FROM WOOD COMBUSTION

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Dissertação apresentada à Universidade de Aveiro para cumprimento dos requisitos necessários à obtenção do grau de Mestre do *JointEuropeanMasterinEnvironmentalStudies*, realizada sob a orientação científica da Doutora Célia Alves, Investigadora Auxiliar no Centro de Estudos do Ambiente e do Mar da Universidade de Aveiro e co-orientação da Doutora Ruth Pereira, Investigadora Auxiliar no Centro de Estudos do Ambiente e do Mar da Universidade de Aveiro e do Doutor Wolfgang Ahlfda *Hamburg UniversityofTechnology*.

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keywords Residential wood combustion, $PM_{2.5}$, polycyclic aromatic hydrocarbons, mutagenicity, Ames test.

abstract Polycyclic aromatic hydrocarbon (PAH) extracts of $PM_{2.5}$ collected from combustion of seven wood species and briquettes were tested for mutagenic activities using Ames test with *Salmonella typhimurium* TA98 and TA100. The woods were*Pinuspinaster* (maritime pine), *Eucalyptus globulus* (eucalypt), *Quercussuber* (cork oak), *Acacia longifolia* (golden wattle), *Quercusfaginea* (Portuguese oak), *Oleaeuropea* (olive), and *Quercus ilex rotundifolia* (Holm oak). Burning experiments were done using woodstove and fireplace, hot start and cold start. A mutagenic/weak mutagenic response was recorded for all species except golden wattle. The extracts with indirect acting mutagenicity were mainly obtained from fireplace and cold start conditions. The strong mutagenic extracts were not correlated with high emission factors of carcinogenic PAHs. Several samples were weak mutagens at low concentration of PAHs. The negative result recorded for the golden wattle extracts is positive since after confirmation, this species can be recommended for domestic use.

Table of contents

List of Tables

List of Abbreviations

ANOVA	one-way analysis of variance
BaP	benzo[a]pyrene
DMSO	Dimethyl sulfoxide
EC	elementary carbon
EOM	extractable organic matter
IARC	International Agency for Research on Cancer
IPCC	Intergovernmental Panel on Climate Change
OC	organic carbon
OECD	Organisation for Economic Co-operation and Development
PAH	polycyclic aromatic hydrocarbon
PC	positive control
PCA	principal component analysis
PM	particulate matter
S. typhimurium	*Samonella typhimurium*
TC	total carbon

Part I: General introduction

1. Introduction

There is a growing awareness that current energy systems with their heavy reliance on fossil fuels are not sustainable. In Europe, fossil fuels account for 78.7% of the primary energy supply (European Commission Energy, 2009). Renewable energy sources can play a fundamental role in the transition to more sustainable energy systems.

Biofuel combustion is an important source of renewable energy. Among biomass fuels, wood is one of the most dominant sources and therefore, it is consumed in a variety of uses. In addition to the use in advanced biofuel combustion power plants, wood is extensively employed in the residential sector for cooking and heating purposes. Its use is recommended, as it is renewable source of energy. Wood is the most dominant fuel for domestic combustion in Portugal (Borrego et al., 2010). It was estimated that around 390,000 tonnes of wood are annually used in domestic combustion appliances (Dias, 2002).

Residential wood combustion is one of the major sources of fine particles in the neighbourhood and the region, especially in wintertime. Biomass combustion contributes significantly to aerosol in United States (Holden et al., 2011) and in Europe in wintertime (Gelencser et al., 2007; Puxbaum et al., 2007). In addition, residential wood combustion has been assessed to be major source of organic compounds, including particulate polycyclic aromatic hydrocarbons (PAHs) and other volatile organic compounds, which contribute to the formation of secondary particles (Baek et al., 1998; Claxon et al., 2004; Gaeggeler et al., 2008). Houses utilising wood combustion appliances contain higher concentration of particles associated with inorganic and organic compounds such as metals, butadiene, benzene and PAHs in comparison to houses without these devices (Bølling et al., 2009).

Fine particles ($PM_{2.5}$ - particulate matter that has aerodynamic diameter equal to or below 2.5 μm) are one of the most important pollutants in the outdoor air. Due to their small size, fine particles are capable of entering and residing in lung, causing health problems, from mild, acute symptoms to triggering or exacerbating chronic conditions. The toxicity of particles is controlled by their capacity to deposit and retain inside the lung, which in turn is governed by the physicochemical properties of the particles such as aerodynamic size, morphology, chemical composition, and solubility (Bølling et al., 2009).

Wood smoke has been linked to cardiovascular and pulmonary diseases (Barregard et al., 2006; Torres-Duque et al., 2008). The International Agency for Research on Cancer

(IARC, 2010) classified wood smoke as a probably carcinogen to humans, and in terms of its chemical content, of primary concern are PAHs and their derivatives. The consistent use of fireplaces and woodstoves causes an increase in indoor mutagenic activity (Alfheim and Ramdahahl, 1984; van Houdt et al., 1986). Residential combustion is also one of the sources of mutagens in outdoor atmosphere (Claxon et al., 2004).

In addition, it is well known that atmospheric aerosols have impact on climate (Intergovernmental Panel on Climate Change-IPCC, 2001). The soot generated in burning activities strongly adsorbs the solar radiation and warms the atmosphere (Ramanathan and Carmichael, 2008). However, the fine particles primarily cool the atmosphere because smouldering combustion at low combustion temperatures produces an aerosol that is enriched in organic carbon. Organic carbon primarily scatters visible light and increases the fraction of incoming solar radiation scattered back to space, which causes a negative climate forcing (McMeeking, 2008).

1.1. Residential wood combustion emissions

Wood burning, always partially incomplete, releases particles and a number of chemicals including both organic and inorganic, containing C, H, O, and other elements. Carbon content in particles is categorised into elementary carbon (EC) and organic carbon (OC). The aggregation of EC and OC gives total carbon (TC).

Based on chemical composition and morphology of particles generated by domestic wood burning, they are divided in three main groups: organic carbon particles, soot particles and inorganic particles. The organic carbon particles are originated from the thermal degradation of wood composition such as cellulose and lignin at low burning temperature. Soot particles, characterised by high EC/TC, are formed during combustion at higher temperature. The inorganic particles are dominated by the inorganic fraction with very low organic carbon content resulted from virtually complete burning of wood at high temperature. In addition to the difference in the chemical constituents, those particles vary in size. Measurements by microscope suggest the diameter of organic carbon particles, soot particles, and inorganic particles are in the ranges of 50 – 600 nm, 20 – 50 nm, and 50 – 125 nm, respectively (Bølling et al., 2009).

The emissions from residential wood burning are governed by a number of factors such as wood type and characteristics (e.g. moisture and ash contents), air supply, combustion appliance, etc. (Fine et al., 2004a,b; Gonçalves et al., 2011). Researchers have established particle emission factors (i.e. particulate matter (PM) mass per kg wood burnt) and detailed chemical profiles for a number of wood species commonly used in residential combustion in several regions worldwide (Alves et al., 2011; Fine et al., 2001; Fine et al., 2002; Fine et

al., 2004a,b; Gonçalves et al., 2011; Johansson et al., 2004; McDonnald et al., 2000; Schmidl et al., 2008).

Airflow supply, in combination with burning temperature, is chief variable that influences on the content of emissions from residential biomass burning. In incomplete combustion at low temperatures with air deficiency coupled with poor biofuel condition (high moisture content), organic carbon particles are the dominant. In combustion reaction at higher combustion temperature (800-900ºC), the solid carbon aggregate (soot) accounts for the majority of emission particles. In modern burning appliance with better air supply and escalated combustion temperature (>900ºC), the emissions contain mostly inorganic ash particles (Bølling et al., 2009; Boman et al., 2004). Particle emissions from incomplete low-temperature combustion conditions are dominated by spherical OC particles and low levels of EC (EC/TC ≈ 0.01-0.11), while sootand high EC/TC ratios (≈ 0.50-0.80) characterise emissions from combustion at higher temperatures (Bølling et al., 2009).

In addition, burning appliance is one of the essential factors deciding the chemical composition, physical properties and amount of emissions from residential wood combustion (Fine et al., 2004b; Gonçalves et al., 2011). Although there are modern devices that have been developed to better control the combustion, fireplace and conventional woodstove are common burning appliances used in many developed countries. However, both are highly inefficient combustion devices. Particle emission factors are significantly higher when uncontrolled burning appliances, such as fireplace or woodstove, are used in comparison to well-controlled devices (Johansson et al., 2004; Borrego, 2010). Recent studies revealed that modern residential biomass combustion appliances, such as automated pellet and logwood boilers, show significantly reduced particulate emissions compared to old stoves, which underlines the technological improvements achieved (Brunner et al., 2008).

1.2. Polycyclic aromatic hydrocarbons formation

PAHs are comprised of a variety of chemical substances with two or more aromatic rings, generated in incomplete combustion or high temperature pyrolysis of biofuels (fossil fuel, biomass). The major contribution of PAHs in atmosphere is from anthropogenic sources including mobile sources (e.g. petrol and diesel engines) and stationary sources (e.g. residential combustion, industrial activities, power generation). The stationary sources can account up to 90% annual PAHs in some regions (Baek et al., 1991). Among those, wood combustion is one of the dominant sources of PAHs (Baek et al., 1991; Bari et al., 2010; Wang et al., 2010). In residential wood combustion, PAH compounds are hypothetically formed from polymerisation of light organic compounds or from incomplete combustion of

pyrolysis gases (Lu et al., 2009; Mohan et al., 2006). More quantity of PAHs is produced at higher combustion temperatures as the organic pyrolysis products formed at lower temperatures are undergone changes and form aromatic hydrocarbons at higher temperatures (Bølling et al., 2009; and references therein).

The majority of PAHs are initially generated in gaseous phase, and then incorporated into particulates by means of condensation and absorption. The attachment of PAHs onto the particles depends on a number of factors including the PAHs vapour pressure and particle size. The least volatile PAHs can attach onto the surface of particles shortly after emission. PAHs tend to incorporate into particles with respirable size, which means less than 5 μm in aerodynamic diameter (Baek et al., 1991).

Although PAHs represent a minor mass fraction of smoke particles from residential combustion (Fine et al., 2004a,b; Gonçalves et al., 2011; Schmidl et al., 2008), their potential mutagenic and carcinogenic activity may pose a significant human health threat. PAH emission factors from traditional woodstoves are higher than those from fireplaces (Fine et al., 2004b). The high temperature in woodstoves favours the production of PAHs (Bølling et al., 2009). The main constituents of PAHs in wood combustion emission are acenaphthylene, naphthalene, phenanthrene (McDonald et al., 2000), benzo[a]pyrene (BaP) (Johansson et al., 2004), fluoranthene, pyrene, and retene (Fine et al., 2001; Fine et al., 2002; Fine et al., 2004b). Gustafson et al. (2008) found that concentrations of BaP - in homes using woodstove was four times higher than that in homes without woodstove and higher than the medium outdoor levels, while phenanthrene was the largest contributor of total PAHs both indoors and outdoors.

In the atmosphere, PAHs can undergo chemical and photochemical changes before removal by dry or wet deposition. The interaction of PAHs with O_3, NO_X, SO_X, OH and other chemical agents produces derivatives of PAHs (Arey and Atkinson, 2003; Baek et al., 1991; Kaiser et al., 2011).

2. Polycyclic aromatic hydrocarbons carcinogenicity and mutagenicity

PAHs are one of the first classesof atmospheric pollutants that were identified as carcinogens (Baek et al., 1991). A study conducted in Xuan Wei, China, linked the presence of three- to four-ring alkylated PAHs with the incidence of lung cancer in woman in that area who exposed to smoke from cooking (Chuang et al., 1992). Particulate phase PAHs contribute significantly to the mutagenicity of airborne organics (Claxon et al., 2004). BaP and flouranthene are two PAHs with high carcinogenic potency. They may account for 60% and 20%, respectively, of the total cancer potency of PAHs (Gustafson et al., 2008). Other carcinogenic/mutagenic PAHs released by wood combustion are

benzo[a]fluoranthene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[i]fluoranthene, benzo[e]pyrene, benzo[ghi]perylene, chrysene, dibenzo[ah]anthracene, indeno[1,2,3-cd]pyrene, perylene and pyrene (IARC 1998). In the atmosphere, PAHs can be transformed to other products that are more toxic than the parent compounds. Some derivatives produced from reaction between PAHs and NO_X, O_3 and other chemical agents have direct mutagenic activity (Claxton et al., 2004).

Carcinogenic and mutagenic potency of wood smoke and PAHs has been determined by several tests, both in vivo and in vitro. In vivo test includes long-term rodent carcinogenicity on mice and rats through inhalation, oral or dermal exposures (Organisation for Economic Co-operation and Development - OECD 1981). The increase in incidence of lung cancer in male and female Kunming mice was observed when those mice underwent inhalation exposure to a high concentration of emissions generated from wood combustion (Liang et al., 1988). Administration of wood smoke extracts under skin increased the incidence of pulmonary cancer in male Kunming mice (Liang et al., 1984). Wood smoke extracts applied on skin increased the incidence of skin cancer in female Kunming mice and SENCAR mice (Liang and Wang, 1987; Mumford et al., 1990). In addition, wood smoke has been proven to be carcinogenic by means of in vitro test on mammalian cell, causing an increase in the sister chromatid exchange frequency in Chinese hamster ovary (Alfheim et al., 1984; Hytönen et al., 1983; Salomaa et al., 1985),morphological transformation in Syrian hamster embryo (Alfheim et al., 1984), and DNA damage in comet assay (Karlsson et al., 2006). Those tests need to be carried out in months and years and require meticulous attention. Therefore, a simple, short-term bioassay using *Salmonella* or Ames test is used frequently to determine the mutagenicity and carcinogenic potential of wood smoke and particularly PAHs (Alfheim et al., 1984; Asita et al., 1991; Bell and Kamens, 1990; van Houdt et al., 1986; Oanh et al., 2002; Mukherji et al., 2002; Nielsen and Jensen 1991; Yang et al., 2010).

The dominance of Ames test in determining the carcinogen/mutagen potential of air samples was recently demonstrated by Claxton et al. (2004) in their review paper. The Ames assay test, a plate incorporation test that is based on genetically engineered microorganisms, offers a quick method for mutagenicity assessment and has been used widely as a screening tool for detecting mutagens and possible carcinogens (Maron and Ames, 1983; Mortelmans and Zeiger, 2000). Ames test uses a variety of *Salmonella* strains with pre-existing mutations that disable the capacity of the cells to synthesise histidine and therefore, unable to grow and form colonies in the absence of this amino acid. The interaction of the mutagenic chemical and the cell forms new mutation at the site of or nearby the pre-existing mutations, which can reverse the function of the gene and the bacteria to produce histidine. The cells for which the mutation was reversed by a

mutagenic compound, therefore, are able to grow and form colonies in environment without histidine. For this reason, the test is called reversion assay or *Salmonella* assay.

Ames assay uses various *Salmonella* strains possessing distinct mutations in the histidine operon. Each bacteria strain therefore can identify mutagens that induce the mutation through a specific mechanism. TA98 and TA100 are the common used bacteria strains with high sensitivity to carcinogens. TA98 identifies frame shift mutation mechanism through the reversion of the existing mutation -1 frame shift on reading frame of a nearby repetitive –C–G–C–G–C–G–C–G– sequence. This mutation is reversed to the wild-type mutagens that cause +1 frame shift mutagens. The mutation in TA100 is the substitution of a leucine (GAG/CTC) by a praline (GGG/CCC). Reversion of this mutation back to the wild-type state is induced by various mutagens that cause base-pair substitution mutations at one of the GC pairs(Maron and Ames, 1983; Mortelmans and Zeiger, 2000).

Besides the chemicals that directly cause the mutation, there are chemicals that remain inactive unless they are metabolised to active form. In animals, the metabolism of these chemicals is carried out by cytochrome-based P450 metabolic oxidation system in liver and other organs. Since bacteria lack this metabolic system, a rodent metabolic activation system, usually a rat liver homogenate (S9 microsomal fraction), needs to be introduced into the test system. A mixed-function oxidise inducer Aroclor 1254 is applied to the animals to enhance the level of metabolizing enzymes on the liver, before obtaining the S9 fraction(Maron and Ames, 1983; Mortelmans and Zeiger, 2000).

By means of *Salmonella* test, organic content of airborne particles have been detected to be mutagenic/carcinogenic. Many researches have been carried out to elucidate the contribution of PAHs in mutagenicity of particles and gases emitted in residential wood combustion (Asita et al., 1991; Bell and Kamens, 1990; Oanh et al., 2002; Mukherji et al., 2002; Nielsen and Jensen, 1991; Yang et al., 2010). A correlation between the amount of PAHs emitted and the mutagenicity of wood combustion emission was found by Nielsen and Jensen (1991). Yang et al. (2010) showed that fifteen PAHs accounted for 38% of the indirect-acting mutagenicity in smoke particles from three wood types. Yet, no quantitative relation between PAH concentration and mutagenicity of wood smoke has been established (Asita et al., 1991; Bell and Kamens, 1990; Oanh et al., 2002).

In order to characterise and evaluate the residential biofuel combustion in Portuguese context, the study was designed to determine the mutagenicity of $PM_{2.5}$ associated with combustion of eight different biofuels under two burning conditions in two household burning appliances (fireplace and woodstove) commonly used in Southern Europe. There are plenty of Ames tests, due to the variety of bacteria strains and test protocols. However, the one that is recommended for testing of chemical in general is a tier approach, using

strains TA98 and TA100 in the absence and presence of metabolic activation (Mortelmans and Zeiger, 2000). The two strains were commonly used in other comparative studies because the testing results are comparable and reproducible (Claxton et al., 2004). In addition, the pre-incubation assay, distinguished from other Ames test protocols by the exposure of the tester strain, for a short period of time, in a small volume containing the test agent with buffer or S9 mix prior to plating on glucose minimal agar medium, is believed to enhance the reaction between mutagenic metabolites and the tester strain (Mortelmans and Zeiger, 2000). Therefore in this study, the pre-incubation test in a tier approach is employed to determine the mutagenicity of the PAHs in this study. The indirect-acting mutagenicity of each set of PAHs has been assayed by the Ames test using *Salmonella typhimurium*(*S. typhimurium*) TA98 and TA100 with S9 mix. The direct-acting mutagenicity of each set of PAHs has been assayed by the Ames test using the same strain, but without in vitro metabolic activation. Such study can determine which fuel is least harmful, which combustion stage and which burning device poses the least mutagenic risk.

The second part of this thesis will be presented in the form of an article to be submitted to *Environmental Pollution*, including a narrow introduction of the work, material and methods, results, discussion, and conclusion sections.

The last part is the discussion on limitations and recommendations for future research.

References

Alfheim, I., Ramdahl, T., 1984.Contribution of wood combustion to indoor air pollution as measured by mutagenicity in *Salmonella* and polycyclic aromatic hydrocarbon concentration. Molecular Mutagenesis 6, 121-130.

Alfheim, I., Becher, G., Hongslo, J.K., Lazaridis, G., Lofroth, G., Ramdahl, T., Rivedal, E., Salomaa, S., Sanner, T., Sorsa, M., 1984. Short-term bioassays of fractionated emission samples from wood combustion. Teratogenesis Carcinogenesis Mutagenesis 4, 459-475.

Alves, C.A., Vicente, A., Monteiro, C., Gonçalves, C., Evtyugina, M., Pio, C., 2011.Emission of trace gases and organic components in smoke particles from a wildfire in a mixed – evergreen forest in Portugal.Science of the Total Environment 409, 1466-1475.

Arey, J., Atkinson, R., 2003. Photochemical Reactions of PAHs in the Atmosphere, in PAHs: An Ecotoxicological Perspective (ed. Douben, P.E.T.). John Wiley & Sons, Ltd, Chichester, UK. doi: 10.1002/0470867132.ch4

Asita, A.O., Matsui, M., Nohmi, T., Matsuoka, A., Hayashi, M., Ishidate Jr., M., Sofuni, T., Koyano, M., Matsushita, H., 1991. Mutagenicity of wood smoke condensates in the Salmonella/microsome assay. Mutation Research 264, 7-14.

Baek, S.O., Field, R.A., Goldstone, M.E., Kirk, P.W., Lester, J.N., Perry, R., 1991.A review of atmospheric polycyclic aromatic□hydrocarbons □ sources, fate and behaviour. Water Air and Soil Pollution 60, 279□300.

Bari, M.A., Baumbach, G., Kuch, B., Scheffknecht, G., 2010. Particle-phase concentrations of polycyclic aromatic hydrocarbons in ambient air of rural residential areas in southern Germany. Air Quality and Atmospheric Health 3, 103-116.

Barregard, L., Sällsten, G., Gustafson, P., Andersson, L., Johansson, L., Basu, S., Stigendal, L., 2006. Experimental exposure to wood-smoke particles in healthy humans: effects on markers of inflammation, coagulation, and lipid peroxidation. Inhalation Toxicology 18, 845-853.

Bell, D., Kamens, R., 1990. Evaluation of the mutagenicity of combustion particles from several common biomass fuels in the Ames/Salmonella microsome test.Mutation Research Letters 254, 177-183.

Bølling, A.K., Pagels, J., Yttri, K., E., Barregard, L., Sallsten, G., Schwarze, P.E., Boman, C., 2009. Health effects of residential wood smoke particles: the importance of combustion conditions and physicochemical particle properties. Particle and Fibre Toxicology 6:29.

Boman, C., Nordin, A., Boström, D., Öhman, M., 2004. Characterization of inorganic particulate matter from residential combustion of pelletized biomass fuels. Energy and Fuels 18, 338-348.

Borrego, C., Valente, J., Carvalho, A., Sá, E., Lopes, M., Miranda, A.I., 2010.Contribution of residential wood combustion to PM_{10} levels in Portugal. Atmospheric Environment 44, 5, 642-651.

Brunner, T., Bärnthaler, G., Obernberger, I., 2008.Evaluation of parameters determining PM emissions and their chemical composition in modern residential biomass heating appliances. In: Proceedings of the International Conference World BIOENERGY 2008, Jönköping, Sweden, pp. 81-86, Swedish Bioenergy Association (Ed.). Stockholm, Sweden.

Chuang, J.C., Wise, S.A., Cao, S., Mumford, J.L., 1992. Chemical characterization of mutagenic fractions of particles from indoor coal combustion: a study of lung cancer in Xuan Wei, China. Environmental Science and Technology 26, 999-1004.

Claxton, L.D., Matthews, P.P., Warren, S.H., 2004. The genotoxicity of ambient outdoor air, a review: Salmonella mutagenicity. Mutation Research 567, 347-399.

Dias, J.J.M., 2002. Utilização da biomassa: avaliação dos resíduos e utilização de pellets emcaldeirasdomésticas. MSc Thesis. Superior Technical Institute of the Technical University of Lisbon.

European Commission Energy 2009. EU energy and transport in figures. http://ec.europa.eu/energy/publications/statistics/doc/2009_energy_transport_figures.pdf

Fine, P.M., Cass, G.R., Simoneit, B.R.T., 2001.Chemical characterization of fine particle emissions from fireplace combustion of woods grown in the northeastern United States. Environmental Science and Technology 35, 2665-2675.

Fine, P.M., Cass, G.R., Simoneit, B.R.T., 2002.Chemical characterization of fine particle emissions from fireplace combustion of woods grown in the southern United States. Environmental Science and Technology 36, 1442-1551.

Fine, P.M., Cass, G.R., Simoneit, B.R.T., 2004a.Chemical characterization of fine particle emissions from fireplace combustion of woods grown in the Midwestern and western United States.Environmental Engineering Science 21, 387-409.

Fine, P.M., Cass, G.R., Simoneit, B.R.T., 2004b. Chemical characterization of fine particle emissions from the woodstove combustion of prevalent United States tree species. Environmental Engineering Science 21, 705-721.

Gaeggeler, K., Prevot, A.S.H., Dommen, J.,Legreid, G., Reimann, S., Baltensperger, U., 2008. Residential wood burning in an Alpine valley as a source for oxygenated volatile organic compounds, hydrocarbons and organic acids. Atmospheric Environment42, 35, 8278- 8287.

Gelencsér, A., May, B., Simpson, D., Sanchez-Ochoa, A., Kasper-Giebl, A., Puxbaum, H., Caseiro, A., Pio, C., Legrand, M., 2007.Source apportionment of $PM_{2.5}$ organic aerosol over Europe: primary/secondary, natural/anthropogenic, fossil/biogenic origin. Journal of Geophysical Research, 112, D23, D23S04.

Gonçalves, C., Alves, C., Fernandes, A.P., Monteiro, C., Tarelho, L., Evtyugina, M., Pio, C., 2011. Organic compounds in $PM_{2.5}$ emitted from fireplace and woodstove combustion of typical Portuguese wood species. Atmospheric Environment. In Press.

Gustafson, P., Ostman, C., Sallsten, G., 2008. Indoor levels of polycyclic aromatic hydrocarbons in homes with or without wood burning for heating. Environmental Science and Technology 42, 5074-5080.

Holden, A.S., Sullivan, A.P., Munchak, L.A., Kreidenweis, S.M., Schichtel, B.A., Malm, W.C., Collett Jr., J.L., 2011. Determining contributions of biomass burning and other sources to fine particle contemporary carbon in the western United States. Atmospheric Environment 45, 1986-1993.

vanHoudt, J.J., Daenen, C.M.J., Boleij, J.S.M., Alink, G.M., 1986. Contribution of woodstoves and fire places to mutagenic activity of airborne particulate matter inside home. Mutation Research 171, 91-98.

Hytönen, S., Alfheim, I. Sorsa, M., 1983. Effect of emissions from residential woodstoves on SCE induction in CHO cells.Mutation Research 118, 1-2, 69-75.

IARC, 1998.Polynuclear aromatic compounds.Part 1, chemical, environmental and experimental data.IARC Monographs on the evaluation of the carcinogenic risk of chemical to humans, 32, 1-453. International Agency for Research on Cancer, Lyon.

IARC, 2010.Household use of solid fuels and high-temperature frying.IARC Monographs on the Evaluation of Carcinogenic Risks to Humans 95, 1-430.International Agency for Research on Cancer, Lyon.

IPCC, 2001. Climate Change 2001: The Scientific Basis. Contribution of Working Group I to the Third Assessment Report of the Intergovernmental Panel on Climate Change [Houghton, J.T., Ding, Y., Griggs, D.J., Noguer, M., van der Linden, P.J., Dai, X., Maskell, K., Johnson, C.A. (eds.)]. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, 881pp. Intergovernmental Panel on Climate Change.

Johansson, L.S., Leckner, B., Gustavsson, L., Cooper, D., Tullin, C., Potter, A., 2004. Emission characteristics of modern and old-type residential boilersfired with wood logs and wood pellets. Atmospheric Environment38, 4183-4195.

Kaiser, C., Riemer, N., Knopf, D.A., 2011. Detailed heterogeneous oxidation of soot surfaces in a particle-resolved aerosol model. Atmospheric Chemistry and Physics 11, 4505-4520.

Karlsson, H.L., Ljungman, A.G., Lindbom, J., Möller, L., 2006. Comparison of genotoxic and inflammatory effects of particles generated by wood combustion, a road simulator and collected from street and subway. Toxicology Letters 165, 203-211.

Liang, C.K., Quan, N.Y., Ma, F., Zhang, Y., Wang, E.M., Yin, X.R., 1984. Carcinogenicity of extract of soot from Xuan Wei County administering subcutaneously to mice.*Environmental Science Research* 31, 826-827;as cited in IARC 2010.

Liang, C.K., Wang, W., 1987. Kunming mouse skin tumor-initiating activity of extracts of inhalable particles in indoor air. Zhonghua Yu Fang Yi XueZaZhi 21, 6, 316-318; as cited in IARC 2010.

Liang, C.K., Quan, N.Y., Cao, S.R.,He, X. Z., Ma, F., 1988. Natural inhalation exposure to coal smoke and wood smoke induces lung cancer in mice and rats. Biomedical and environmental sciences 1, 1, 42-50.

Lu, H., Zhu, L., Zhu, N., 2009. Polycyclic aromatic hydrocarbon emission from straw burning and the influence of combustion parameters.Atmospheric Environment 43, 4, 978-983.

Maron, D.M., Ames, B.N, 1983. Revised methods for the Salmonella mutagenicity test.Mutation Research 113, 173-215.

McDonnald, J. D., Zielinska, B., Fujita, E.M., Sagebiel, J.C., Chow, J.C., Watson, J.G., 2000. Fine particle and gaseous emission rates from residential wood combustion. Environmental Science and Technology 34, 2080-2091.

McMeeking, G.R., 2008. The optical, chemical, and physical properties of aerosols and gases emitted by the laboratory combustion of wildland fuels. PhD Thesis. Colorado State University.

Mohan, D., Pittman, C.U., Steele, P.H., 2006. Pyrolysis of wood/biomass for bio-oil: a critical review. Energy and Fuels 20, 848-889.

Mortelmans, K, Zeiger, E., 2000. The Ames *Salmonella*/microsome mutagenicity assay.Mutation Research 455, 29-60.

Mukherji, S., Swain, A.K., Venkataraman, C., 2002.Comparativemutagenicityassessmentofaerosolsin emissions frombiofuelcombustion.AtmosphericEnvironment 36, 5627-5635.

Mumford, J.L., Helmes, C.T., Lee, X.M., Seidenberg, J., Nesnow, S., 1990. Mouse skin tumorigenicity studies of indoor coal and wood combustion emissions from homes of residents in Xuan Wei, China with high lung cancer mortality.Carcinogenesis 11, 3, 397-403.

Nielsen, P.A., Jensen, L., 1991. Emission of PAH and mutagenic activity from furnaces fuelled with cereal straw or wood chips. Chemosphere 23, 6, 723-735.

Oanh, N.T.K., Nghiem, L.H., Phyu, Y.L., 2002. Emission of polycyclic aromatic hydrocarbons, toxicity, and mutagenicity from domestic cooking using sawdust briquettes, wood, and kerosene. Environmental Science and Technology 36, 833-839.

OECD, 1981. Test Guideline 451. Carcinogenicity Studies. In: OECD Guideline for the Testing of Chemicals. Organisation for Economic Co-operation and Development. Paris, France.

Puxbaum, H., Caseiro, A., Sanchez-Ochoa, A., Kasper-Giebl, A., Clayes, M., Gelencser, A., Legrand, M., Preunkert, S., Pio, C., 2007.Levoglucosan levels at background sites in Europe for assessing the impact of biomass combustion on the European aerosol background. Journal of Geophysical Research 112 (D23) D23S05/1-D23S05/11.

Ramanathan, V., Carmichael, G., 2008.Global and regional climate changes due to black carbon.Nature Geoscience 1, 221-227.

Salomaa, S., Sorsa, M., Alfheim, I., Leppänen, A., 1985. Genetoxic effects of smoke emissions in mammalian cells. Environmental International 11, 2-4, 311-316

Schmidl, C., Marr, I.L., Caseiro, A., Kotianova, P., Berner, A., Bauer, H., Kasper-Giebl, A., Puxbaum, H., 2008. Chemical characterisation of fine particle emissions from woodstove combustion of common woods growing in mid-European Alpine regions.Atmospheric Environment 42, 126-141.

Torres-Duque, C., Maldonado, D., Pérez-Padilla, R., Ezzati, M., Viegi, G., 2008. Biomass fuels and respiratory diseases: a review of the evidence. Proceedings of the American Thoracic Society 5, 577-590.

Wang, X.Y., Li, Q.B., Luo, Y.M., Lian, Q.D., Xi, M., Ma, J.M., Li, Y., Liu, Y.P., Cheng, C.L., 2010.Characteristics and sources of atmospheric polycyclic aromatic hydrocarbons (PAHs) in Shanghai, China. Environmental Monitoring and Assessment 165, 295-305.

Yang, X.Y., Igarashi, K., Tang, N., Lin, J.M., Wang, W., Kameda, T., Toriba, A., Hayakawa, K., 2010. Indirect- and direct-acting mutagenicity of diesel, coal and wood burning-derived particulates and contribution of polycyclic aromatic hydrocarbons and nitro polycyclic aromatic hydrocarbons. Mutation Research 695, 29-34.

Part II: Article

Mutagenicity assessment of aerosols in emissions from wood combustion in Portugal

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Abstract:Polycyclic aromatic hydrocarbon (PAH) extracts of $PM_{2.5}$ collected from combustion of seven wood species and briquettes were tested for mutagenic activities using Ames test with *Salmonella typhimurium* TA98 and TA100. The woods were*Pinuspinaster* (maritime pine), *Eucalyptus globulus* (eucalypt), *Quercussuber* (cork oak), *Acacia longifolia* (golden wattle), *Quercusfaginea* (Portuguese oak), *Oleaeuropea* (olive), and *Quercus ilex rotundifolia* (Holm oak). Burning experiments were done using woodstove and fireplace, hot start and cold start.A mutagenic/weak mutagenic response was recorded for all species except golden wattle. The extracts with indirect acting mutagenicity were mainly obtained from fireplace and cold start conditions. The strong mutagenic extracts were not correlated with high emission factors of carcinogenic PAHs. Several samples were weak mutagensat low concentration of PAHs. The negative result recorded for the golden wattle extracts is positive since after confirmation, this species can be recommended for domestic use.

Key words: Wood smoke, mutagenicity, polycyclic aromatic hydrocarbon, Ames test

1. Introduction

Residential biofuel combustion for cooking and heating has posed several environmental problems and health risks (Bølling et al., 2009;Zielinska and Samburova, 2011). Burning of wood and other biofuels for domestic application is one of the major sources of regional and local air pollution, principally regarding the production and release of particulate matter and organic compounds (Caseiro et al., 2009; Fine et al., 2004a,b**;** Gaeggeler et al., 2008**;** Gonçalves et al., 2010). Biomass combustion is one of the major sources of aerosol in United States (Holden et al., 2011) and in Europe during the winter (Gelencser et al., 2007**;** Puxbaum et al., 2007). In Portugal, it was estimated that this source accounts, on average, for 18% of the atmospheric aerosol mass (Borrego et al., 2010). The particulate matter borne from domestic combustion is dominated by submicron particles, which, through inhalation, enter and reach the upper and lower regions of the respiratory tract and induce public health problems. The health implications by fine aerosol from domestic wood combustion may be more serious than has previously been thought (Bølling et al., 2009;Boman et al., 2003). On the other hand, particulate matter interferes with the radiative properties of the atmosphere, supporting or offsetting the greenhouse gas effects depending on their size and their organic and inorganic content (Aunan et al., 2009**;** Danny and Kaufmann, 2002). In addition to climate change and air quality models, source apportionment methodologies applied to ambient data require detailed biomass burning emission factors. Due to the lack of specific data, biomass burning profiles obtained for the United States (e.g. Fine et al., 2001, Fine et al., 2002, Fine et al., 2004a,b), Scandinavian countries (Johansson et al., 2004), and mid-European Alpine regions (Schmidl et al., 2008) have been applied in source apportionment studies carried out in Southern European countries. However, the emission of pollutants from domestic combustion is a function of many variables, among others, fuel, burning appliance and moisture content (Johansson et al., 2004). To overcome the lack of emission factors for the combustion of major biofuels in Portugal, a series of source tests were conducted to establish the chemical composition of particulate matter that has an aerodynamic diameter smaller than 2.5 μm ($PM_{2.5}$) emitted from the burning in woodstove and fireplace devices (Alves et al., 2011**;** Gonçalves et al., 2011).

The organic chemicals associated with airborne particles, especially polycyclic aromatic hydrocarbons (PAHs) from extractable organic matter (EOM) have been widely investigated in studies exploring the mutagenic and potentially carcinogenic activity of ambient particulate matter (Atkinson and Arey, 1994; Baek et al., 1991; IARC, 1998). Many research using source apportionment techniques have pointed out that wood smoke and mobile source emissions contribute to a substantial amount of airborne extractable

organics and mutagenicity of ambient air (Atkinson and Arey, 1994; Baek et al., 1991; Bari et al., 2010; Claxton et al., 2004; Wang et al., 2010). Studies show that PAHs and their derivatives generated from domestic cooking and domestic heating are mutagenic agents, with mutagenic potential varying due to combustion appliances, fuels and extraction methods (Claxton et al., 2004; can Houdt et al., 1986; Mukherji et al., 2002; Oanh et al., 2002).

The Ames assay, a plate incorporation test which is based on genetically engineered microorganisms, offers a quick method for mutagenicity assessment and has been used widely as a screening tool for detecting mutagens and possible carcinogens (Maron and Ames, 1983; Mortelmans and Zeiger, 2000). Two bacterial strains of *Salmonella typhimurium* - strain TA98 and strain TA100 - are used to determine frameshift mutations and base – pair substitution mutations, respectively. Metabolic activation by the S9 mix, consisting of a rat liver microssomal fraction obtained after induction of drug metabolizing enzymes with an exposure to Aroclor-1254 (Mortelmans and Zeiger, 2000), is introduced to the test for the purpose of detecting the toxicity of metabolic products of the testing chemicals (Maron and Ames, 1983;Mortelmans and Zeiger, 2000).

Taking into account that emission profiles of domestic burning in Portugal have not been created before, there are no studies about the toxic effects of emitted PAHs from domestic combustion in typical appliances. In order to characterise and evaluate the residential biofuel combustion in Portuguese context, the study was designed to determine the mutagenicity of $PM_{2.5}$ associated with combustion of eight different biofuels under two burning conditions in two household burning appliances (fireplace and woodstove) commonly used in Southern Europe. In a tier approach, the Ames assay performed in this study has used strains TA98 and TA100 in the absence and presence of metabolic activation as recommended in Mortelmans and Zeiger (2000). The two strains were commonly used in other comparative studies because the testing results are comparable and reproducible (Claxton et al., 2004). In addition, the pre-incubation assay, distinguished from other Ames test protocols by the exposure of the tester strain for a short period of time in a small volume containing the test agent with buffer or S9 mix prior to plating on glucose minimal agar, is believed to enhance the reaction between mutagenic metabolites and the tester strain (Mortelmans and Zeiger, 2000). The indirect – acting mutagenicity of each set of PAHs has been assayed by the Ames test using *S. typhimurium* TA98 and TA100 with S9 mix. The direct – acting mutagenicity of each set of PAHs has been assayed by the Ames test using the same strains, but without in vitro metabolic activation. Such study can determine which fuel is least harmful, which combustion stage and which burning device poses the least mutagenic risk.

2. Material and methods

2.1. Biomass fuel selection

According to the Portuguese Forest Inventory (2005), the top seven most prevalent tree species in Portugal are *Pinuspinaster* (maritime pine), *Eucalyptus globulus* (eucalypt), *Quercussuber* (cork oak), *Acacia longifolia* (golden wattle), *Quercusfaginea* (Portuguese oak), *Oleaeuropea* (olive), and *Quercus ilex rotundifolia* (Holm oak). These tree species were selected for this study. In addition, biomass briquettes commonly used in domestic heating nowadays, made of wastes from forest cleaning activities and/or from local wood processing industries, were also included in this study.

2.2. Sampling method

The burning tests were performed at the combustion facilities of the Department of Environment, University of Aveiro,Portugal. The facility structure and operation conditions were described in detail elsewhere (Calvo et al., 2011; Fernandes, 2009; Tarelho et al., 2011). Two types of residential biomass combustion appliances were selected for the source tests: i) a cast iron woodstove (Solzaima®, model Sahara) with handheld control of combustion air (primary air underbed feed), and ii) a traditional Portuguese brick open fireplace with no control of combustion air. Both of them were operated manually in batch mode. Two experiment protocols were performed for each appliance and wood type: “cold start” and “hot start” to evaluate the influence of the temperature and fuel ignition process on the combustion fuel emission profile. The cold start experiments represent the start-up combustion phase. On the other hand, the hot start experiments were initiated by loading the combustion chamber with a batch of fuel, which was already at a temperature of around 100ºC and with the presence of a small amount of burning char from a batch of fuel burned previously.

Each combustion experiment lasted between 45 minutes to 90 minutes, burning about 6 kg of wood. The wood was burnt as split logs of 30 – 50 cm in length and around 10 cm in diameter. Fires were ignited with pinecones and small kindling pieces cut from the same wood being burnt.

$PM_{2.5}$ was collected in a dilution tunnel coupled to the woodstove or fireplace chimneys. Dilution tunnel was employed because it stimulates the rapid cooling and dilution that occurs as exhaust mixes with the atmosphere (Lipsky and Robinson, 2006). The dilution tunnel consisted of a cylindrical tube with 0.20 m internal diameter and 11 m length. The sampling of $PM_{2.5}$ was made at a dilution ratio of 25:1. $PM_{2.5}$ were collected using an Echo sampling head connected to a TECORA sampler (model 2.004.01, Italy) operating at a

flow of 2.3 m^3h^{-1}, onto quartz fibre filters (47 mm diameter) located 10 m downstream the dilution tunnel entrance.

2.3. Polycyclic aromatic hydrocarbons extraction method

The quartz fibre filters were previously fired at 500ºC to eliminate organic contaminants and weighed before and after sampling to determine the mass of collected $PM_{2.5}$. The result was calculated from average of three measurements, when variation was less than 5%. The weighing was performed by a microbalance (Sartorius M5P) after 24h equilibrium in a room with controlled temperature and humidity.

Elemental carbon (EC) and organic carbon (OC) in quartz fibre filters were analysed by a thermal – optical transmission technique following a short multi – step temperature protocol, first in inert N_2 and then in an oxidising atmosphere (N_2/O_2) (Alves et al., 2011).

Half of the area of each quartz filter was sequentially extracted with dichloromethane and methanol (Fisher Scientific). The total organic extract was divided into five fractions by flash chromatography with silica gel and a variety of solvents of increasing polarity. After separation, each fraction was vacuum concentrated and evaporated by ultra pure nitrogen stream. Details on the methodology for the extraction of organic compounds have been reported elsewhere (Alves et al., 2011).

The fractionated extracts were then analysed by gas chromatography – mass spectrometry (GC model 6890, quadrupole MSD 5973 from Hewlett – Packard and GC Trace Ultra, quadrupole DSQ II from Thermo Scientific). The protocol of GC – MS analysis and identification of PAH compounds have been presented in Gonçalves et al. (2011).

The $PM_{2.5}$ particle emission factor was determined by particle mass and mass of fuel burnt in dry basis, taking into account the dilution applied to the exhaust. The PAH emission factors were calculated as a mass fraction of total OC, which, in turn, was expresses as a mass fraction of total $PM_{2.5}$ emitted. After separated from other organic fractions, PAH extracts obtained from each combustion experiment were dissolved in 1.6 mL of DMSO. Dilutions of these extracts were tested in the Ames assays.

2.4. Mutagenicity Testing Method

The total PAH extracts from each combustion test were tested for mutagenicity performing the Ames assay with *S. typhimurium* TA98 and TA100 strains with and without metabolic activation by the S9 fraction (Trinova Biochem® GmbH, Giessen, Germany) in order to assess the indirect and direct – acting, frame – shift and base – pair substitution mutagens.

Each sample was tested in four to five doses in range of µg/L, decreasing in two – fold magnitude, with two to three replicates per dose.

The test was performed in plates containing two distinct layers of agar: 25 mL of the bottom agar (glucose minimal agar) to provide support media and 2 mL of top agar to deliver the extract concentration, the S9 mix (in assays with S9) or buffer (in assays without S9) and the tester strain to the bottom agar. All reagents were prepared according to Maron and Ames (1983) and Mortelmans and Zieger (2000). The tester strain was pre-incubated over night in Oxoid nutrient Broth number 2. The rat liver microsomal fractions for metabolic activation S9 was obtained in lyophilised form, purchased from Trinova Biochem® GmbH. In each test, a negative control consisting of DMSO solvent blank (50 µL/plate) was included to determine the spontaneous revertant. In addition, a positive control consisting of known mutagens was used to confirm the reversion properties and specificities of each strain, activity of the S9 mix and other components presented in the assay. For experiment without S9, 2 – nitrofluorene (10 µg/plate) and sodium azide (10 µg/plate) were used in positive control for test with *S. typhimurium* TA98 and TA100, respectively. For assays with the inclusion of S9, 2-aminoanthracene (10 µg/plate) was used in positive control for both bacterial strains, TA98 and TA100. After hardening, the plates were incubated at 37ºC for 48 hours, and subsequently, the number of revertant colonies formed in each plate was counted.

2.5. Statistical analysis

Principal component analysis (PCA) was performed to reveal patterns among extracts, obtained from different burning devices and conditions, based on all the variables assessed, which could not be found assessing for each variable individually (Quinn and Keough, 2002).

Results were considered positive, either when a dose-related increase in the number of revertant colonies was observed or when the average number of revertant colonies in the plates was two times greater than those recorded in the negative control plates (Mortelmans and Zeiger, 2000). According to OECD guidelines (1997), this last criterion is sufficient to assume mutagenic potential, even without a dose-effect relationship. When suspicions arouse about the potential toxicity of some extracts, one-way analysis of variance (ANOVA) followed by a Dunnet test were performed to check for a significant decrease in the number of revertant colonies on plates in comparison with the negative control.

3. Results

Table 1presents the emission factors of $PM_{2.5}$ and PAHs from combustion of each wood species by woodstove and fireplace, during the start-up phase and subsequently with hot combustion chamber.

Twenty one PAHs have been detected in the wood smoke, including 13 carcinogenic/mutagenic PAHs characterised by IARC (1998). The majority of wood smoke samples contain 17-20 PAHs. Wood smoke from hot combustion of maritime pine by woodstove contains the least number of PAHs, only eight. Emission factors of 13 carcinogenic/mutagenic PAHs (µg/kg wood burned, dry basis) in wood smoke are also included in Table 1. Eucalypt, Holm oak, olive, Portuguese oak and cork oak have the highest emissions of those PAHs.

Table 1also shows the emission factors of fluoranthane andbenzo[a]pyrene(BaP) (µg/kg wood burned, dry basis)obtained in each combustion experiment, as those PAHs account for the majority of mutagenicity of PAHs emitted by wood combustion (Gustafson et al., 2008). Combustion of olive, Holm oak and golden wattle releases the largest quantity of these two PAHs.

According to the PCA analysis (Figure 1), the first component was mainly correlated with carcinogenic PAHs and with BaP and fluoranthene emission factors. This component was also responsible by joining almost all the extracts in the negative part setting them apart from extracts obtained from Holm oak, olive, eucalyptus and Portuguese oak, almost all from cold start. However, this analysis did not reveal a clear pattern in the distribution of samples related to burning devices and conditions. The first component of the PCA accounted for 99.9% of the total variability among extracts. The extract obtained from Holm oak burned on a fireplace with a hot start was the one more correlated with carcinogenic PAHs emission factors.

The number of revertant colonies obtained from the mutagenicity tests of PAH extracts of particles emitted in combustion of the seven wood species and briquettes by woodstove and fireplace at two burning conditions (hot start and cold start) are presented in Table 2 and

Table 3. A sample is assessed as mutagen when one or two of the following conditions are achieved: i) the presence of dose-related increase in all tested concentrations, and ii) the emergence in testing samples of more than two times revertant colony numbers in negative control.

Fourteen in total mutagenic tests of this study have dose-related increase in the number of revertant colonies. They are samples obtained from combustion of briquettes, eucalypt, Holm oak, maritime pine, olive, and Portuguese oak. The mutagenic potency (rev/ng of PAH mixture) of the samples with regression coefficient higher than 0.6 is presented in Table 4.

When the two-fold principle was applied, five samples showed positive result in tests with strain TA98 and TA100 in the absence of S9 mixture (Table 4). The positive samples in Ames with strain TA98 are Holm oak (woodstove, hot start), cork oak (fireplace, cold start), olive (fireplace, hot start) and Portuguese oak (fireplace, cold start). The only wood species whose combustion released an extract mutagenic in Ames test with TA100 was eucalypt, using woodstove in hot start condition. Test results of all of these samples, excluding cork oak, were also detected as positive according to dose-related rule.

According to the PCA biplot, all the samples for which a strong positive result was obtained in the Ames test without S9, where not those more correlated with component 1 mainly explained by carcinogenic/mutagenic PAHs (except for samples from Portuguese cork and oak, obtained from the fireplace with cold start) (Figure 1).

The remaining samples that were not determined as mutagen/weak mutagen are inclusive for two reasons: there are scattered elevated revertant counts and the testing concentration is far below the maximum concentration 5 to 10 mg/platesuggested by Mortelman and Zieger (2000) for pure compounds.

Six samples were statistically proven havingsignificant decrease in the number of revertant colonies on plates in comparison with the negative control by one-way ANOVA followed by a Dunnet test ($p<0.05$). All of them are in test with metabolic activation. The detailed statistical results of those samples are presented in Table 5. Since these samples have proved to be toxic to one of the *S.typhimurium* strains, it is not feasible to conclude about their genotoxicity/mutagenicity.

Table 1: Emission factors of $PM_{2.5}$, total PAHs, 13 carcinogenic/mutagenic PAHs, and fluoranthene and benzo[a]pyrene for domestic combustion

Wood species	$PM_{2.5}$ Emission factor (g/kg wood burned, dry basis) [1]				PAH emission factor(mg/kg wood burned, dry basis) [2]				13 carcinogenic/mutagenicPAH emission factor(µg/kg wood burned, dry basis) [3]				Fluoranthene and benzo[a]pyrene emission factor(µg/kg wood burned, dry basis) [4]			
	Woodstove		Fireplace		Woodstove		Fireplace		Woodstove		Fireplace		Woodstove		Fireplace	
	Hot start	Cold start	Hot start	Cold start	Hot start	Cold start	Hot start	Cold start	Hot start	Cold start	Hot start	Cold start	Hot start	Cold start	Hot start	Cold start
Briquettes	3.36	14.69	18.36	9.14	10.18	99.22	43.84	25.88	1504	2995	836	1703	289	722	220	466
Cork oak	12.05	18.75	11.99	28.99	19.03	29.11	22.62	68.83	2958	3554	2337	11997	339	781	672	2127
Eucalypt	10.12	19.26	11.68	19.06	13.58	29.41	6.11	31.10	3628	12648	737	2347	1080	2921	216	640
Golden wattle	9.12	11.55	0.84	10.45	17.99	30.97	1.76	22.40	4908	5356	377	5180	1233	1278	120	1221
Holm oak	4.69	14.15	21.71	14.81	10.36	35.28	78.42	31.46	3808	13969	37122	3938	1000	3584	9182	918
Maritime pine	1.66	5.62	5.94	8.11	17.70	47.59	23.91	81.05	2039	2642	2042	6476	139	630	521	485
Olive	7.66	22.29	20.22	26.01	33.31	70.49	45.26	112.24	5348	17751	2560	19213	1474	4189	622	4487
Portuguese oak	16.01	25.83	5.85	27.93	16.03	74.51	14.17	49.12	1742	3151	560	18549	226	676	181	4025

[1] Gonçalves et al., 2011.

[2] PAH emission factor = (total PAHs/OC)*(OC/$PM_{2.5}$)*($PM_{2.5}$ emission factor)

[3] 13 carcinogenic/mutagenic PAH emission factor = (13PAHs/OC)*(OC/$PM_{2.5}$)*($PM_{2.5}$ emission factor)

[4] Fluoranthene and benzo[a]pyrene emission factor =[(fluoranthene+BaP)/OC]*(OC/$PM_{2.5}$)*($PM_{2.5}$ emission factor)

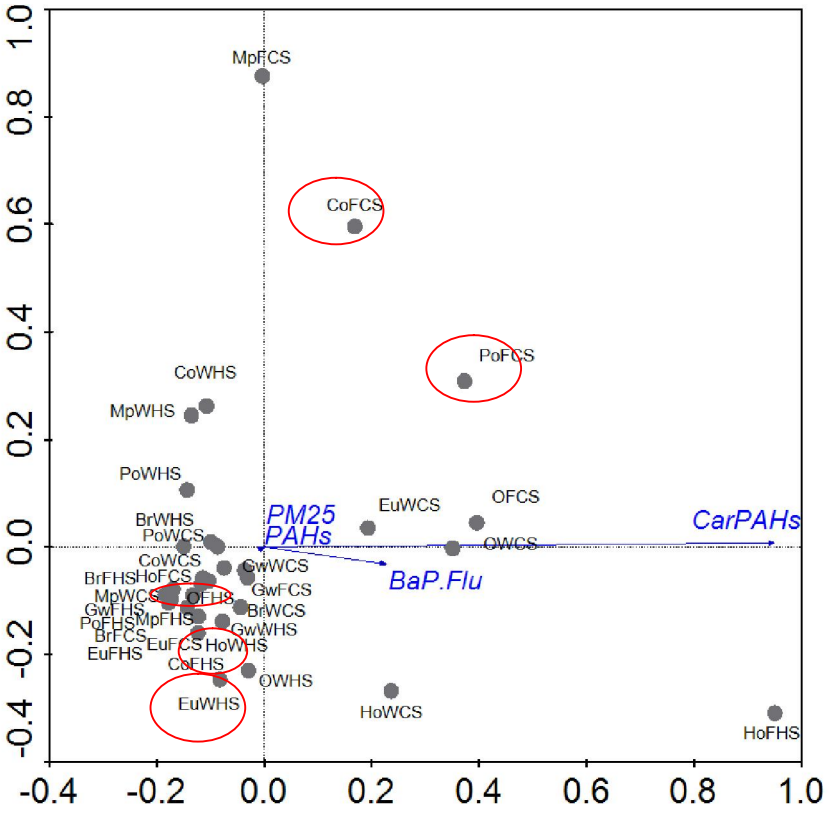

Figure 1: PCAbiplot of all the emission extracts based on emission factors of $PM_{2.5}$, total PAHs, 13 carcinogenic/mutagenic PAHs, and fluoranthene and benzo[a]pyrene. Br-briquettes; Co-cork oak; Eu-eucalypt; Gw-golden wattle;Ho-Holm oak; Mp-maritime pine; O-olive; Po-Portuguese oak;W-woodstove; F-fireplace; HS–hot start, CS-cold start. Circles surrounding samples for which a positive result (based on the criteria of an induction factor greater than 2, comparatively to the control) was recorded in the Ames assay without S9.

Table 2: Mutagenicity of PAH extracts of particles emitted in domestic wood combustion by woodstove, hot start and cold start, to *S. typhimurium* TA98, TA100 in the absence and presence of S9 mixture

Wood species	Hot start					Cold start				
	ng PAHs /plate	Number of revertants (mean±SD)[1]				ng PAHs /plate	Number of revertants (mean±SD)			
		TA98	TA100	TA98/S9	TA100/S9		TA98	TA100	TA98/S9	TA100/S9
Briquettes	225	23.5±2.1(1.91)[2]	364.3±162(0.65)	21.9±2.9(1.00)	87.0±7.0(0.99)	1001	12.0±2.8(1.33)	121.7±31.0(1.25)	18.7±6.0(0.94)	123.0±7.2(1.25)
	113	13.0±4.2(1.05)	455.3±22.3(0.81)	19.0±1.4(0.86)	96.0±24.4(1.10)	500	10.0±0.0(1.11)	116.7±17.0(1.20)	17.3±4.2(0.87)	123.7±24.2(1.26)
	56	14.0±2.8(1.04)	432.0±96.6(0.77)	17.5±0.7(0.80)	86.7±15.2(0.99)	250	14.7±8.1(1.66)	145.3±38.9(1.49)	17.0±4.7(0.85)	117.3±30.0(1.19)
	28	12.5±2.1(1.01)	504.7±208(0.90)	14.0±3.5(0.64)*	98.3±9.1(1.12)	125	14.0±3.6(1.56)	156.3±28.3(1.61)	16.3±1.5(0.82)	120.0±8.9(1.22)
	14	12.5±4.9(1.01)	372.7±98.0(0.58)	12.7±3.1(0.58)*	89.7±3.1(1.02)	63	11.3±5.9(1.26)	158.3±19.3(1.63)	19.0±5.3(0.95)	134.0±3.0(1.36)
	0	12.3±3.5	561.7±47.4	22.0±1.0	87.7±11.6	0	9.0±5.6	97.3±31.9	20.0±2.6	98.3±10.1
	PC	136.3±2.5	1482.0±139	279.7±25.8	360.0±43.6	PC	213.3±8.7	568.3±120.7	364.3±111.7	405.0±36.3
Cork oak	240	16.0±1.0(0.96)	147.0±20.7(1.51)	15.0±1.7(0.75)	96.7±4.7(0.98)	235	15.5±0.7(1.26)	142.5±3.5(1.19)	20.7±4.6(0.94)	105.0±13.0(1.20)
	120	17.0±2.6(1.02)	148.3±14.4(1.52)	12.3±0.6(0.62)	108.7±11.0(1.11)	117	13.0±1.4(1.05)	138.0±12.7(1.16)	15.7±4.7(0.73)	99.0±16.1(1.13)
	60	13.7±5.7(0.82)	143.0±13.1(1.47)	16.7±6.4(0.84)	120.7±14.2(1.23)	59	11.0±2.8(0.89)	137.5±17.7(1.17)	20.3±2.1(0.92)	90.7±8.1(1.03)
	30	17.3±7.5(1.04)	144.7±5.7(1.49)	13.7±2.5(0.69)	93.7±18.6(0.95)	29	8.0±0.0(0.65)	102.5±13.4(0.86)	19.3±8.5(0.88)	112.0±2.6(1.28)
	15	16.0±1.7(0.96)	157.0±26.6(1.61)	25.7±6.5(1.29)	104.3±10.6(1.06)	15	10.0±1.4(0.81)	181.0±67.9(1.51)	16.7±2.3(0.76)	105.3±10.1(1.20)
	0	16.7±4.2	97.3±31.9	20.0±2.6	98.3±10.1	0	12.3±3.5	119.3±33.1	22.0±1.0	360.0±43.6
	PC	121.7±36.3	568.3±120.7	364.7±11.7	405.0±36.3	PC	136.3±2.5	610.7±12.9	279.7±25.8	87.7±11.6
Eucalypt	236	12.5±0.7(0.75)	207.5±54.4 **(2.13)**[3]	20.5±2.1(1.03)	114.3±18.5(1.16)	567	15.0±2.8(1.22)	289.7±45.5(1.12)	21.3±1.5(1.42)	74.0±3.6(0.84)
	118	11.7±2.1(0.70)	184.7±20.6 (1.90)	19.5±3.5(0.98)	99.0±18.2(1.01)	283	9.5±0.7(0.77)	277.7±19.5(1.07)	17.3±4.7(1.15)	91.0±9.6(1.04)
	59	12.3±3.2(0.74)	196.7±27.2 **(2.02)**	18.0±0.0(0.90)	102.7±16.0(1.04)	142	14.0±2.8(1.14)	330.3±31.5(1.28)	17.3±5.7(1.15)	77.7±2.5(0.89)
	29	15.7±3.2(0.94)	153.3±33.1 (1.58)	14.7±1.5(0.74)	100.3±2.9(1.02)	71	9.5±3.5(0.77)	297.7±22.4(1.15)	15.7±4.9(1.05)	80.7±7.0(0.92)
	14	10.0±1.7(0.60)	168.0±22.0 (1.73)	18.0±5.6(0.90)	111.7±19.9(1.14)	35	13.0±0.0(1.05)	268.3±19.6(1.04)	15.3±0.6(1.02)	78.3±19.9(0.89)
	0	16.7±4.2	97.3±31.9	20.0±2.6	98.3±10.1	0	12.3±3.5	259.0±48.3	15.0±1.0	87.7±11.6
	PC	121.7±36.3	568.3±120.7	364.3±111.7	405.0±36.3	PC	136.3±2.5	823.0±61.5	259.7±27.3	360.0±43.6
Golden wattle	302	15.0±1.4(1.22)	257.7±34.6(0.99)	19.0±2.6(1.27)	84.7±6.1(0.97)	314	16.5±2.1(1.33)	323.0±14.9(1.25)	21.7±2.1(1.45)	94.0±19.8(1.20)
	151	11.0±2.8(0.89)	332.3±86.8(1.28)	16.0±1.7(1.07)	74.7±10.0(0.85)	157	23.0±4.2(1.86)	299.0±24.6(1.15)	16.7±4.5(1.11)	47.3±6.8(0.60)*
	75	9.5±2.1(0.77)	270.7±13.7(1.05)	17.3±3.2(1.15)	87.0±8.5(0.99)	78	13.3±2.3(1.13)	329.3±19.5(1.27)	18.7±8.5(1.25)	61.3±7.6(0.78)
	38	14.5±2.1(1.18)	244.0±25.2(0.94)	12.3±0.6(0.82)	86.0±6.6(0.98)	39	14±1.4(1.14)	269.0±33.7(1.04)	21.7±2.1(1.45)	63.3±0.6(0.81)
	19	8.5±6.4(0.69)	378.0±64.2(1.46)	18.7±3.5(1.25)	96.7±10.4(1.10)	20	7.0±1.4(0.57)	259.7±62.6(1.00)	20.0±3.6(1.33)	63.3±12.6(0.81)
	0	12.3±3.5	259.0±48.3	15.0±1.0	87.7±11.6	0	12.3±3.5	259.0±48.3	15.0±1.0	78.3±4.6
	PC	136.3±2.5	823.0±61.5	259.7±27.3	360.0±43.6	PC	136.3±2.5	823.0±61.5	259.7±27.3	331.7±76.1
Holm	138	25.0±0.0**(2.08)**	263.3±43.9 (1.02)	16.7±4.5(1.11)	71.7±16.3(0.91)	388		451.5±44.5(0.80)	26.5±0.7(1.20)	88.7±4.5(1.01)

oak	69	23.0±3.0(1.92)	294.3±8.1 (1.14)	19.0±4.6(1.27)	67.3±6.0(0.86)	194	10.0±5.7(0.81)	519.3±90.7(0.92)	18.0±4.2(0.82)	87.0±10.8(0.99)
	34	17.3±3.2(1.44)	303.3±44.4 (1.17)	21.0±3.0(1.4)	68.3±5.1(0.87)	97	10.5±0.7(0.85)	537.0±61.9(0.96)	15.0±4.4(0.68)*	96.0±6.6(1.10)
	17	15.0±2.0(1.25)	317.3±17.8 (1.23)	16.0±1.0(1.07)	71.3±4.2(0.91)	49	13.5±3.5(1.09)	450.7±97.6(0.80)	12.3±0.6(0.56)*	81.0±12.3(0.92)
	9	18.7±3.2(1.56)	244.7±38.8 (0.94)	13.7±2.5(0.91)	65.3±4.7(0.83)	24	19.5±3.5(1.58)	612.7±172.6(1.09)	13.3±4.0(0.60)*	97.5±19.1(1.11)
	0	12.0±5.6	259.0±48.3	15.0±1.0	78.3±4.6	0	12.3±3.5	561.7±47.4	22.0±1.0	87.7±11.6
	PC	149.3±17.8	823.0±61.5	259.7±27.3	331.7±76.1	PC	136.3±2.5	1482.0±139	279.7±25.8	360.0±43.6
	474	14.3±2.9(1.19)	311.1±8.0(1.20)	18.3±3.2(1.22)	56.7±5.5(0.72)*	1064	12.7±3.8(0.76)	152.7±10.7(1.57)	19.0±1.0(0.95)	125.0±22.1(1.27)
	237	17.3±5.5(1.44)	301.7±46.9(1.16)	18.7±1.2(1.23)	79.0±18.4(1.01)	532	15.3±4.6(0.92)	146.7±1.5(1.51)	18.3±4.9(0.92)	121.0±2.6(1.23)
Mariti-	119	14.3±1.5(1.19)	344.7±76.2(1.33)	15.3±1.2(1.02)	72.3±2.1(0.92)	266	18.0±4.2(1.08)	145.0±8.2(1.49)	18.3±8.3(0.92)	106.0±7.2(1.08)
me	59	20.7±1.5(1.73)	326.3±107.0(1.26)	17.7±2.1(1.18)	72.3±4.0(0.92)	133	14.5±0.7(0.87)	144.3±18.8(1.48)	15.0±3.5(0.75)	109.0±7.8(1.11)
pine	30	12.7±1.5(1.06)	305.3±50.8(1.18)	15.7±3.2(1.05)	60.3±4.2(0.77)	66	18±1.4(1.08)	146.3±30.7(1.50)	18.3±5.7(0.92)	91.7±10.6(0.93)
	0	12.0±5.6	259.0±48.3	15.0±1.0	78.3±4.6	0	16.7±4.2	97.3±31.9	20.0±2.6	98.3±10.1
	PC	149.3±17.8	823.0±61.5	259.7±27.3	331.7±76.1	PC	121.7±36.3	568.3±120.7	364.7±11.7	405.0±36.3
	618	14.0±1.0(1.17)	280.3±29.0(1.08)	19.0±3.5(1.27)	78.0±39.9(1.00)	630	11.7±8.3(0.95)	643.0±40.7(1.14)	26.7±4.7(1.21)	106.7±9.5(1.22)
	309	15.3±5.5(1.28)	280.7±5.9(1.08)	21.3±7.2(1.42)	64.7±6.7(0.83)	315	11.0±2.8(0.89)	577.7±81.1(1.03)	24.0±7.2(1.09)	99.3±5.7(1.13)
	154	14.0±2.6(1.17)	290.0±40.0(1.12)	16.0±1.7(1.07)	70.0±2.6(0.89)	158	11.0±4.2(0.89)	429.3±54.6(0.76)	17.7±3.5(0.80)	93.0±6.2(1.06)
Olive	77	18.3±3.2(1.52)	253.7±26.3(0.98)	14.7±3.8(0.98)	64.7±7.5(0.83)	79	13.5±0.7(1.09)	353.7±78.2(0.63)	19.3±0.6(0.88)	91.7±19.6(1.05)
	39	13.7±3.1(1.14)	304.0±39.0(1.17)	21.3± 2.1(1.42)	63.7±7.2(0.81)	39	12.5±7.1(1.01)	504.0± 80.1(0.90)	16.5±4.9(0.75)	96.3±16.6(1.10)
	0	12.0±5.6	259.0±48.3	15.0±1.0	78.3±4.6	0	12.3±3.5	561.7±47.4	22.0±1.0	360.0±43.6
	PC	149.3±17.8	823.0±61.5	259.7±27.3	331.7±76.1	PC	136.3±2.5	1482.0±139	279.7±25.8	87.7±11.6
	234	23.5±2.1(1.91)	323.7±72.5(1.25)	15.3±1.5(1.02)	100.3±10.8(1.14)	685	16.3±3.8(0.98)	169.7±10.0(1.74)	21.5±4.9(1.08)	132.0±9.5(1.34)
	117	13.0±4.2(1.05)	309.3±20.3(1.19)	16.7±4.5(1.11)	89.0±6.1(1.02)	343	10.0±5.6(0.60)	159.3±3.5(1.64)	17.0±2.6(0.85)	128.0±37.6(1.30)
Portu-	59	14.0±2.8(1.14)	278.7±17.4(1.08)	13.7±0.6(0.91)	70.3±5.5(0.80)	171	16.0±7.0(0.96)	171.0±1.7(1.76)	18.3±2.5(0.92)	117.7±16.0(1.20)
guese	29	12.5±2.1(1.01)	287.7±12.7(1.11)	15.7±1.2(1.05)	85.7±21.1(0.98)	86	12.7±4.5(0.76)	156.0±24.5(1.60)	15.0±3.6(0.75)	130.0±9.6(1.32)
oak	15	12.5±4.9(1.01)	310.3±39.3(1.20)	20.7±0.6(1.38)	78.0±2.6(0.89)	43	13.3±2.9(0.80)	167.7±20.3(1.72)	22.0±1.0(1.1)	138.3±25.1(1.41)
	0	12.3±3.5	259.0±48.3	15.0±1.0	87.7±11.6	0	16.7±4.2	97.3±31.9	20.0±2.6	98.3±10.1
	PC	136.3±2.5	823.0±61.5	259.7±27.3	360.0±43.6	PC	121.7±36.3	568.3±120.7	364.7±11.7	405.0±36.3

[(1)] Values are means± Standard Deviation of 2-3 plates; [(2)] Numbers in parenthesis are the ratios of test values to negative control (in DMSO) values. [(3)] Ratios of test values to negative control equal to or above 2 are marked in **bold**; 0: negative control in DMSO; PC: positive control; * values significantly different from the negative control (p<0.05), n=3

Table 3: Mutagenicity of PAH extracts of particles emitted in domestic wood combustion by fireplace, hot start and cold start, to *S. typhimurium* TA98, TA100 in the absence and presence of S9 mixture

Wood species	Hot start					Cold start				
	ng PAHs /plate	Number of revertants (mean±SD) [1]				ng PAHs /plate	Number of revertants (mean±SD)			
		TA98	TA100	TA98/S9	TA100/S9		TA98	TA100	TA98/S9	TA100/S9
Briquettes	599	16.0±5.2(0.96) [2]	171.3±4.5(1.76)	20.5±0.7(1.34)	123.0±2.6(1.25)	378	9.5±0.7(1.06)	133.5±3.5(1.12)	18.7±1.5(0.85)	105.3±9.3(1.20)
	300	19.0±2.6(1.14)	178.0±24.6(1.83)	14.7±3.8(0.98)	115.7±27.0(1.18)	189	10.3±4.0 (1.14)	121.0±22.6(1.01)	18.0±1.0(0.81)	94.5±6.4(1.08)
	150	14.3±3.5(0.86)	178.3±26.7(1.83)	18.0±5.3(1.2)	119.7±6.1(1.22)	95	11.0±3.6 (1.22)	102.0±17.0(0.85)	17.0±5.3(0.77)	94.7±15.0(1.08)
	75	13.7±3.5(0.82)	175.7±14.0(1.81)	17.0±1.0(1.13)	128.3±11.9(1.31)	47	11.7±1.2(1.3)	142.0±31.11.19)	16.7±1.5(0.76)	117.0±10.5(1.33)
	37	14.7±2.9(0.88)	156.7±6.7(1.61)	17.0±1.0(1.13)	105.3±5.5(1.07)	24	12.7±4.6(1.41)	142.0±2.8(1.19)	15.0±3.6(0.68)	106.0±12.7(1.21)
	0	16.7±4.2	97.3±31.9	15.0±1.0	98.3±10.1	0	9.0±5.6	119.3±33.1	22.0±1.0	87.7±11.6
	PC	121.7±36.3	568.3±120.7	259.7±27.3	405.0±36.3	PC	213.3±8.7	610.7±12.9	279.7±25.8	360.0±43.6
Cork oak	442	12.7±4.0(1.41)	126.0±12.7(1.06)	26.0±2.8(1.18)	113.5±9.2(1.29)	811	12.3±0.6(1.37)	135.0±0.0(1.13)	21.0±4.2(1.26)	108.0±4.6(1.23)
	221	8.7±3.2(0.97)	101.0±11.3(0.85)	17.7±2.9(0.80)	103.7±17(1.18)	406	13.7±5.5(1.52)	142±45.3(1.19)	16.0±1.4(0.96)	97.7±17.9(1.11)
	110	10.0±1.7(1.11)	115.5±3.5(0.97)	15.7±4.5(0.71)*	128.0±20(1.46)	203	18.7±4.0**(2.08)**	109.0±8.5(0.91)	19.0±5.7(1.14)	114.0±11.5(1.30)
	55	8.3±2.5(0.92)	119.5±6.4 (1.00)	21.3±2.9(0.97)	94.0±5.6(1.07)	101	12.7±3.2(1.41)	114.5±0.7(0.96)	12.0±4.1(0.72)	106.0±14.1(1.21)
	28	10.3±3.8(1.14)	111.5±19.1(0.93)	21.5±0.7(0.98)	125.7±23(1.43)	51	13.7±1.5(1.52)	134.5±6.4(1.13)		105.0±21.0(1.20)
	0	9.0±5.6	119.3±33.1	22.0±1.0	87.7±11.6	0	9.0±5.6	119.3±33.1	22.0±1.0	87.7±11.6
	PC	213.3±8.7	610.7±12.9	279.7±25.8	360.0±43.6	PC	213.3±8.7	610.7±12.9	279.7±25.8	360.0±43.6
Eucalypt	83	16.0±0.0(1.30)	129.3±1.2(1.33)	26.5±3.5(1.77)	109.7±8.4(1.12)	360	18.3±1.5(1.10)	345.0±120(1.33)		72.0±1.0(0.92)
	42	15.5±2.1(1.26)	145.0±22.9(1.49)	19.0±4.2(1.27)	110.0±20.7(1.12)	180	17.6±2.6(1.05)	258.3±24.5(1.00)	19.7±3.2(1.31)	72.7±7.6(0.93)
	21	14.5±7.8(1.18)	146.7±21.4(1.51)	26.5±13(1.77)	106.7±15.0(1.08)	90	17.3±2.1(1.04)	295.3±57.7(1.14)	19.0±3.6(1.27)	65.3±8.6(0.83)
	10	17.0±7.1(1.38)	168.3±34.0(1.73)	14.0±3.0(0.70)	105.6±16.0(1.07)	45	14.7±5.1(0.88)	434.3±36.0(1.68)	18.7±1.5(1.25)	63.7±1.5(0.81)
	5	12.5±0.7(1.01)	152.7±11.2(1.57)	15.0±7.2(0.75)	120.7±2.1(1.23)	23	15.0±6.1(0.90)	299.7±61.7(1.16)	16.7±4.6(1.11)	94.0±38.1(1.2)
	0	12.3±3.5	97.3±31.9	20.0±2.6	98.3±10.1	0	16.7±4.2	259.0±48.3	15.0±1.0	78.3±4.6
	PC	136.3±2.5	568.3±120.7	364.7±11.7	405.0±36.3	PC	121.7±36.3	823.0±61.5	259.7±27.3	331.7±76.1
Golden wattle	272	11.0±4.6(1.22)	103.5±19.1(0.87)	22.0±3.6(1.00)	103.7±5.1(1.18)	684	12.0±2.6(1.33)	472.3±119(0.84)	26.7±3.5(1.21)	122.3±3.5(1.24)
	136	10.7±3.5(1.19)	107.0±0.0(0.90)	24.3±4.9(1.10)	77.5±3.5(0.88)	342	10.7±3.2(1.19)	475.7±29.9(0.85)	27.0±4.0(1.23)	144.0±61.6(1.46)
	68	8.7±5.7 (0.97)	110.5±6.4(0.93)	25.7±5.9(1.17)	90.0±8.7(1.03)	171	9.7±1.5(1.08)	571.0±101(1.02)	20.3±0.6(0.92)	81.0±6.6(0.82)
	34	10.0±5.6(1.11)	459.7±0.0(0.95)	23.3±2.9(1.06)	83.7±12.3(0.95)	85	10.0±2.6(1.11)	498.0±85.5(0.89)	19.0±2.8(0.86)	100.7±2.1(1.02)
	17	12.0±3.6(1.33)	96.5±2.1(0.81)	19.0±2.6(0.86)	94.7±11.9(1.08)	43	10.7±2.1(1.19)	620.3±38.9(1.10)	26.0±1.7(1.18)	114.0±10.4(1.16)
	0	9.0±5.6	119.3±33.1	22.0±1.0	87.7±11.6	0	9.0±5.6	561.7±47.4	22.0±1.0	98.3±10.1
	PC	213.3±8.7	610.7±12.9	279.7±25.8	360.0±43.6	PC	213.3±8.7	1482.0±139	279.7±25.8	405.0±36.3
Holm	333	14.3±1.2(1.19)	302.3±49.7(1.17)	14.7±4.6(0.98)	70.0±8.7(0.89)	473	12.0±0.0(1.33)	113.0±18.2(1.16)	16.5±6.4(0.75)	105.7±24.8(1.07)

oak	166	18.3±2.9(1.53)	368.3±118(1.42)	19.7±1.5(1.31)	69.0±14.7(0.88)	237	15.7±3.5(1.74)	166.0±42.3(1.71)	19.7±4.5(0.90)	106.3±14.2(1.08)
	83	13.7±1.5(1.14)	271.7±16.1(1.05)	16.0±2.6(1.07)	64.3±11.0(0.82)	119	10.3±2.9(1.14)	117.0±19.9(1.20)	21.3±5.0(0.97)	98.3±14.6(1.00)
	42	14.0±6.6(1.17)	281.3±45.0(1.09)	16.0±1.7(1.07)	47.7±15.0(0.61)	59	10.0±2.6(1.11)	83.0±0.0(0.85)	23.3±0.6(1.06)	109.7±6.41.12)
	21	22.3±7.1(1.86)	266.3±28.5(1.03)	15.0±2.6(1.00)	57.0±7.0(0.73)	30	12.7±8.1(1.41)	150±70.7(1.54)	16.3±5.5(0.74)	107.3±19.9(1.09)
	0	12.0±5.6	259.0±48.3	15.0±1.0	78.3±4.6	0	9.0±5.6	97.3±31.9	22.0±1.0	98.3±10.1
	PC	149.3±17.8	823.0±61.5	259.7±27.3	331.7±76.1	PC	213.3±8.7	568.3±120.7	279.7±25.8	405.0±36.3
Mariti-me pine	461	21.7±4.0(1.81)	313.3±24.5(1.21)	18.0±2.0(1.20)	83.7±15.9(1.07)	1124	15.3±3.2(0.92)	147.0±20.1(1.51)	21.7±2.5(1.05)	127.3±7.1(1.29)
	231	21.7±1.2(1.81)	303.3±27.3(1.17)	16.3±5.9(1.08)	63.0±15.5(0.80)	562	19.0±1.0(1.14)	161.3±35.1(1.66)	20.7±2.3(1.04)	107.3±4.6(1.09)
	115	20.5±3.5(1.71)	332.3±50.0(1.28)	20.7±6.4(1.38)	69.7±5.5(0.89)	281	14.7±5.1(0.88)	162.0±19.3(1.66)	14.7±3.1(0.74)*	100.7±8.1(1.02)
	58	18.0±10(1.50)	279.7±32.1(1.08)	16.7±4.6(1.11)	56.0±8.7(0.71)	140	15.0±5.0(0.90)	164.3±18.9(1.69)	15.0±2.6(0.75)	105.7±13.3(1.07)
	29	12.0±2.8(1.00)	338.0±51.9(1.31)	16.7±3.8(1.11)	87.7±23.1(1.12)	70	16.7±2.1(1.00)	157.3±15.4(1.62)	19.0±1.0(0.95)	110.0±13.9(1.12)
	0	12.0±5.6	259.0±48.3	15.0±1.0	78.3±4.6	0	16.7±4.2	97.3±31.9	20.0±2.6	98.3±10.1
	PC	149.3±17.8	823.0±61.5	259.7±27.3	331.7±76.1	PC	121.7±36.3	568.3±120.7	364.7±11.7	405.0±36.3
Olive	603	27.0±1.4**(2.19)**[3]	159.3±10.7(1.64)	19.0±1.7(0.95)	91.7±3.5(0.93)	1040	11.7±4.0(1.3)	115.0±7.1(0.96)	26.7±4.7(1.21)	98±9.8(1.12)
	302	19.0±2.8(1.54)	180.0±4.4(1.85)	25.3±5.5(1.27)	95.3±11.6(0.97)	520	11.0±7.0(1.22)	109.0±18.4(0.91)	24.0±7.2(1.09)	108.0±20.0(1.23)
	151	19.5±3.5(1.58)	152.7±28.0(1.57)	22.7±5.0(1.14)	90.0±4.6(0.92)	260	15.7±1.5(1.74)	112.5±17.7(0.94)	17.7±3.5(0.80)	126.3±22.2(1.44)
	76	20.0±2.8(1.62)	152.7±35.9(1.57)	13.5±2.1(0.68)	90.0±15.4(0.92)	130	11.0±0.0(1.22)	110.0±2.8(0.92)	19.3±0.6(0.87)	113.0±8.0(1.29)
	38	9.5±0.7(0.77)	193.3±4.0(1.99)	13.0±2.8(0.65)	97.7±17.6(0.99)	65	15.3±5.0(1.7)	104.5±10.6(0.87)	16.5±4.3(0.75)	119.3±22.5(1.36)
	0	12.3±3.5	97.3±31.9	20.0±2.6	98.3±10.1	0	9.0±5.6	119.3±33.1	22.0±1.0	87.7±11.6
	PC	136.3±2.5	568.3±120.7	364.7±11.7	405.0±36.3	PC	213.3±8.7	610.7±12.9	279.7±25.8	360.0±43.6
Portugu-ese oak	528	12.3±2.1(1.37)	126.0±12.7(1.06)	34.0±4.2(1.55)	102.3±15.1(1.17	674	34.3±16.2**(2.86)**	327.7±39.3(1.27)	19.7±6.4(1.31)	92.3±13.6(1.18)
	264	16.3±4.2(1.81)	101.0±11.3(0.98)	26.3±2.1(1.20)	99.3±21.5(1.13)	337	25.3±5.5**(2.11)**	284.7±40.0(1.10)	20.7±2.5(1.38)	79.0±10.5(1.01)
	132	17.3±4.9(1.92)	115.5±3.5(0.97)	30.7±6.4(1.40)	100.7±17.8(1.15	168	18.3±4.2(1.53)	300.7±30.4(1.16)	20.7±5.9(1.38)	81.3±13.0(1.04)
	66	13.7±4.7(1.52)	119.5±6.4(1.00)	24.0±3.5(1.09)	98.3±16.9(1.12)	84	14.7±7.6(1.23)	309.3±35.9(1.19)	16.7±2.9(1.11)	72.3±8.4(0.92)
	33	13.0±2.6(1.44)	98.0±2.8(0.82)	22.3±2.1(1.01)	95.0±9.9(1.08)	42	20.7±5.5(1.73)	320.7±32.2(1.24)	16.3±4.2(1.09)	75.0±15.1(0.96)
	0	9.0±5.6	119.3±33.1	22.0±1.0	87.7±11.6	0	12.0±5.6	259.0±48.3	15.0±1.0	78.3±4.6
	PC	213.3±8.7	610.7±12.9	279.7±25.8	360.0±43.6	PC	149.3±17.8	823.0±61.5	259.7±27.3	331.7±76.1

[1] Values are means± Standard Deviation of 2-3 plates; [2] Numbers in parenthesis are the ratios of test values to negative control (in DMSO) values. [3] Ratios of test values to negative control equal to or above 2 are marked in **bold**; 0: negative control in DMSO; PC: positive control; * values significantly different from the negative control (p<0.05), n=3

Table 4: Mutagenicity potency for biomass combustion PAHs based on Ames test with tester strain TA98 and TA100

Fuel	Burning appliance	Direct-acting mutagenicity		Indirect-acting mutagenicity	
		Slope (rev/ngPAHs)	r^2	Slope (rev/ngPAHs)	r^2
Briquettes	Woodstove Hot start	0.050 (1)	0.83		
Eucalypt	Woodstove Cold start			0.010 (1)	0.95
	Woodstove Hot start	0.189 (2)*	0.62		
	Fireplace Cold start			0.015 (1)	0.67
Holm oak	Woodstove Hot start	0.068 (1)*	0.77		
Maritime pine	Woodstove Cold start			0.062 (2)	0.73
	Fireplace Cold start			0.019 (2)	0.67
Olive	Woodstove Cold start			0.022 (2)	0.84
	Fireplace Hot start	0.021(1)*	0.65		
Portuguese Oak	Fireplace Cold start	0.027 (1)*	0.87	0.027 (2)	0.86
	Fireplace Hot start	0.048 (1)	0.83	0.019 (1) 0.010(2)	0.67 0.62

(1) Result of test with strain TA98
(2) Result of test with strain TA100
* Samples with revertant/plate two times higher than that of negative control

Table 5: Samples with average number of revertants on plates significant below that on the negative control plates

Wood species/ Bacteria strain(1)	Burning condition	*F*	*df*	*p*
Briquettes TA98	Woodstove Hot start	6.992	5, 10	0.005
Cork oak TA98	Fireplace Hot start	3.904	5, 10	0.032
Golden wattle TA100	Woodstove Cold start	7.281	5, 11	0.003
Holm oak TA98	Woodstove Cold start	8.137	5, 10	0.003

Maritime pine TA100	Woodstove Hot start	3.648	5, 12	0.031
Maritime pine TA98	Fireplace Cold start	4.417	5, 12	0.016

(1)All samples were in test with the presence of metabolic agent

4. Discussion

4.1. Polycyclic aromatic hydrocarbons emissions

$PM_{2.5}$ emissions were higher during the combustion experiments in the fireplace than in the woodstove (Table 1). In general, emission factors of particulate matter, total PAHs, the 13 mutagenic PAHs, and flouranthene and BaP were higher during the experiments with cold start for both appliances. Lower temperatures result in a lower degree of conversion (oxidation) of the biomass (solid and pyrolysis products), thus originating a higher emission of unburned chemical compounds. The particulate matter emissions obtained from the combustion of maritime pine, the only softwood burned, were the lowest among all, whether in the woodstove or in the fireplace. Emissions from the woodstove at higher temperatures included a higher PAH content in terms of mass ratio to OC (Gonçalves et al., 2011) than those of the fireplace, whereas comparable average total PAH emissions were obtained in both combustion appliances during the start-up phase. It is known that PAH amounts increase with the increase of temperatures and oxygen content in supplied air (Lu et al., 2009; and references therein). In the present study, the fireplace wasoperated at lower temperatures than those of the woodstove, but the O_2 content in supplied air was around 20%, while values lower than 15% were provided to the woodstove (Gonçalves et al., 2011).

4.2. Mutagenic activities of different wood species

Seven in total eight wood species used in this study had emissions with mutagenic/weak mutagenic activity in Ames assay with *S.typhymirium*TA98 and/or TA100. They were briquettes, cork oak, eucalypt, Holm oak, maritime oak, olive, and Portuguese oak. The opposite was recorded only for the Golden wattle. Fourteen samples of those wood species observed dose-related increase in the number of revertant colonies; however, only four of them had the average number of revertant colonies in platemore than two times of the one recorded in corresponding negative control, which indicated that those samples were mutagen (Mortlemans and Zieger, 2000). They are extracts of particulate matter emitted from combustion of olive by fireplace, hot start; Portuguese oak by fireplace, cold start; and Holm oak and eucalypt by woodstove, hot start. All of them are mutagenic in the absence of metabolic agent. The remaining ten samples had the average revertant of

colonies not exceed two times of that recorded in negative control plate were characterised as weak mutagens (Mortelmans and Zieger, 2000). Theoretically, those samples should be considered for additional test with some changes in the test design (Mortelmans and Zieger, 2000); however, due to the insufficient sample, no additional test was performed.

Eight of the fourteen samplesdisplayed a positive result (as weak mutagens) when the S9 mixture was added, suggesting the presence of indirect-acting mutagens. They were extracts of particulate matter obtained from combustion by fireplace in cold start of eucalypt, maritime pine and Portuguese oak, combustion by woodstove in cold start of eucalypt and maritime pine, and combustion by fireplace in hot start of Portuguese oak. Potential higher risks of mutagenic effects resulting from exposures to particulate matter of these species under the mentioned burning conditions could be expected to mammal species, man included. This suspicion should be the trigger force to complement the evaluation of these extracts with a complementary battery of mutagenicity/carcinogenicity assays (Claxton and Woodall, 2007). The remaining six positive samples were in tests without metabolic agent, indicating the direct-acting mutagenicity of those samples. They were extracts of particulate matter from combustion of briquettes, eucalypt and Holm oak by woodstove in hot start condition; combustion of olive and Portuguese oak by fireplace in cold start condition; and combustion of Portuguese oak by fireplace in hot start condition. Portuguese oak was the only one wood species having mutagenic PAHs adsorbed on $PM_{2.5}$ emitted upon combustion in both tests with and without metabolic activation. The only mutagenic sample without regression line was cork oak (fireplace, cold start, without S9), imposing direct mutagenicity. Direct-acting mutagenicity in extracts of combustion products of wood has been linked to the presence of polar fraction such as nitroarenes (Claxton et al., 2004); however it is not applicable in all the cases, such as coconut smoke (Bell and Kamens et al. 1990).

Six out of seven direct-acting mutagenic samples and three out of eight indirect-acting mutagenic samples imposed direct mutagenicity towards TA98, suggesting the shift mutation mechanism (Mortelmans and Zieger, 2000). The remaining samples were mutagenic to TA100, which implies the base-pair substitution mutation mechanism. Direct-acting mutagenic samples seemed to be more sensitive towards TA98 than TA100. On the other hand, samples that were mutagenic in indirect-acting procedure are more sensitive towards TA100.

In the case of Portuguese oak (fireplace, hot start), when S9 was introduced to the test, the mutagenic activity decreased in assay TA98+S9, and increased in assay TA100+S9. This suggests that this sample contained indirect acting base-pair substitution mutagens that are activated in the presence of metabolic agent, and direct acting frameshift mutagens that

loose their mutaginicity after being homogeneized by enzymes from the S9 liver fraction. Similar trend was observed in the study of Oanh et al. (2002) where the mutagenicity of organic extract from wood combustion decreased when S9 was added. Other samples induced mutagenicity, if had, in one of four possible mechanisms: direct/indirect-acting frameshift/base-pair substitution, either restrained by or reinforced by metabolic activation.

PAH extracts in $PM_{2.5}$ from combustion of eucalypt (woodstove, hot start) were the mightiest mutagens with mutagenic potency of 0.189 revertant/ng PAHs (Table 4). Following was Holm oak, maritime pine, briquettes, Portuguese oak and olive. In general, mutagenic activity of direct-acting mutagenic samples was higher than that of indirect-acting mutagenic samples.

Studies have been carried out to compare mutagenicity of different wood species. McCrillis et al. (1992) concluded that emissions from burning pine were more mutagenic than those from burning oak. This is contrast to the results in this study, where combustion emissions of Holm oak were more potent than those of pine. Therefore the conclusion of McCrillis et al. (1992) cannot generalise for all oak and pine species. Mukherji et al. (2002) did a research comparing mutagenicity of organic matter extracts of $PM_{2.5}$ from burning of wood (*Acasianilotica*), dung cake and briquettes and found out that wood was less mutagenic compared to briquettes. This difference in mutagenicity was not observed in this study, in which briquettes emissions were found out to have comparable mutagenicity as some wood species.

The mutagenic potency of EOM of burning other wood species has been established (Asita et al., 1991; Bell and Kamens, 1990; Mukherji et al., 2002; Oanh et al., 2002). Most of them were expressed in mutagenicity emission factors (revertant/kg wood burnt or revertant/g extracts). However, the mutagenicity emission factors were not calculated in this study, due to the fact that only PAH fraction was collected and tested in Ames assay. In addition to PAH fraction, the mutagenicity of organic extracts is due to an extensive number of compounds of various chemical classes, including oxygen-containing compounds, nitrogen-containing compound, halogen-containing compounds and so forth (Claxton el al., 2004). Although in some cases the PAH might contribute 12-25% S9 mutagen to indirect mutagenicity of wood smoke (Bell and Kamens, 1986), there was not sufficient data to generalise the mutagenicity of this faction into the emission factor of the whole organic extracts and total emissions. Moreover, there are various factors need to be taken carefully into account when comparing the results of mutagenicity tests between different studies such as the extraction method, bioassay, wood composition, moisture content, burning condition (burning rate, burning temperature), air supply, stove design

(Claxton et al., 2004). Therefore, the comparison of mutagenic activity of combustion emissions of wood species in this study to other wood species was not carried out.

Five wood species showed an average number of revertants on the plates significantly below (p<0.05) that recorded on the negative control plates, which recommended the toxicity of the samples upon the bacteria strain. They are briquettes, cork oak, golden oak, Holm oak, and Maritime pine. The toxicity restrained the growth of bacteria and might mask the mutagenicity of those samples. Theoretically, repeats of those Ames tests at different range of concentration should be carried out in order to clarify that doubt; however, due to the limit in the sample quantity, those tests were not performed.

4.3. Influence of burning appliance and burning condition on the mutagenicity of emissions

Mild (cold start) combustion in start-up phase resulted in more mutagen samples (eight samples) in comparison to hot start combustion (seven samples). Combustion in hot start condition induced more direct-acting mutagenicity (five in six direct-acting mutagenic samples), whereas combustion in start-up phase resulted in more indirect-acting mutagens (six in eight indirect-acting mutagenic samples), which is believed more dangerous to mammals in general and humans in particular, as the S9 fraction mimics the role of the liver in the metabolism of organic contaminants. In opposition, burning by fireplace produced more mutagenic samples (nine samples), particularly indirect-acting mutagens (five samples), comparing to combustion carried out in woodstove (six samples, three indirect-acting mutagens). This is in an agreement with the results of van Houdt et al. (1986), who found out that fireplace led to an increase in direct and indirect mutagenicity of bioduel emission in comparison to wood stove.

4.4. Correlation between polycyclic aromatic hydrocarbon emissions and mutagenicity

In general, there were no evident relations between PAH emissions and the mutagenicity of the samples. In fact, as it was observed by the PCA biplot only two of the strongest mutagenic extracts were correlated with carcinogenic PAHs. Such fact suggests that organic compounds other than PAHs analysed may have been responsible for the mutagenicity of these samples. The most potent mutagens corresponded to samples with moderate emission factors of $PM_{2.5}$, PAHs, 13 carcinogenic/mutagenic PAHs,fluoranthene and BaP.Those samples had strong direct-acting mutagenicity, which is contrast to the reported indirect mutagenic activities of the 13 carcinogenic/mutagenic PAHs (IARC, 1998). On the other hand, when taking into account only samples with indirect-acting

mutagenic activities, those samples were virtually the ones with highest emission factors of total PAHs, 13 carcinogenic/mutagenic PAHs, and fluoranthane and BaP. However, the sequence of mutagenic potency of those samples was not perfectly corresponding to emissions factors of total PAHs, 13 mutagenic PAHs and the two PAHs. Samples from burning of Portuguese oak by fireplace, hot start had lower emissions factors of those PAHs but were the most potent indirect mutagens.As mentioned above, this suggests again a possibility that the PAH mixture contained other mutagens than 13 identified PAHs, which acting by means of direct or indirect way.

As far as we know, no study has investigated the mutagenic activity of PAHs obtained from domestic combustion of biofuel. Many researchers have carried out Ames test for the obtained particles or the extractable organic matterfrom particles and have attempted to link their mutagenicity and the PAH content (Asita et al., 1991; Mukherji et al., 2002; Oanh et al, 2002). The mass of particles, organic extracts, and PAH content tested in those studies was much higher than in this study.

Asita et al. (1991) tested the mutagenicity of smoke condensates obtained from burning of five wood species. Mass of smoke condensates in each *Salmonella* test was from 0 to 3000 μg/plate, many of which were mutagen in the absence and presence of metabolic agent. TA98 strain was more sensitive to the condensates than the TA100. They attempted to determine the relative contribution of PAHs to the total mutagenicity of the smoke condensates by means of Ames assay with individual PAH, including BaP and pyrene, at dose of 250-4000 ng/plate; however no mutation was induced. This concentration is considerably higher than PAHs tested in this study (maximum 1124 ng/plate). This suggests the higher mutagenicity of samples tested in this study, even though the mutagenicity of the mixture can be different from the sum of individual test. Mukherji et al. (2002) tested the mutagenic activity of organic extractsof $PM_{2.5}$ particles emitted by burning of wood, dung cake and biofuel briquettes by a number of traditional woodstoves. The test doses were 2, 3, and 5 μg extract/plate. The largest contributor to the total mutagenicity of mixture was direct acting component (more than 70%).Oanh et al. (2002) tested the mutagenicity of organic extracts of particulate matter,with the mass of PAH in in range of 28.7-6160 mg, released from combustion of wood (*Pterocarpusindicus*), saw dust, and kerosene by different burning appliances,.The organic extract from burning of wood by Thailand ceramic woodstove was the most mutagenic one. This extract presented mutagenicity in the absence, as well as in the presence, of S9 mix.

5. Conclusion

PAH extracts of $PM_{2.5}$ collected from combustion of seven wood species and briquettes were tested for mutagenic activities using Ames test with *Salmonella typhimurium* TA98 and TA100. The woods were *Pinuspinaster* (maritime pine), *Eucalyptus globulus* (eucalypt), *Quercussuber* (cork oak), *Acacia longifolia* (golden wattle), *Quercusfaginea* (Portuguese oak), *Oleaeuropea* (olive), and *Quercus ilex rotundifolia* (Holm oak). Burning experiments were done using woodstove and fireplace, hot start and cold start. In general, emissions from the woodstove at higher temperatures include a higher PAH content than those of the fireplace, whereas comparable average total PAH emissions were obtained in both combustion appliances during the start-up phase. Although, a mutagenic/weak mutagenic response was recorded for different species (except for Golden wattle), burning appliances and burning start conditions, the extracts that have proved to have indirect acting mutagens, were mainly obtained from fireplace and cold start conditions. These extracts represent those with potential higher risks to humans exposed to these smoke emissions. The strong mutagenic extracts were not correlated with higher emission factors of carcinogenic PAHs, suggesting that PAHs or other organic compounds, not assessed may be responsible for their mutagenicity. Nevertheless, and despite the low concentration of PAHs recorded in the extracts, comparatively to other studies, several samples have proved to be weak mutagens. Such fact increases our concern about the consequences to humans of continuous exposures to such emissions, especially during the winter, justifying further evaluation. In an ecological point of view, the negative result recorded for the Golden wattle extracts, is positive since after confirmation, this species, which is not an autochthonous species, can be recommended for domestic use, reducing the harvesting of the other species.

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References

Alves, C.A., Vicente, A., Monteiro, C., Gonçalves, C., Evtyugina, M., Pio, C., 2011.Emission of trace gases and organic components in smoke particles from a wildfire in a mixed – evergreen forest in Portugal.Science of the Total Environment 409, 1466-1475.

Asita, A.O., Matsui, M., Nohmi, T., Matsuoka, A., Hayashi, M., Ishidate Jr., M., Sofuni, T., Koyano, M., Matsushita, H., 1991. Mutagenicity of wood smoke condensates in the Salmonella/microsome assay. Mutation Research 264, 7-14.

Atkinson, R., Arey, J., 1994. Atmospheric chemistry of gas – phase polycyclic aromatic hydrocarbons: Formation of atmospheric mutagens. Environmental Health Perspectives, 102 (suppl 4), 117-126.

Aunan, K., Berntsen, T.K., Myhre, G., Rypdal, K., Streets, D.G., Jung-Hun Woo, Smith, K.R., 2009. Radiative forcing from household fuel burning in Asia.Atmospheric Environment43, 35, 5674-5681

Baek, S.O., Field, R.A., Goldstone, M.E., Kirk, P.W., Lester, J.N., Perry, R., 1991.A review of atmospheric polycyclic aromatic□hydrocarbons □ sources, fate and behaviour. Water Air and Soil Pollution 60, 279□300.

Bari, M.A., Baumbach, G., Kuch, B., Scheffknecht, G., 2010. Particle-phase concentrations of polycyclic aromatic hydrocarbons in ambient air of rural residential areas in southern Germany. Air Quality and Atmospheric Health 3, 103-116.

Bell, D., Kamens, R., 1986. Photodegradation of wood smoke mutagens under low NO_x conditions. Atmospheric Environment 20, 2, 317-321.

Bell, D., Kamens, R., 1990. Evaluation of the mutagenicity of combustion particles from several common biomass fuels in the Ames/Salmonella microsome test.Mutation Research Letters 254, 177-183.

Bølling, A.K., Pagels, J., Yttri, K., E., Barregard, L., Sallsten, G., Schwarze, P.E., Boman, C., 2009. Health effects of residential wood smoke particles: the importance of combustion conditions and physicochemical particle properties. Particle and Fibre Toxicology, 6, 29, doi:10.1186/1743-8977-6-29.

Boman, B.C., Forsberg, A.B., Järvholm, B.G., 2003. Adverse health effects from ambient air pollution in relation to residential wood combustion in modern society Scandinavian Journal of Work, Environment and Health 29, 4, 251-260.

Borrego, C., Valente, J., Carvalho, A., Sá, E., Lopes, M., Miranda, A.I, 2010.Contribution of residential wood combustion to PM_{10} levels in Portugal. Atmospheric Environment 44, 5, 642-651.

Calvo, A.I., Tarelho, L.A.C., Duarte, M., Evtyugina, M., Alves, C.A., 2011. Operating conditions of a Portuguese woodstove. Proceedings of the 4*th* International Congress of Energy and Environment Engineering and Management (CIIEM), Merida, Spain.

Caseiro, A., Bauer, H., Schmidl, C., Pio, C.A., Puxbaum, H., 2009.Wood burning impact on PM_{10} in three Austrian regions.Atmospheric Environment43, 2186-2195.

Claxton, L.D., Matthews, P.P., Warren, S.H., 2004. The genotoxicity of ambient outdoor air, a review: Salmonella mutagenicity. Mutation Research 567, 347-399.

Claxton, L.D., Woodall Jr., G.M., 2007. A review of the mutagenicity and rodentcarcinogenicity of ambient air.Mutation research 636, 36-94.

Danny, L.D.H., Kaufmann, R.K., 2002. Simultaneously constraining climate sensitivity and aerosol radiative forcing. Journal of Climate 15, 2837-2861.

Fernandes, A.P.S., 2009. Emission of $PM_{2.5}$ and gases from domestic appliances burning.Master's thesis.University of Aveiro (in Portuguese: http://biblioteca.sinbad.ua.pt/Teses/2010000392).

Fine, P.M., Cass, G.R., Simoneit, B.R.T., 2001.Chemical characterization of fine particle emissions from fireplace combustion of woods grown in the northeastern United States. Environmental Science and Technology 35, 2665-2675.

Fine, P.M., Cass, G.R., Simoneit, B.R.T., 2002.Chemical characterization of fine particle emissions from fireplace combustion of woods grown in the southern United States. Environmental Science and Technology 36, 1442-1551.

Fine, P.M., Cass, G.R., Simoneit, B.R.T., 2004a.Chemical characterization of fine particle emissions from fireplace combustion of woods grown in the Midwestern and western United States.Environmental Engineering Science 21, 387-409.

Fine, P.M., Cass, G.R., Simoneit, B.R.T., 2004b. Chemical characterization of fine particle emissions from the woodstove combustion of prevalent United States tree species. Environmental Engineering Science 21, 705-721.

Gaeggeler, K., Prevot, A.S.H., Dommen, J.,Legreid, G., Reimann, S., Baltensperger, U., 2008. Residential wood burning in an Alpine valley as a source for oxygenated volatile organic compounds, hydrocarbons and organic acids. Atmospheric Environment42, 35, 8278- 8287.

Gelencsér, A., May, B., Simpson, D., Sanchez-Ochoa, A., Kasper-Giebl, A., Puxbaum, H., Caseiro, A., Pio, C., Legrand, M., 2007.Source apportionment of $PM_{2.5}$ organic aerosol over Europe: primary/secondary, natural/anthropogenic, fossil/biogenic origin. Journal of Geophysical Research, 112, D23, D23S04.

Gonçalves, C., Alves, C., Evtyugina, M., Mirante, F., Pio, C., Caseiro, A., Schmidl, C., Bauer, H., Carvalho, F., 2010.Characterisation of PM_{10} emissions from woodstove combustion of common woods grown in Portugal.Atmospheric Environment 44, 4474-4480.

Gonçalves, C., Alves, C., Fernandes, A.P., Monteiro, C., Tarelho, L., Evtyugina, M., Pio, C., 2011. Organic compounds in $PM_{2.5}$ emitted from fireplace and woodstove combustion of typical Portuguese wood species. Atmospheric Environment. In Press.

Gustafson, P., Ostman, C., Sallsten, G., 2008. Indoor levels of polycyclic aromatic hydrocarbons in homes with or without wood burning for heating. Environmental Science and Technology 42, 5074-5080.

Holden, A.S., Sullivan, A.P., Munchak, L.A., Kreidenweis, S.M., Schichtel, B.A., Malm, W.C., Collett Jr., J.L., 2011. Determining contributions of biomass burning and other sources to fine particle contemporary carbon in the western United States. Atmospheric Environment 45, 1986-1993.

IARC, 1998.Polynuclear aromatic compounds.Part 1, chemical, environmental and experimental data. In: IARC Monographs on the evaluation of the carcinogenic risk of chemical to humans, Vol. 32. International Agency for Research on Cancer, Lyon, 453.

Johansson, L.S., Leckner, B., Gustavsson, L., Cooper, D., Tullin, C., Potter, A., 2004. Emission characteristics of modern and old-type residential boilersfired with wood logs and wood pellets. Atmospheric Environment38, 4183-4195.

Lighty, J.S., Veranth, J.M., Sarofim, A.F., 2000. Combustion aerosols: factors governing their size and composition and implications to human health. Journal of the Air and Waste Management Association 50, 1565-1618.

Lipsky, A.M., Robinson, A.L., 2006. Effects of dilution on fine particle mass and partitioning of semivolatile organics in diesel exhaust and wood smoke. Environmental Science and Technology 40, 155-162.

Lu, H., Zhu, L., Zhu, N., 2009. Polycyclic aromatic hydrocarbon emission from straw burning and the influence of combustion parameters.Atmospheric Environment 43, 4, 978-983.

Maron, D.M., Ames, B.N, 1983. Revised methods for the Salmonella mutagenicity test.Mutation Research 113, 173-215.

McCrillis, R.C., Watts, R.R., Warren, S.H., 1992. Effects of operating variables on PAH emissions and mutagenicity of emissions from woodstoves.Journal of the Air and Waste Management Association 42, 5, 691-694.

McDonnald, J. D., Zielinska, B., Fujita, E.M., Sagebiel, J.C., Chow, J.C., Watson, J.G., 2000. Fine particle and gaseous emission rates from residential wood combustion. Environmental Science and Technology 34, 2080-2091.

Mortelmans, K, Zeiger, E., 2000. The Ames *Salmonella*/microsome mutagenicity assay.Mutation Research 455, 29-60.

Mukherji, S., Swain, A.K., Venkataraman, C., 2002.Comparativemutagenicityassessmentofaerosolsin emissions frombiofuelcombustion.AtmosphericEnvironment 36, 5627-5635.

Oanh, N.T.K., Nghiem, L.H., Phyu, Y.L., 2002. Emission of polycyclic aromatic hydrocarbons, toxicity, and mutagenicity from domestic cooking using sawdust briquettes, wood, and kerosene. Environmental Science and Technology 36, 833-839.

OECD, 1997. Test Guideline 471. Bacteria Reverse Mutation. In: OCED Guidelines for the Testing of Chemicals. Organisation for Economic Co-operation and Development. Paris, France.

Portuguese Forest Inventory, 2005.DirecçãoGeral de Florestas, Lisbon.

Puxbaum, H., Caseiro, A., Sanchez-Ochoa, A., Kasper-Giebl, A., Clayes, M., Gelencser, A., Legrand, M., Preunkert, S., Pio, C., 2007.Levoglucosan levels at background sites in Europe for assessing the impact of biomass combustion on the European aerosol background. Journal of Geophysical Research 112 (D23) D23S05/1 – D23S05/11.

Quinn, G.P., Keough, M.J., 2002. Experimental design and data analysis for biologists.Cambridge University Press, Cambridge, UK.

Rogge, W.F., Hildemann, L.M., Mazurek, M.A., Cast, G.R., Simoneit, B.R.T., 1998. Sources of fine organic aerosol. 9. Pine, oak, and synthetic log combustion in residential fireplaces. Environmental Science and Technology 32, 13-22.

Schmidl, C., Marr, I.L., Caseiro, A., Kotianova, P., Berner, A., Bauer, H., Kasper-Giebl, A., Puxbaum, H., 2008. Chemical characterisation of fine particle emissions from woodstove combustion of common woods growing in mid – European Alpine regions.Atmospheric Environment 42, 126-141.

Tarelho L.A.C., Gordaliza A.I.C., Neves D.S.F., Alves C.A., Matos M.A.A., 2011. Characteristics of wood combustion in a Portuguese fireplace and stove. In: Proceedings of the European Biomass Conference and Exhibition, 6-10 June, Berlin, Germany.

Wang, X.Y., Li, Q.B., Luo, Y.M., Lian, Q.D., Xi, M., Ma, J.M., Li, Y., Liu, Y.P., Cheng, C.L., 2010.Characteristics and sources of atmospheric polycyclic aromatic hydrocarbons (PAHs) in Shanghai, China. Environmental Monitoring and Assessment 165, 295-305.

Zielinska, B., Samburova, V., 2011. Residential and non-residential biomass combustion: impact on air quality. Encyclopedia of Environmental Health 819-827.

Part III: Perspectives

This study investigated the mutagenicity of PAH fraction in $PM_{2.5}$ collected from combustion of seven wood species and briquettes. Due to the limit in collected samples, some recommended additional assays were not performed to attain an utmost conclusion about the mutagenic activities of some samples. This problem occurs in many environmental studies, and some researchers have been searching for alternative test designs, which have not been standardised, to reduce the amount of sample required (Claxton et al., 2004).

PAHs contribute significantly to the mutagenic activity of wood smoke; however they are not the only fraction responsible for the total mutagenicity of the wood smoke. There are contributions of oxygenated compounds (e.g. methoxyphenols), nitrogenated compounds, halogenated compounds and organic fractions, which were extracted from the wood smoke. In order to achieve a wider picture of the mutagenicity of emissions from wood combustion, additional studies on other fractions than PAHs should be carried out.

The study focused on two common burning appliances in Portugal: woodstove and fireplace. The same experiment design can be applied for emissions from combustion of the same wood species by other burning devices, such as the modern eco-labelled woodstoves and log boilers, which have better combustion efficiency, to draw a conclusion whether these new technologies actually lessen the mutagenicity of burning emissions. Further studies can also extend the investigation to other wood species and wood composition (e.g. distinct moisture contents) to establish more data on the mutagenicity of combustion emissions in various conditions.

Aiming at establishing targeted strategies to reduce wood smoke emissions, more research is needed regarding the physicochemical properties of the wood smoke particles we are exposed to and the influence of these properties on the induced biological effects. To attain this, there is need for a stronger partnership between the diverse fields of research including epidemiology, toxicology, combustion science and aerosol science.

References

Claxton, L.D., Matthews, P.P., Warren, S.H., 2004. The genotoxicity of ambient outdoor air, a review: Salmonella mutagenicity. Mutation Research 567, 347-399.