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Chemical composition and toxicity of emissions from burning five vegetation types of Western Australia under experimental combustion conditions

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Chemical composition and toxicity of emissions from burning five vegetation types of Western Australia under experimental combustion conditions

This thesis is presented in fulfilment of the requirements for the degree of

Doctor of Philosophy

By

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ABSTRACT

This study investigated the emission factors (EFs) for inorganic gases (CO_2, CO, SO_2, NO and NO_2), carbonyls (formaldehyde, acetaldehyde, acetone, propionaldehyde, butyraldehyde and benzaldehyde), volatile organic compounds (VOCs) and particulate matter ($PM_{2.5}$ and PM_{10}) from laboratory-based fires of vegetation from five typical vegetation types of Western Australia. Species burnt were three grasslands (Spinifex represented by *Triodia basedowii*, Kimberley grass represented by *Sehima nervosum* and *Heteropogon contortus,* and an invasive grass represented by *Ehrharta calycina* (Veldt grass)), Banksia woodland and Jarrah forest under different combustion conditions. Chemical composition (water-soluble metals and polycyclic aromatic hydrocarbons – PAHs) and *in vitro* toxicity of $PM_{2.5}$ were also measured. Vegetation samples were burnt in a ceramic chamber in varying combustion conditions altered by controlling the vegetation moisture content (<10%, 12–16% and 20–25%) and the air flow rate (0, 1.25 and 2.94 $m.s^{-1}$). Burns of woodland (Banksia) and forest (Jarrah) had significantly higher EFs for CO, SO_2 and $PM_{2.5}$ compared with those from grassland (Spinifex). Emissions of temperate grass (Veldt) fires were significantly different from those of the tropical grass (Spinifex and Kimberley grasses), with lower EF_{CO2} and higher EFs for CO, carbonyls and $PM_{2.5}$. EFs for SO_2, NO and NO_2 were variable between different vegetation types, indicating variation in the nitrogen and sulphur content of the fuels. The EFs for most carbonyls were similar between most vegetation types, with the exception of Veldt grass. Functions which may be useful to predict emissions of infrequently measured carbonyls (acetaldehyde, acetone and propionaldehyde) from the EF for formaldehyde, a commonly measured and reported substance, were also proposed. Fifteen VOCs were identified in the smoke, but concentrations were too low to be quantified. Benzene, toluene, styrene and indene were the most frequently detected VOCs.

Moisture content did not strongly influence the modified combustion efficiency (MCE) and EFs for gaseous pollutants, but significantly affected the EF for $PM_{2.5}$ with higher emissions from burns of moister vegetation. Increasing the air flow rate significantly increased the emissions of most pollutants. However, combustion conditions did not strongly affect the $PM_{2.5}$ chemical composition.

The MCE, EFs for CO and CO_2 results in this study were similar to values reported from field measurements for similar vegetation types in Australia, indicating the applicability of these laboratory-based results. Emission factors were different to the profiles generated from vegetation fires in other parts of the world.

Toxicity of $PM_{2.5}$ on human lung epithelial (A549) cells was assessed using cell viability and cytokine production measurements. Responses on cell viability were associated with K and Na concentrations in $PM_{2.5}$, whilst the cytokine production of cells was more affected by the $PM_{2.5}$-bound PAH, Al, Cu and Mn concentrations. Toxicity between vegetation types was different, which might be due to the differences in chemical composition of $PM_{2.5}$. $PM_{2.5}$ emitted from Jarrah burns appeared to have the highest toxicity on

epithelial cells, followed by those from Banksia, Veldt grass and Spinifex. The findings of this study on toxicity of $PM_{2.5}$ demonstrate the adverse impact on human health of particulate from bushfires and emphasise the importance of vegetation type on toxicological outcomes of bushfire-derived $PM_{2.5}$.

The EFs obtained in this study can be used in models to estimate the emissions from bushfires in Australia, particularly Western Australia. Results on toxicity of $PM_{2.5}$ provide information for relevant government agencies to preliminarily evaluate the risk to human health, especially for firefighters and communities in close proximity to bushfire events.

DECLARATION

I certify that this thesis does not, to the best of my knowledge and belief:

i. *incorporate without acknowledgment any material previously submitted for a degree or diploma in any institution of higher education;*

ii. *contain any material previously published or written by another person except where due reference is made in the text of this thesis; or*

iii. *contain any defamatory material;*

Signed: 

Dong, Thi Thu Trang

March 15h, 2019

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LIST OF ABBREVIATIONS

A549	:	Human lung epithelial respiratory cells
ANOVA	:	Analysis of variance
ATP	:	Adenosine triphosphate
BEAS-2B	:	Human bronchial epithelial cells
BTEX	:	Benzene, toluene, ethylbenzene and xylene
CE	:	Combustion efficiency
DCM	:	Dichloromethane
DMSO	:	Dimethyl sulfoxide
DNPH	:	Dinitrophenylhydrazine
DTT	:	Dithiothreitol
EC	:	Elemental carbon
EEA	:	European Environment Agency
EF	:	Emission factor
ESCAPE	:	European Study of Cohorts for Air Pollution Effects
FBS	:	Fetal Bovine Serum
FTIR	:	Fourier transform infrared
GSH	:	Glutathione
HBE	:	Human bronchial epithelial cells
HPAECs	:	Human pulmonary artery endothelial cells
HRGC/LRMS	:	High resolution gas chromatography/low-resolution mass spectrometry
HPLC	:	High performance liquid chromatography
ICP-MS	:	Inductively coupled plasma mass spectrometry
ICP-OES	:	Inductively coupled plasma optical emission spectrometry
IL	:	Interleukin
LDH	:	Lactate dehydrogenase
MCE	:	Modified combustion efficiency

MCN	:	Micronuclei
MTT	:	3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyl-tetrazolium bromide
NA	:	Not applicable
NDIR	:	Non-dispersive infrared
NIOSH	:	National Institute for Occupational Safety and Health
NMHC	:	Non-methane hydrocarbon
PAHs	:	Polycyclic aromatic hydrocarbons
PERMANOVA:		Permutational multivariate ANOVA
PM	:	Particulate matters
PMI	:	Personal Modular Impactor
PTFE	:	Polytetra-fluoroethylene
PTR-MS	:	Proton-transfer reaction mass spectrometry
PVC	:	Polyvinyl chloride
QA/QC	:	Quality assurance/Quality control
RAW.264.7	:	Mouse macrophage cells
ROS	:	Reactive oxidative species
SRB	:	Sulforhodamine B
THP-1	:	Human monocytes
TNF	:	Tumour necrosis factor
US	:	United States of America
USEPA	:	US Environmental Protection Agency
VOCs	:	Volatile organic compounds
WA	:	Western Australia
WHO	:	World Health Organization

RESEARCH OUTPUTS

Dong, T. T. T., Hinwood, A. L., Callan, A. C., Zosky, G., & Stock, W. D. (2017). **In vitro assessment of the toxicity of bushfire emissions: A review.** Science of the Total Environment, 603-604, 268–278. http://doi.org/10.1016/j.scitotenv.2017.06.062.

Chapter 1. INTRODUCTION

1.1. Background

Bushfires, also known as vegetation fires or forest fires (including wildfires and prescribed burns), along with other types of biomass burning are now considered one of the most significant emission sources of pollutants to the atmosphere (Chen et al., 2007; McMeeking et al., 2009; Vicente et al., 2012). Many studies investigating bushfire emissions have been conducted across the globe, both in countries impacted by long-range transport of bushfire-derived pollutants from neighbouring territories and those experiencing many and increasing bushfire events.

Researchers have found that bushfire smoke consists of many compounds, generated in two phases: gaseous and particulate (Reisen and Brown, 2009; Weinhold, 2011). The gaseous phase includes pollutants such as carbon dioxide (CO_2), carbon monoxide (CO), nitrogen oxides (NO_x), sulphur dioxide (SO_2), aldehydes, polycyclic aromatic hydrocarbons (PAHs), volatile organic compounds (VOCs) and several other organic and inorganic compounds (Barboni and Chiaramonti, 2010; Koppmann et al., 2006; Reisen et al., 2011; Sinha et al., 2003). The particulate phase contains different sizes of particulate matter (PM), commonly divided into two categories: PM_{10} – particulates with mass mean aerodynamic diameter of less than 10 µm; and $PM_{2.5}$ – particulates with mass mean aerodynamic diameter of less than 2.5 µm. Particulate matter is an aggregate of extremely tiny particles, liquid droplets and many pollutants adhering to the surface or absorbed into the particles. The concentration of PM_{10} in the ambient air has been reported to increase 1.2 to 10 times during bushfire smoke-affected periods or locations when compared with non-fire conditions (Liu et al., 2014). $PM_{2.5}$ has been estimated to contribute more than 90% of the mass of PM_{10} generated (McMeeking et al., 2009; Radojevic, 2003).

Emissions of pollutants from bushfires depend on many factors including fuel type, fuel load, weather and topographic conditions (Christian et al., 2003; Gu et al., 2008; Reisen and Brown, 2009; Youssouf et al., 2014). Many pollutants in bushfire smoke are known to be toxic and/or carcinogenic such as CO, formaldehyde, benzene and some PAHs that have been associated with health effects (Bell & Adams, 2009). These pollutants can cause adverse impacts to firefighters who usually have direct exposure to the smoke (Barboni & Chiaramonti, 2010). Many health effects have been associated with these pollutants including short-term effects such as coughing, eye irritation, shortness of breath, headaches, fatigue, dizziness and nausea (Reisen and Brown, 2009; Weinhold, 2011) and long-term respiratory and cardiovascular chronic diseases and cancer (Barboni & Chiaramonti, 2010).

Rationale for this research

Australia has millions of hectares of forest, bushland, and grassland which contribute to its unique and rich biodiversity, but this also carries a high risk of bushfire during dry seasons because of their fire-prone nature. Thousands of natural bushfires occur in Australia annually, in addition to thousands of prescribed fires for purposes such as regenerating forests, managing wildlife habitats, and reducing the accumulation of flammable litter and understorey fuels to minimise the density and frequency of accidental fires (Wain et al., 2009). In Western Australia, there were more than 60,000 bushfires from 2000 to 2007 which destroyed millions of hectares of vegetation (Bryant, 2008) and this state experienced more than 3,800 bushfire events in the single season 2012–2013 (Climate Council of Australia, 2015). In recent years, there has been an increase in the number of studies in Australia investigating the effects of bushfire emissions on the air shed and human health outcomes as well as characterising bushfire smoke pollutants. These studies have sought to determine the relationships between bushfire events and cardiorespiratory diseases and mortality risks to members of the community (Henderson and Johnston, 2012; Johnston et al., 2011, 2007, 2006). Other studies have focused on exposure of firefighters in individual firefighting efforts (Reisen and Brown, 2009; Reisen et al., 2011, 2006a). In order to estimate the emissions of bushfires to the airshed as well as to predict their potential health effect, emission factors for pollutants (defined as mass of pollutants generated from combustion of a mass unit of dry fuel) have also been determined for fires of savanna and temperate forests in the northern and south-eastern regions of Australia, but no such values have been reported for Western Australian vegetation (Desservettaz et al., 2017; Guérette et al., 2018; Paton-Walsh et al., 2014, 2005; Smith et al., 2014; Wang et al., 2017a). Studies investigating how fuel type influences the composition of pollutants in smoke are also limited. McMeeking et al. (2009) in the United States (US) identified differences in emission profiles with fuel types, however, the application of these measurements to Australian vegetation and conditions may not be appropriate due to differences in vegetation type and combustion conditions (De Vos et al., 2009). Furthermore, very few studies have been undertaken to identify the toxicity of vegetation fire emissions and the toxicity of emissions from different types of vegetation fires remains to be examined (Weinhold, 2011).

It is predicted that the number and intensity of bushfires in Australia will increase in the future due to the effects of a changing climate (Climate Council of Australia, 2015). Climate change has made many parts of Australia drier and hence increased the risk of bushfire and this trend is continuing. To decrease the risk of natural bushfire, the number of prescribed burns will also increase so as to reduce the combustible vegetation load (Hughes and Steffen, 2013). With a projected increase in

wildfire and prescribed burns and their subsequent impacts on human health and the environment, it is necessary to have a better understanding of the emissions of bushfires from different vegetation types and their potential impacts. Hence, this study was conducted to investigate the chemical composition and toxicity of emissions from burning different vegetation types in Western Australia. The results provide data on the pollutant composition of smoke emitted from burning biomass for bushfire management agencies to better target efforts at protecting human health from burns in particular types of ecosystems. These data can be used in the development and modification of models simulating and estimating the emissions of bushfires as well as the development of exposure standards for bushfire smoke emissions.

1.2. Aims of the study

My study aimed to investigate the chemical composition of emissions (both gaseous and particulate phases) from burning typical vegetation types of Western Australia and the effects of combustion conditions on the chemical composition and *in vitro* toxicity of $PM_{2.5}$ in the smoke. This study also aimed to investigate the relationship between *in vitro* toxicity associated with fuel type and $PM_{2.5}$-bound chemical composition. The study specifically aimed to answer the following questions.

1. How different is the chemical composition of smoke resulting from the burning of different vegetation types?
2. Are the emissions of air pollutants from burning Western Australian typical vegetation types similar to those reported in other Australian and international studies?
3. How much do combustion conditions (fuel moisture and air flow rate) influence the chemical composition of smoke?
4. Is the toxicity of $PM_{2.5}$ dependent on vegetation type?
5. Which chemical components of $PM_{2.5}$ are associated with the toxicity?

1.3. Structure of this thesis

This thesis consists of seven chapters.

Chapter 1 provides an introduction to the current knowledge about bushfires and their toxicity. A rationale for the study is provided and the questions needed to be answered to address the stated aims of the project are given.

Chapter 2 is a literature review which summarises knowledge about bushfires, the chemical composition of smoke and *in vitro* toxicity of particulate matter from bushfire smoke. A part of this chapter (section 2.3) has already been published.

The next three chapters focus on the results of emissions testing of different pollutants in smoke, including inorganic gases (Chapter 3), volatile and semi-volatile organic compounds (Chapter 4), and particulate matter (Chapter 5). These chapters have been prepared as papers, one of which has been submitted for publication. The general experimental design and methods of this study are outlined in Chapter 3. Specific methodological aspects are outlined in the relevant subsequent chapters.

Chapter 6 discusses the *in vitro* toxicity of $PM_{2.5}$ and its measurements depending on the vegetation type burned and PM chemical composition. This chapter has been also prepared as an article manuscript.

The final chapter (Chapter 7) is a synthesis of the major findings of this study, a discussion of the implications of the research findings and it then goes on to recommend some future research needs.

Chapter 2. LITERATURE REVIEW

2.1. Factors that influence the combustion of vegetation and emissions of pollutants

Vegetation combustion is defined as the burning of living and dead plant matter (Cole, 2001). Combustion of vegetation includes a series of chemical and physical processes and is often divided into three stages: ignition, flaming and smouldering (Koppmann et al., 2006; Strezov and Evans, 2014). In the ignition stage, the drying of the vegetation material occurs and this where most water evaporates from the material before the fuel can be ignited. Fuel characteristics including moisture content, size and density strongly influence the length of the ignition stage (Koppmann et al., 2006). Volatilisation of several VOCs also occurs during the drying process (Urbanski et al., 2009). After that combustion proceeds to the flaming stage, where the main components of vegetation including cellulose, hemicellulose, lignin, extractives and trace minerals are burned. Pyrolysis and char oxidation are the main processes occurring during the flaming phase, which rapidly reduce the fuel volume and thus reduce the flaming, leading to the smouldering phase (Urbanski et al., 2009). The smouldering phase mainly involves the combustion of remaining char in the absence of flame (Koppmann et al., 2006). The composition of biomass combustion emissions varies depending on the different combustion stages (Vicente et al., 2012). Many highly volatile organic chemicals are released during the ignition stage, while carbon monoxide is the predominant emission in the smouldering stage. During the flaming phase, the major burning product in a complete biomass combustion process is carbon dioxide from oxidisation reactions of the carbon compounds in the biomass. However, under conditions of oxygen deficiency incomplete combustion may occur which generates carbon monoxide and other organic compounds (Demirbas, 2007; Koppmann et al., 2006). Particulates are also emitted mostly in the flaming phase (Urbanski et al., 2009).

In the field, the combustion of vegetation is commonly separated into only two stages, namely flaming and smouldering (Urbanski et al., 2009). These two combustion stages can be identified from calculations of combustion efficiency (CE) or modified combustion efficiency (MCE). CE of a fire is calculated by dividing the carbon amount emitted as carbon dioxide (CO_2) by the total amount of carbon emitted from that fire (Ward and Hardy, 1991). Carbon from a fire is emitted in many forms including CO_2, carbon monoxide (CO), methane (CH_4), non-methane hydrocarbon (NMHC) and particulate matter (Koppmann et al., 2006). Since it is hard to identify and measure all fire products containing carbon and most of the carbon (>95%) is emitted as CO_2 and CO, CE

is modifed to MCE which is the ratio of carbon amount emitted as CO_2 to the sum of carbon emitted as CO_2 and CO (Ward and Radke, 1993). CEs or MCEs of more than 0.9 or less than 0.8 indicate fires dominated by flaming or smouldering phases, respectively. Fires with CEs or MCEs from 0.8 to 0.9 are a mixture of flaming and smouldering phases (Sinha et al., 2003).

The vegetation combustion process is influenced by many factors involving fuel properties (composition, size and load) and environmental factors (air distribution, temperature, burning time, etc.) Among them, vegetation composition including ash (inorganic material) content, moisture content, extractive content (organic combustible component), element content (determined by ultimate analysis) and structural content (determined by proximate analysis) are the key factors affecting the combustion process (Demirbas, 2007). These factors vary depending on species of vegetation and growing conditions (Jenkins et al., 1998). Sami et al. (2001) investigated the effects of biomass moisture and ash content on flame temperature and found that the higher the moisture and/or ash content, the lower the flame temperature. Air distribution or more commonly in the field wind speed is the principal environmental factor that influences the biomass combustion process since any increase in air provision may reduce the temperature of flame, which affects emissions (Strezov and Evans, 2014).

2.2. Chemical composition of bushfire smoke

2.2.1. Major pollutants in bushfire emissions

Many studies addressing bushfire smoke emissions have been conducted across the globe, including in Germany, Finland, France, Portugal, Africa, the United States, South America, Singapore, Australia, Indonesia, China and Japan (Andreae and Merlet, 2001; Barboni and Chiaramonti, 2010; Chen et al., 2007; Christian et al., 2003; Hänninen et al., 2009; Karthikeyan et al., 2006; Koppmann et al., 2006; Miranda et al., 2010; Qin and Xie, 2011; Sinha et al., 2003; Takeuchi et al., 2013; Wain et al., 2009). Emission factors (EF), which are defined as mass of a pollutant generated from combustion of a mass unit of fuel on a dry basis (e.g. $g.kg^{-1}$ dry fuel) (Ward and Radke, 1993), are commonly reported to quantify emissions of pollutants from vegetation fires. Emission factors can be used to estimate emissions from vegetation fires but can also be used to compare emissions from different fuel sources (Simões Amaral et al., 2016). Major air pollutants from bushfires such as CO_2, CO, NO_x, SO_2, carbonyls, VOCs and PM have been measured and reported in many studies and the characteristics of each pollutant are discussed below.

a) Carbon dioxide (CO_2)

CO_2 is the main product of biomass burning due to the high carbon content of biomass. CO_2 is considered to be a significant contributor to the greenhouse effect (Takeuchi et al., 2013) but is not usually considered in terms of its impact on health. It is estimated that 6564 to 9093 Tg of CO_2 are emitted to the atmosphere annually from open biomass burning (Jain et al., 2006). The average EF for CO_2 generated from vegetation burning has been found to be in the range of 1500 to 1800 $g.kg^{-1}$ dry fuel (Akagi et al., 2011; Andreae and Merlet, 2001) and varies slightly depending on vegetation type and combustion stage (Chen et al., 2007).

b) Carbon monoxide (CO)

CO is a by-product of incomplete combustion and may be present in high concentrations in bushfire smoke depending on combustion conditions such as fuel moisture, burning temperature and stage of the fire (Koppmann et al., 2006; Reisen and Tiganis, 2007). The emission factor for CO from vegetation fires ranges broadly depending on fuel type and is especially dependent on the phase of combustion (Andreae & Merlet, 2001; Christian et al., 2003; McMeeking et al., 2009; Soares Neto et al., 2011). In a study measuring the emission of pollutants from Australian tropical savanna fires, Desservettaz et al. (2017) observed a wide range of EF for CO which varied from 15 to 147 $g.kg^{-1}$ dry fuel. Jain et al. (2006) estimated that about 438 to 658 Tg $CO.yr^{-1}$ are emitted from open biomass fires. When inhaled into the body, this colourless and odourless gas can bind to, and inhibit, the oxygen-carrying function of haemoglobin (Hb) in the blood (Gerostamoulos et al., 2011). This can be harmful to human health due to the reduction of oxygen (human brain function can be affected by COHb levels of over 10% in the blood) (World Health Organization – WHO, 2010) and may even cause death in cases of extreme exposure (COHb levels in blood of over 50%) (Nelson, 1987). CO has been found to be significantly correlated with other pollutants generated during the smouldering stage of fires such as aldehydes and VOCs (Koppmann et al., 2006; Reisen et al., 2006a). Studies on firefighters' exposure during bushfires have shown that firefighters are sometimes exposed to higher concentrations of CO than the regulated occupational concentrations for workers (Miranda et al., 2010; Reisen et al., 2011).

c) Nitrogen oxides (NO_x)

NO_x is a family of seven oxides of nitrogen, including nitrous oxide (N_2O), nitric oxide (NO), dinitrogen dioxide (N_2O_2), dinitrogen trioxide (N_2O_3), nitrogen dioxide (NO_2), dinitrogen tetroxide (N_2O_4) and dinitrogen pentoxide (N_2O_5) (US. Environmental Protection Agency – USEPA, 1999). Hurst et al. (1994) found that 15 ± 8% of nitrogen in vegetation is emitted in the form of NO_x when burned. As noted, NO_x is a product of the flaming phase of vegetation fires and it is highly

correlated with the emission of CO_2 (Burling et al., 2010; Lobert et al., 1991). NO_x in vegetation fire emissions has also been reported to be positively correlated with fuel nitrogen content (Burling et al., 2010). Therefore EFs for NO_x are highly variable (Akagi et al., 2011; Andreae and Merlet, 2001; Urbanski et al., 2009). In the NO_x group, NO_2 is the dominant form in the atmosphere and NO is the most abundant form emitted from the combustion of vegetation (Andreae and Merlet, 2001). NO_2 is a primary pollutant and also a component of ozone production, when reacting with NMHCs in the presence of sunlight (Jaffe and Wigder, 2012). NO_2 is a pulmonary toxicant and has been demonstrated to have adverse impacts on lung resistance (WHO, 2010). NO has a similar mechanism for absorbing oxygen in blood to carbon monoxide, but is not a significant threat to human health (except for infants and very sensitive people) (USEPA, 1999).

d) Sulphur dioxide (SO_2)

SO_2 is also generated from burning the sulphur component of biomass (Andreae and Merlet, 2001). Emission of SO_2 from vegetation fires is also found to be associated with the flaming phase (Lobert et al., 1991). However, some studies have observed negative correlations between SO_2 and MCE with more SO_2 emitted from burns with a higher proportion of the smouldering phase (Burling et al., 2010; Sinha et al., 2003). Compared with the other inorganic gases (CO_2, CO and NO_x), SO_2 is the least reported in studies of emissions from bushfires. Emission factors for SO_2 range from 0.1 to 1.0 $g.kg^{-1}$ dry fuel depending on the types of vegetation (Andreae and Merlet, 2001; McMeeking et al., 2009). The water-soluble nature of SO_2 and the formation of sulphuric acid explains why SO_2 exposure may adversely affect the respiratory system by causing chronic bronchitis or asthma symptoms (Bell and Adams, 2009). The reaction of SO_2 and other atmospheric compounds can form small particles, such that SO_2 is considered a major precursor of ambient $PM_{2.5}$ concentration (European Environment Agency, 2014). These particles can penetrate deeply into the lungs and cause more serious respiratory diseases (Feng et al., 2016).

e) Volatile organic compounds (VOCs) and semi-volatile compounds

VOCs from bushfires are generated during the ignition phase, while drying and low-temperature pyrolysis processes occur, and also during the smouldering phase (Urbanski et al., 2009). VOC emissions are strongly related to the duration of the different combustion stages, and have been identified in significantly higher concentrations in the smouldering stage compared with the flaming stage in prescribed burns (Barboni and Chiaramonti, 2010). Barboni and Chiaramonti (2010) reported the concentrations of benzene, toluene, ethylbenzene and xylene of 21±4.2 $mg.m^{-3}$ in the flaming stage and 34±4.3 $mg.m^{-3}$ in the smouldering stage. Yokelson et al.(2007) identified emission factors for benzene, toluene, ethylbenzene and xylene compounds from tropical forest fires, which were 0.26 $g.kg^{-1}$, 0.2 $g.kg^{-1}$, 0.08 $g.kg^{-1}$ and 0.13 $g.kg^{-1}$, respectively. VOCs in the

troposphere are degraded and transformed under several physical and chemical processes. The photochemical transformation of VOCs with the presence of NO_x produces more ozone (O_3) in the atmosphere (Sinha et al., 2003). Benzene is a major VOC detected in bushfire smoke (Reisen et al., 2006a) and it is a genotoxic compound which has been associated with increased risk of acute myeloid leukaemia and cancer in children (Li et al., 2015).

Carbonyls are a group of semi-volatile organic compounds for which emissions are associated with incomplete combustion conditions in the smouldering stage of fires (Urbanski et al., 2009). Formaldehyde and acrolein have been found to be the prominent aldehydes that firefighters are exposed to during bushfires (De Vos et al., 2009; Garcia-Hurtado et al., 2014; Reisen et al., 2006a). Other studies have shown formaldehyde and acetaldehyde to be dominant in vegetation fire smoke (Christian et al., 2003; Vicente et al., 2011; Yokelson et al., 2008). Of the carbonyls, emission factors for formaldehyde and acetaldehyde are most commonly reported and they vary depending on vegetation types. Formaldehyde is categorised as a carcinogenic substance (Group I) to humans by the International Agency for Research on Cancer (IARC, 2012) and it also causes irritation to the eyes and upper respiratory tract, asthma and eczema (WHO, 2010). Exposure to high concentrations of formaldehyde has been shown to cause nasal tumours in experimental rats (Leikauf and Katz, 2005). Formaldehyde emitted from bushfires also has a significant influence on the hydroxide (OH^-) balance and ozone production in the atmosphere due to the process of photolysis (Radojevic, 2003; Sinha et al., 2003). Acetaldehyde has been found to cause cancer in rats following inhalation exposure and is categorised as a possible human carcinogen due to its ability to interfere with DNA synthesis and repair (IARC, 2010). Acrolein can irritate mucous membranes, airways and even skin at high concentrations (Faroon et al., 2008). In a study of firefighters' exposure during prescribed burns in Australia, Reisen et al. (2006) reported that 28% of monitored firefighters were exposed to formaldehyde concentrations that exceeded the proposed occupational exposure standard.

g) Particulate matter (PM)

It is estimated that less than 5% of the carbon in vegetation is emitted in the form of particulate matter (PM) when burned (Reid et al., 2005b). PM_{10} (particulates with diameter of less than 10 µm) in smoke from bushfires consists of a high proportion of particulates with diameter of less than 2.5µm ($PM_{2.5}$) which can penetrate deeply into the lungs and impact the health of populations and individuals (Alves et al., 2010a; Feng et al., 2016; Reid et al., 2005b). Several epidemiological studies have used time-series analysis to investigate the relationship between the increase in concentrations of PM_{10} in ambient air during bushfire events/seasons and the number of patients admitted to local hospitals or the number of deaths from diseases associated with air pollution.

These diseases includes asthma, chronic obstructive pulmonary disease (COPD), bronchitis, emphysema, pneumonia and ischaemic heart disease (Crabbe, 2012; Henderson and Johnston, 2012; Morgan et al., 2010; Tham et al., 2009). When investigating the hospital admissions for respiratory illness in Brisbane, Australia in the period from 1997 to 2000, Chen et al. (2006) found that the PM_{10} level in the ambient air had a positive relationship with the daily number of patients admitted and this association was more pronounced during bushfire episodes. On days on which bushfires occurred with ambient PM_{10} concentration exceeding 15 $\mu g.m^{-3}$, the number of people admitted to hospital due to respiratory issues increased by 9 to 19 % compared with normal atmospheric conditions (Chen et al., 2006). A comprehensive time-series study that analysed hospital admissions over a period of eight years in Sydney, Australia found that bushfire PM_{10} had greater impacts on COPD admissions of elderly people and asthma complaints in adults compared with background PM_{10} concentrations (Morgan et al., 2010).

Bushfire particulates are comprised of carbon (50 – 70% mass, separated into organic carbon and black carbon), elements associated with carbon compounds including oxygen, hydrogen and nitrogen (20 – 30%) and inorganic species (10%). The composition of these components of PM varies depending on many factors such as vegetation type, combustion phase, and fire variability (Reid et al., 2005b). Average PM_{10} emission factors from tropical forest fires were measured at 17.8 $g.kg^{-1}$ (Yokelson et al., 2007), whilst $PM_{2.5}$ emission factors were more variable and ranged from 5.4 $g.kg^{-1}$ to 29.4 $g.kg^{-1}$ depending on vegetation type (Akagi et al., 2011; McMeeking et al., 2009).

Polycyclic aromatic hydrocarbons (PAHs) in PM (PM-PAHs): PAHs have been found in bushfire smoke with significant fractions of high-molecular PAHs associated with the particulate phase. Many PAHs are defined as mutagenic and/or carcinogenic compounds that can cause lung cancer (Choi et al., 2010). Alves et al. (2010a) found that the dominant PAHs in shrubland burning particles were alkylated compounds, benzo(a)anthracene, pyrene, phenanthrene, fluoranthene and chrysene. Higher concentrations of these PAHs were present in finer-sized particulates (Alves et al., 2010a). When studying PAH emissions from different firewood types in Australia, Zou et al. (2003) reported that most genotoxic PAHs were present in the particulate phase.

Metals in PM (PM-metal): K and Ca are metals found in bushfire-derived PM and mostly are present in the core of particulates, in which K accounts for about 2 to 5% of the $PM_{2.5}$ mass (Reid et al., 2005b). Garcia-Hurtado et al. (2014) found that other major trace metals in $PM_{2.5}$ emitted from shrub wildfire in Spain were Cu, Zn, Zr, Pb, Ti, and Ba. A comparison of metals in aerosols in Singaporean ambient air under normal conditions and in a period affected by smoke from biomass burning was conducted by Pavagadhi et al. (2013). The study showed that higher

concentrations of metals including Al, Cr, Fe, Mn, Co, Ni, Zn, Cu, Cd and Pb were observed in $PM_{2.5}$ during the smoke-affected period compared with those in normal conditions. Some first-row transition metals such as Fe, Ni, and Cu absorbed in PM have been suggested to produce free radicals that may cause oxidative stress when accumulated in the body (Carter et al., 1997; Jiang et al., 2014). This phenomenon has negative impacts on human health because it may cause chronic illness such as lung damage and cancer (Pham-Huy et al., 2008; Truong-Tran et al., 2001).

2.2.2. The influence of vegetation type on the emissions of pollutants emitted in smoke

Each vegetation type has a specific fuel structure and chemical content, thus generating different chemical compounds in the smoke when being burned. Some studies have analysed and compared the chemical composition of emissions from combusting different types of biomass. Two hundred and fifty-five laboratory burns for 33 vegetation species from five specific ecosystems in the US were undertaken and the emissions of several gas-phase and particle-phase compounds in the emitted smoke (including CO_2, CO, CH_4, C_{2-4} hydrocarbons, NH_3, SO_2, NO, NO_2, HNO_3, organic carbon, elemental carbon, SO_4^{2-}, NO_3^-, Cl^-, Na^+, K^+ and NH_4^+) were measured (McMeeking et al., 2009). Emission factors for these compounds were then calculated, providing a comprehensive data set on the chemical composition of smoke generated from vegetation fires in the US. Burling et al. (2010) conducted a similar laboratory-based study investigating the emissions of wildfire fuels from two regions (south-eastern and south-western) of the US. In addition to the gaseous compounds that were examined by McMeeking et al. (2009), a study by Burling et al. (2010) focused on other trace gases such as carbonyl compounds (formaldehyde, acetaldehyde, acetone), acetic acid, furan and nitrous acid.

Other smaller-scale studies investigating emissions from burning different fuel types were conducted for Indonesian, African and Brazilian fuels (Christian et al., 2003; Soares Neto et al., 2011). Christian et al. (2003) measured 26 compounds in smoke produced from burning 16 common fuel types, while Soares Neto et al. (2011) burned typical native vegetation species of the Amazon forest and evaluated the EFs for CO_2, CO, NO_x and unburned hydrocarbons.

In recent years a few studies have been carried out in Australia to determine whether there are significant differences in emissions from burning different vegetation types. Possell and Bell (2012) compared the EFs of six Australian grass species and found a much higher EF for CO_2 when combusting grasses from the Northern Territory compared with those from the Australian Capital Territory. Because the burned species had similar moisture content, this difference might be caused by the specific “chemical composition of plant biomass” due to the soil and nutrient conditions of each region (Possell and Bell, 2012). A preliminary evaluation of emissions from

prescribed burns of some forest fuel types in Australia was conducted by Reisen et al. (2006a) who compared firefighters' exposure to smoke from fires in eucalypt forests, grassland heathland, mallee heathland and tropical forests in some Australian states. Higher concentrations of CO, respirable particles and formaldehyde were found in smoke from mallee heathland burns whilst higher concentrations of VOCs were measured in grassland fire emissions. However, the higher emission of VOCs from grassland observed in that study might be attributable to the sampling conditions, whereby the grassland samples were collected in denser smoke compared with samples from fires of other vegetation types (Reisen et al., 2006a). Another study by Wardoyo et al. (2006) investigated the particulate emissions from the combustion of five tree species of Queensland including spotted gum, blue gum, bloodwood, iron bark and stringybark. Their study found that the EFs for $PM_{2.5}$ were different between types of fuel, with hard wood burns emitting higher EF for particle number than soft wood burns. Differences in EFs were also reported between different parts of the trees, with EFs for $PM_{2.5}$ from burning leaves and branches significantly higher than those from wood burns (Wardoyo et al., 2006).

2.2.3. Influence of combustion conditions on the chemical composition of vegetation fire smoke

Combustion conditions such as fire temperature, wind speed, fuel conditions (age, load and moisture) and topological factors have a significant influence on the behaviour of vegetation fires and the chemical composition of the resulting smoke (Maleknia et al., 2009; Reisen and Tiganis, 2007). Some laboratory-based studies have investigated the influence of fuel moisture content and air speed on vegetation fires. Chen et al. (2010) examined the effects of moisture content on emissions of carbon and nitrogen species in smoke of five types of wildland fuels (including litter, duff, soil, leaves and stems) and found that the MCE decreased with an increase in fuel moisture content, resulting in higher emissions of pollutants associated with the smouldering phase such as CO, organic carbon, NH_3 and other particulate nitrogen. Possell and Bell (2013) examined the effect of fuel moisture on the chemical composition of smoke generated from burning eucalyptus leaves. They also found that the MCE had a negative correlation with fuel moisture, and the emission factors for VOCs from burning leaves with greater moisture were higher than those from drier leaves.

Surawski et al. (2015) studied the emissions of greenhouse gases including CO_2, CH_4 and N_2O of eucalyptus litter under different wind directions, mimicking three fire spread modes: heading fire (move with the wind), flanking fire (at right angles to the wind), and backing fire (against the wind). Their study found that flanking and backing fires generated lower EFs for CO_2 and CO than

did heading fires. Fuel was burned rapidly and the rapid transition of the fires from flaming to smouldering combustion was the reason for the higher CO emissions from heading fires (Surawski et al., 2015). Zou et al. (2003) investigated PAH emissions from burning firewood using a residential woodburner under fast and slow burns generated by different air flows and found that the PAH concentrations emitted from slow-burn conditions were higher than those from the fast-burn conditions. In a study investigating $PM_{2.5}$ emissions from the combustion of different tree species, Wardoyo et al. (2006) found that the influence of wind speed (fast burn – air supplied at rate of 20 $m.s^{-1}$ and slow burn – no air supply) on EF for PM was different depending on fuel type. Whilst the wind speed did not affect the $EF_{PM2.5}$ of hard wood burns, it was found to significantly influence the $PM_{2.5}$ emissions of soft wood combustion (Wardoyo et al., 2006).

2.2.4. Studies on emissions from bushfires in Australia

Some of the early studies investigating emissions from bushfires in Australia were conducted by Hurst et al. (1994a, 1994b). These studies characterised and reported emissions of trace gases from tropical savanna fires using aircraft on-board instruments and Teflon bag grab sampling. In recent years, many more studies on bushfire emissions have been conducted (Desservettaz et al., 2017; Guérette et al., 2018; Lawson et al., 2015; Paton-Walsh et al., 2014, 2010, 2005; Possell et al., 2015; Shirai et al., 2003; Smith et al., 2014; Surawski et al., 2015; Wang et al., 2017b; Wardoyo et al., 2006). All of them, except for the studies by Wardoyo et al. (2006) and Surawski et al. (2015), were also field-based studies which used infrared (IR) and/or solar absorption spectroscopy techniques to measure excess mixing ratio of pollutants in the smoke. Trace gases emissions were investigated in all these studies. Only two field-based studies examined and reported the emission factors for particulate matters and/or their characteristics (Desservettaz et al., 2017; Lawson et al., 2015) which was likely due to the difficulty in sampling and measuring particulates in the field. Another study which focused on investigating PM emissions was laboratory-based (Wardoyo et al., 2006). Most of the studies have focused on emissions from fires of the two most common Australian ecosystems: tropical savanna and temperate forest. Only one study has reported the emissions of pollutants from coastal heathland and woodland fires (Lawson et al., 2015). Furthermore, the majority of these studies have focused on fires occurring in northern and south-eastern regions of Australia, with fires in other parts of the country including Western Australia yet to be studied in detail (Table 2.1).

Table 2-1. Summary of information from studies investigating the emissions from bushfires in Australia. Studies are sorted chronologically based on year of publication

Authors	Type of vegetation	Measured pollutants	Type of study	Sampling platform	Region of Australia
Hurst et al. (1994a)	Tropical savanna	CO_2, CO, CH_4, acetylene (C_2H_2), benzene (C_6H_6), formaldehyde (CH_2O), acetaldehyde (CH_3CHO), NO_x, ammonia (NH_3), hydrogen cyanide (HCN), and acetonitrile (CH_3CN)	Field-based	Aircraft	Northern
Hurst et al. (1994b)	Tropical savanna	CO_2, CO, CH_4, NMHC, CH_3CHO, NO_x, NH_3, N_2O, HCN and sulphur (S)	Field-based	Aircraft	Northern
Shirai et al. (2003)	Tropical savanna	CO_2, CO, CH_4, NMHC, methyl halides, dimethyl sulphide (DMS)	Field-based	Aircraft	Northern
Paton-Walsh et al. (2005)	Temperate forest	CO, C_2H_2, ethylene (C_2H_4), ethane (C_2H_6), formic acid (HCOOH), H_2CO, HCN and NH_3	Field-based	Ground	South-eastern
Wardoyo et al. (2006)	Temperate forest	$PM_{2.5}$ (particle number, particle mass)	Laboratory-based	NA	South-eastern
Paton-Walsh et al. (2010)	Tropical savanna	CO, H_2CO, HCN, C_2H_2, and C_2H_6	Field-based	Ground	Northern
Possell and Bell (2012)	Tropical and temperate grassland	CO_2, CO, VOCs	Laboratory-based	NA	Northern and south-eastern
Paton-Walsh et al. (2014)	Temperate forest	CO_2, CO, CH_4, C_2H_4, C_2H_6, CH_2O, methanol (CH_3OH), acetic acid (CH_3COOH), HCOOH, NH_3 and nitrous oxide (N_2O)	Field-based	Ground	South-eastern
Smith et al. (2014)	Tropical savanna	CO_2, CO, CH_4, C_2H_2, C_2H_4, C_2H_6, CH_2O, CH_3OH, CH_3COOH, HCOOH, HCN, and NH_3	Field-based	Ground	Northern
Possell et al. (2015)	Temperate forest	CO_2, CO, $\sum$(CH_4, NMHC, PM)	Field-based	Ground	South-eastern
Lawson et al. (2015)	Coastal heathland and woodland	NMHCs, PM (size distribution and number, black carbon), O_3, CH_4, CO, hydrogen (H_2), CO_2, C_2H_6, HCN, CH_2O, CH_3CN, CH_3CHO, HCOOH, N_2O, furan (C_4H_4O), C_6H_6, toluene (C_7H_8),	Field-based	Ground	Southern

Authors	Type of vegetation	Measured pollutants	Type of study	Sampling platform	Region of Australia
		phenol (C_6H_5OH), xylenes (C_8H_{10}) , methyl chloroform (CH_3CCl_3), carbon tetrachloride (CCl_4), methyl halides			
Surawski et al. (2015)	Temperate forest	CO_2, CO, CH_4 and N_2O	Laboratory-based	NA	South-eastern
Desservettaz et al. (2017)	Tropical savanna	CO_2, CO, CH_4, N_2O, PM_1 (organic content, SO_4^{2-}, NO_3^-, NH_4^+, Cl^-) and gaseous elemental mercury	Field-based	Ground	Northern
Wang et al. (2017)	Tropical savanna and sub-tropical forest	PAHs, semi-volatile organic compounds (polychlorinated biphenyls – PCBs, polychlorinated naphthalene – PCNs, polybrominated diphenyl ethers – PBDEs)	Field-based	Ground	Northern and south-eastern
Guérette et al. (2018)	Temperate forest	CO_2, CO, CH_4, N_2O, C_2H_2, C_2H_4, C_2H_6, CH_3CHO, acetone$(CH_3)_2CO$, CH_3CN, C_6H_6, CH_2O, ethanol (CH_3CH_2OH), HCN, CH_3OH, C_7H_8	Field-based	Ground	South-eastern

NA: Not applicable

2.3. *In vitro* toxicity of biomass burning emission

(This section is a review article titled "In vitro assessment of the toxicity of bushfire emissions: A review" which has been published in the journal Science of the Total Environment, volumes 603–604, pages 268–278, year 2017. I wrote and revised the manuscript (70% work load), whilst other co-authors (Hinwood, A. L., Callan, A. C., Zosky, G., & Stock, W. D) provided comments and edited the manuscript).

Abstract

Bushfires produce many toxic pollutants and the smoke has been shown to have negative effects on human health, especially to the respiratory system. Bushfires are predicted to increase in size and frequency, leading to a greater incidence of smoke and impacts. While there are many epidemiological studies of the potential impact on populations, there are few studies using *in vitro* methods to investigate the biological effects of bushfire emissions to better understand its toxicity and significance. This review focused on the literature pertaining to *in vitro* toxicity testing to determine the state of knowledge on current methods and findings on the impacts of bushfire smoke.

There was a considerable variation in the experimental conditions, outcomes and test concentrations used by researchers using *in vitro* methods. Of the studies reviewed, most reported adverse impacts of particulate matter (PM) on cytotoxic and genotoxic responses. Studies on whole smoke were rare. Finer primary particulates from bushfire smoke were generally found to be more toxic than the coarse particulates and the toxicological endpoints of bushfire PM different to ambient PM. However the variation in study designs and experimental conditions made comparisons difficult. This review highlights the need for standard protocols to enable appropriate comparisons between studies to be undertaken including the assessment of physiologically relevant outcomes. Further work is essential to establish the effect of burning different vegetation types and combustion conditions on the toxicity of bushfire emissions to better inform both health and response agencies on the significance of smoke from bushfires.

Keywords: biomass burning, smoke particulate matter, woodsmoke, *in vitro* toxicity, cytotoxic, genotoxic

Introduction

Bushfires, also known as wildfires, vegetation fires or forest fires, along with other types of biomass burning are now considered one of the most significant emission sources of pollutants to the atmosphere (Chen et al., 2007; McMeeking et al., 2009; Vicente et al., 2012). Many studies investigating bushfire emissions have been conducted across the globe, ranging from the countries where bushfires usually occur to countries concerned about long-range transport of pollutants from neighbouring territories (Jalava et al., 2010; Pavagadhi et al., 2013; Sinha et al., 2003; Vicente et al., 2013). Most of these studies have focused on measuring the concentrations of emitted gases

and particulate matter (PM) known to have adverse biological effects (Garcia-Hurtado et al., 2014; Junquera et al., 2005; Reisen et al., 2006). There has also been considerable effort devoted to developing pollutant emission factors for individual pollutants for use in models to forecast and predict the potential impact of bushfire smoke on an air-sheds or for population exposure studies (Andreae & Merlet, 2001; Chen et al., 2007; Christian et al., 2003; McMeeking et al., 2009). Other studies have also investigated the effects of bushfire smoke on human health, especially to the respiratory system (Chen et al., 2006; Crabbe, 2012; Henderson & Johnston, 2012; Jalaludin et al., 2000; Johnston et al., 2007; Liu et al., 2014; Morgan et al., 2010). These studies have focused on PM with a number of studies reporting adverse impacts on human health with an increase in the number of hospital admissions during bushfire episodes.

Particulate matter is a major pollutant generated from biomass burning, producing both PM_{10} (particulates with a diameter of less than 10 µm) and $PM_{2.5}$ (particulates with diameter of less than 2.5 µm), where $PM_{2.5}$ accounts for most of the particulate matter generated (Alves et al., 2010a; Bell & Adams, 2008; Garcia-Hurtado et al., 2014). $PM_{2.5}$ has adverse impacts on the human respiratory system and can penetrate deeply into the lungs (Feng et al., 2016; Xing et al., 2016). PM also contains toxicants such as polycyclic aromatic hydrocarbons (PAHs) and metals adsorbed onto its surfaces (Cavanagh et al., 2009; Dieme et al., 2012).

In addition to PM, hundreds of gaseous and volatile chemicals have been identified in vegetation fire smoke (Mobley et al., 1976; Weinhold, 2011). In a study summarising the emission characteristics of pollutants in biomass burning, Andreae & Merlet (2001) listed more than 90 compounds commonly found in the smoke. Among the compounds are some pollutants that have been demonstrated to have adverse health impacts in epidemiological and *in-vivo* experimental studies including carbon monoxide (CO), nitrogen oxide (NOx), sulphur dioxide (SO_2), aldehydes, PAHs and volatile organic compounds (VOCs) (Barboni & Chiaramonti, 2010; Koppmann et al., 2006; Reisen et al., 2011; Sinha et al., 2003).

Although the PM derived from bushfires has been demonstrated to adversely impact human health, it is unclear whether the cause of the impact is the higher concentration of PM *per se*, especially the higher proportion of fine particulates, or if it is a consequence of the changes in chemical composition of bushfire emissions.

Both *in-vivo* and *in vitro* approaches have been used to test the toxicity of smoke and PM from bushfire and biomass burning. *In-vivo* studies on a range of species (e.g. rodents, rabbits, dogs) to determine specific biological end points from exposure to the smoke have been used to predict human toxicity (Dubick et al., 2002; Nieman et al., 1995; Thorning et al., 1982). *In vitro* testing typically involves cultured immortalised cell lines or primary cells (Franzi et al., 2011; Jalava et

al., 2012; Leonard et al., 2007; Nakayama Wong et al., 2011; Verma et al., 2009). These *in vitro* approaches have been widely adopted in recent years to identify the potency of inhaled substances (Aufderheide & Scheffler, 2011; Bakand et al., 2006). Owing to the ethical issues associated with *in-vivo* toxicity testing, as well as the higher cost and time consuming nature of this work, the use of *in vitro* methods is predicted to increase, particularly the use of cells and tissues derived from humans (U.S. National Research Council, 2007).

With a predicted increase in bushfire frequency, and intensity, due to the effect of climate change (Hughes & Steffan, 2013), there are increasing concerns about the impact of bushfire emissions on population health. There is also a need to understand whether emissions under different combustion conditions and vegetation types results in differing toxicities. The development of *in vitro* toxicity testing is anticipated to meet the demand for a better understanding of the toxic nature of bushfire emissions on human health by making use of different cell types and physiologically relevant outcomes which will serve to inform agencies involved in the prevention and management of human exposures.

To summarise what is already known and to identify the knowledge gaps, we reviewed the literature to explore the current state of knowledge on cell lines, methodological approaches used and the results obtained from *in vitro* studies that have investigated the toxicity of bushfire smoke.

Approach and methodology

A literature search was undertaken using various online sources of English journal articles including Google Scholar, ScienceDirect, PubMed and Web of Science. The keywords “toxicity”, “toxic”, “cytotoxic”, “genotoxic”, “in vitro” and “cell” were used to search papers related to *in vitro* toxicological research together with the keywords “bushfire”, “wildfire”, “forest fire”, “vegetation fires”, and “biomass burning” to indicate the nature of fires. The references listed in papers found using this approach were also scanned to find all related studies that were not identified by the search engines.

The initial focus of this review paper was bushfires and biomass burning in open large areas with all parts of the plant (leaves, branches, trunks and litter layer) consumed by the fire. However, due to the limited number articles on the topic, studies on *in vitro* toxicity testing of woodsmoke in general, were also included in the review.

Results

From the review outlined above, fifteen articles were identified for inclusion with seven studies focused on emissions of bushfire and open biomass burning, and eight studies focused on woodsmoke. Since PM is a significant emission from bushfires and biomass burning and is relatively easy to collect and store for long periods of time, most of the *in vitro* toxicity studies have focused on investigating the impacts of PM. Investigations of the toxicity of whole woodsmoke on cells is limited to the single study conducted by Leonard et al. (2000).

PM collection method

A summary of sampling methods outlined in the studies reviewed is presented in Table 2.2. In studies that focused on bushfire and open biomass burning, PM was collected in the ambient air of areas that were some distance from the actual bushfire, except for the study by Leonard et al (2007). This study examined the toxicity of PM collected in the field close to a prescribed burn at a distance determined to be safe by firefighters. Meanwhile, the PM arising from woodsmoke was collected directly from the flue gas of experimental burns in furnaces/wood stoves/boilers.

$PM_{2.5}$ was the research focus of most of the identified studies (five of the seven studies on bushfire and open biomass burning and two of three studies on woodsmoke in which information of PM size was available). PM was collected onto filters or other types of substrates such as polyurethane foam and steel plate. Among different types of filters, glass fibre and Teflon filters were most commonly used (Table 2.2).

Table 2-2. Summary of sampling methodology of bushfire smoke and woodsmoke for toxicity testing

Author	Nature of smoke	Types of filter/substrate	Type of PM
Bushfire/open biomass burning			
Jalava et al., 2006	Urban ambient air PM during wildfire and non-wildfire episodes	Polyurethane foam (PUF) sample substrates and glass fibre filters	$PM_{10–2.5}$, $PM_{2.5–1}$, $PM_{1–0.2}$ and $PM_{0.2}$
Leonard et al., 2007	PM collected at field during prescribed burns	Polyvinyl Chlorine (PVC) filters	$PM_{0.042–0.24}$, $PM_{0.42–2.4}$ and $PM_{4.2–24}$
Verma et al, 2009	Urban ambient air PM during wildfire episode	Teflon-coated glass fibre filters	$PM_{2.5}$
Nakayama Wong et al., 2011	Fine rural ambient air during wildfire	Teflon-coated glass fibre filters	$PM_{2.1}$
Franzi et al., 2011	Coarse rural ambient air PM during wildfire	Cascade impactor substrate	$PM_{10.2-2.1}$
Pavagadhi et al., 2013	Urban ambient air during time affected by biomass burning from neighbouring country	Quartz fibre filters and Teflon membrane filters	$PM_{2.5}$
Alves et al., 2014	PM collected in ambient air of an area affected by biomass burning	Teflon filters	PM_{10}
Woodsmoke			
Leonard et al., 2000	Whole smoke from thermolyzing 100g of tree bark in a furnace at 400°C	Smoke was bubbled through saline	Whole smoke
Liu et al., 2005	PM from thermal decomposition of 100 g of dry wood dust in a furnace at 500°C	Glass fibre filters	Not specified
Karlsson et al., 2006	PM from burning dry wood/wood pellet in wood boilers/pellet burners	Glass fibre filters	Not specified
Kubátová et al., 2006	PM from burning bulk wood in an airtight wood stove	Not specified	Not specified
Danielsen et al., 2011	Fine PM from burning 1kg of beech wood in a wood stove	Steel plates used in an electrostatic precipitator	$PM_{4.2}$

Author	Nature of smoke	Types of filter/substrate	Type of PM
Jalava et al., 2010	PM from burning logwood in a masonry heater	Polyurethane foam (PUF) and glass fibre filters	$PM_{1\text{-}0.2}$ and $PM_{0.2}$
Jalava et al., 2012	PM from burning pellet/wood chip/log wood in different styles of boilers/stoves	Glass fibre filters	PM_1
Bølling et al., 2012	PM from burning wood in a small wood stove	Polycarbonate filters	$PM_{0.1\text{-}2.5}$ and $PM_{2.5\text{-}10}$

Toxicity testing methods

In vitro toxicity endpoints

Cytotoxicity is a basic assay in *in vitro* study, and cytotoxicity of a compound is considered as its potential to cause cell death (Eisenbrand et al., 2002). Cytotoxicity is used to interpret and assess other cellular responses (Mahto et al., 2010). Cytotoxicity can be assessed by using different assays such as MTT ((3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyl-tetrazolium bromide), SRB (sulforhodamine B), LDH (lactate dehydrogenase), ATP (adenosine triphosphate). MTT and SBR are compounds having ability to react with some components/chemicals in viable cells to make the cells stained. Then the number of viable cells can be estimated based on colorimetric measurement (Mahto et al., 2010). LDH is an enzyme that is usually secreted when cells are damaged and can be used as an indicator of injury or illness (Chan et al., 2013). ATP in cellular nucleus plays significant role in energy exchange and takes part in several important cellular processes. The cellular ATP level decreases dramatically when the necrosis occurs and therefore ATP level can be used as an indirect marker of cytotoxicity (Mahto et al., 2010). Cytotoxicity assays using MTT or LDH were applied in the majority of the studies on toxicity of PM derived from bushfire smoke or biomass burning conducted to date (Table 2.3).

In addition to cytotoxicity, the toxicity of a compound at the cellular level can also be determined based on other cellular responses such as induction of proteins, generation of oxidative stress, changes in gene expression and DNA damage (Eisenbrand et al., 2002).

Responses and interactions between cells induced by pollutants can be elucidated by using the indirect approach of measuring the production of cytokines and other proteins secreted from cells. Some of the most commonly measured proteins are the pro-inflammatory cytokines interleukin (IL)-6, IL-8, tumour necrosis factor (TNF)-α and macrophage inflammatory protein (MIP)-2 (Eisenbrand et al., 2002). NF-κB is a transcriptional regulatory protein that controls the inflammatory gene expression (Sun & Andersson, 2002) while caspase-3 and 7 are proteases contributing to the regulation of apoptosis and inflammation (Lamkanfi & Kanneganti, 2010; Wolf et al., 1999).

To identify the generation of oxidative stress in cells, concentration of reactive oxidative species (ROS) such as NO, H_2O_2, O_2^-, increase of glutathione (GSH) released, consumption of dithiothreitol (DTT) and generation of lipid peroxidation can be used as biomarkers (Danielsen et al., 2011; Jalava et al., 2006; Kubátová et al., 2006; Liu et al., 2005; Pavagadhi et al., 2013; Verma et al., 2009). GSH is a common antioxidant in cells and can sequester excess ROS generated, in an effort to protect cells from injury. Therefore, the increase in the amount of GSH can indirectly

indicate an increase of ROS (Pavagadhi et al., 2013). Lipid peroxidation occurs when the antioxidant system is overwhelmed and excess ROS degrades the lipids in cell membranes resulting in cell damage (Mylonas and Kouretas, 1999; Poljsak et al., 2013). DTT is a cell-free assay and when added to PM extract, DTT is oxidized by redox active components in the PM extract and the consumption of DTT indicates the oxidative capacity of PM (Charrier & Anastasio, 2012).

Genotoxicity of PM can also be determined. Fragmentation of DNA appears to be the most frequently measured parameter at the genetic level (Danielsen et al., 2011; Jalava et al., 2012; Karlsson et al., 2006; Liu et al., 2005), however other indirect genotoxic endpoints that have been used include damage events occurring in genes such as the presence of micronuclei (MCN), nucleoplasmic bridges and nuclear buds (Bølling et al., 2012; Fenech et al., 2011).

A summary of toxicity endpoints used in the reviewed studies is presented in Table 2.3.

Type of cells used

The main route of exposure of humans to smoke is inhalation and many of the cell types that have been used in *in vitro* toxicity testing of bushfire and biomass burning smoke are from the human respiratory system. These include human bronchial epithelial cells – HBE, human lung epithelial respiratory cells - A549 and human pulmonary arterial endothelial cells (Table 2.4) (Alves et al., 2014; Danielsen et al., 2011; Karlsson et al., 2006; Kubátová et al., 2006; Liu et al., 2005; Nakayama Wong et al., 2011; Pavagadhi et al., 2013). Epithelial cells of the respiratory tract are exposed directly to pollutants when the air/smoke is inhaled into the lung (Bakand et al., 2005, 2006; Nakayama Wong et al., 2011). Endothelial cells are found inside all blood vessels and have a role in selectively exchanging material between the blood and the tissues. These cells also take part in other important processes such as vascular volume regulation, angiogenesis, permeability and immunity (Félétou, 2011), hence any damage to lung endothelial cells may lead to the dysfunction of these cells and cause pulmonary oedema (Liu et al., 2005).

Table 2-3. Summary of toxicity endpoints determined in toxicological studies on PM from bushfire and biomass burning

Author	Toxicity endpoints																	
	Cytotoxicity				Other cellular responses													
	Cell viability				Inflammation production						Oxidative stress					Genotoxicity		
	MTT	LDH	Other assays (Trypan blue, GF-AFC)	Cell apoptosis	IL-6	IL -8	TNF -α	MIP -2	NF-κB	Caspase-3/7	ROS generation (NO, H_2O_2, O_2^- …)	Glutathione (GSH)	Lipid peroxidation	Oxidative-xenobiotic stress genes	Dithiothreitol (DTT)	DNA fragmentation	Generation of MCN, nucleoplasmic bridges and nuclear buds	Activation/ upregulation of inflammatory genes
Bushfire/open biomass burning																		
Jalava et al., 2006	✓			✓	✓		✓	✓			✓					✓		
Leonard et al., 2007											✓		✓					
Verma et al, 2009											✓				✓			
Nakayama Wong et al., 2011														✓				✓
Franzi et al., 2011			✓						✓									
Pavagadhi et al., 2013			✓	✓						✓		✓						
Alves et al., 2014	✓																✓	

Author	Toxicity endpoints																		
	Cytotoxicity				Other cellular responses														
	Cell viability				Inflammation production						Oxidative stress					Genotoxicity			
	MTT	LDH	Other assays (Trypan blue, GF-AFC)	Cell apoptosis	IL-6	IL -8	TNF -α	MIP -2	NF-κB	Caspase-3/7	ROS generation (NO, H_2O_2, O_2^- …)	Glutathione (GSH)	Lipid peroxidation	Oxidative-xenobiotic stress genes	Dithiothreitol (DTT)	DNA fragmentation	Generation of MCN, nucleoplasmic bridges| and nuclear buds	Activation/ upregulation of inflammatory genes
Woodsmoke																✓		
Leonard et al., 2000							✓						✓			✓		
Liu et al., 2005	✓			✓							✓	✓				✓		
Karlsson et al., 2006					✓	✓	✓											
Kubátová et al., 2006		✓										✓				✓		
Danielsen et al., 2011		✓				✓	✓				✓					✓		
Jalava et al., 2010, 2012	✓			✓	✓		✓	✓										
Bølling et al., 2012		✓			✓	✓	✓											

Macrophages and their precursor, monocytes, are significant cells in the immune system and coordinate the response of the inflammatory system to toxicants (Guastadisegni et al., 2010; Jalava et al., 2012). Therefore other *in vitro* toxicity studies in bushfire and biomass burning smoke have used macrophages and monocytes to assess toxicity instead of cells of the respiratory system including mouse macrophage cells – RAW.264.7 (Franzi et al., 2011; Jalava et al., 2006; Kubátová et al., 2006; Leonard et al., 2007) and human monocytes – THP-1 (Bølling et al., 2012; Danielsen et al., 2011). These cells can provide an indication of the reaction of the human immune system when exposed to toxicants.

Extraction methods applied to PM samples

PM collected on filters and other substrates were used to expose cells after being prepared in solutions The toxicity of different components of bushfire or woodsmoke PM have been investigated and the specific component investigated was dependent on the extraction methods used such as PM suspension, organic extract or water extract (Table 2.4). PM suspension can be prepared by simply suspending PM in water and materials used in cell culture (e.g. growth media, PBS) (Danielsen et al., 2011; Franzi et al., 2011; Karlsson et al., 2006; Leonard et al., 2007; Nakayama Wong et al., 2011). Extracting PM (PM extract) can be undertaken by using chemicals or water with the support of extraction enhancing techniques such as sonication or heat and pressure. Some studies have investigated the organic extracts of PM by using chemicals to extract PM such as dichloromethane (DCM), methanol, ethanol and dimethyl sulfoxide (DMSO) (Alves et al., 2014; Danielsen et al., 2011; Jalava et al., 2006, 2010, 2012; Liu et al., 2005). These are organic compounds that can extract the contents in PM more effectively, but they also may have adverse effects on cells (Da Violante et al., 2002; Koop, 2006; Treichel et al., 2003; U.S. Environmental Protection Agency, 2011). The approach taken is to allow the organic solvent(s) to be removed by evaporation prior to exposing the cells (Danielsen et al., 2011; Jalava et al., 2006, 2012). In some cases PM extracts were diluted with cell medium (Liu et al., 2005) to minimise the potential for toxic effects. Kubátová et al. (2006) investigated water extract of woodsmoke PM by using hot pressured water as the extractant. Sonication was applied in most studies to enhance the extraction efficiency but extraction time varied considerably between studies (7 minutes to 2 hours) (Table 2.4).

Exposure methods applied to PM samples

The most common exposure method was to add PM extract/suspension to cultured cells. There was one study (Pavagadhi et al., 2013) which introduced $PM_{2.5}$ collected onto filters directly to

cells by placing the filters upside down and in direct contact with the cells in sample wells for 48 hours.

There was considerable variation in the approaches used to expose cells to PM. The majority of studies used a range of concentrations of PM solution to obtain a dose response in order to determine the dose generating responses (Danielsen et al., 2011; Franzi et al., 2011; Jalava et al., 2006, 2010, 2012; Kubátová et al., 2006; Liu et al., 2005). Most studies used concentrations of greater than 10 $\mu g.mL^{-1}$ in order to obtain significant toxicity responses. One exception is the study by Alves et al. (2014) where doses of less than 1 $\mu g.mL^{-1}$ of PM extract were added to cultured cells. Interestingly, this is also the only study that used organic solvents in both the extraction (DCM) and extract preparation (DMSO) steps.

Cell exposure time varied from 30 minutes to 48 hours depending on the type of toxicological test and cell types used, with a common exposure time of 24 hours (Alves et al., 2014; Danielsen et al., 2011; Franzi et al., 2011; Jalava et al., 2006, 2010, 2012; Karlsson et al., 2006; Leonard et al., 2000; Liu et al., 2005). Generally, a shorter exposure time, varying from 30 minutes to 4 hours, was applied for testing the generation of ROS and lipid peroxidation (Danielsen et al., 2011; Leonard et al., 2000, 2007; Liu et al., 2005; Nakayama Wong et al., 2011). To determine the cell viability, production of cytokines, LDH release and DNA fragmentation, longer exposure periods were used (12 to 48 hours) (Bølling et al., 2012; Franzi et al., 2011; Jalava et al., 2006; Liu et al., 2005; Pavagadhi et al., 2013).

Table 2-4. Summary of some main features of the methods used in in vitro toxicological studies on PM derived from bushfire/biomass burning.

Author	Type of cells	PM extraction/suspension	PM solution preparation before exposure	Extraction time	Exposure concentration	Exposure time
Bushfire/open biomass burning						
Jalava et al., 2006	Mouse macrophage cell line RAW 264.7	Filters/substrate were sonication extracted in methanol, evaporated to remove methanol	The extract was resuspended in non-pyrogenic water using a waterbath sonicator for 30 min	2x30 min	12, 50, 150, 300 µg/mL	24 hours
Leonard et al., 2007	RAW 264.7 mouse peritoneal monocytes	Filters were blended on ice into fine slurry in phosphate buffer saline (PBS), centrifuged to separate the smoke suspension and PM pellet	Not specified	Not specified	100 µg/mL	Based on endpoints: - H_2O_2 measurement: 30 min in an incubator at 37°C - Lipid peroxidation: 1 hour in a shaking waterbath at 37°C; - DNA: 30 min in a shaking waterbath at 37°C
Verma et al, 2009	Rat alveolar macrophage (AM) cells	Not specified	Not specified		Not specified	Not specified
Nakayama Wong et al., 2011	Human bronchial epithelial (HBE) cells	Ambient PM: Sonication in pyrogen-free water for 1 hour; Wildfire PM: suspended in PBS.	Extracts were diluted and dispersed by probe sonication	1 hour	10 µg/mL	3 hours
Franzi et al., 2011	Murine macrophage RAW 264.7 cells	PM was collected by scraping the substrate then suspended at 1-2mg/ mL in water or in PBS	Not specified		10 µg Dose response test: 0 - 25µg for wildfire PM, 0-50 µg for ambient PM	- 30 min, 1 hour, 2 hours, 4 hours, 6 hours and 24 hours - Dose response: 24 hours

Author	Type of cells	PM extraction/suspension	PM solution preparation before exposure	Extraction time	Exposure concentration	Exposure time
Pavagadhi et al., 2013	Human epithelial lung cell line (A549)	Filters were placed upside down and the collected $PM_{2.5}$ contacted directly with the layer of cells				48 hours
Alves et al., 2014	Human alveolar cells A549	Filters was ultrasonically extracted in DCM; concentrated to 5mL	The extracts were dissolved in DMSO	3x10 min	0.1, 0.5 and 1.0 µg/mL	Cell viability: 24 hours; Genotoxic test: 48 hours
Woodsmoke						
Leonard et al., 2000	RAW 264.7 mouse peritoneal monocytes	Bubbling the smoke into 10mL saline for 1min	100 µL of liquid sample was mixed with DMPO (100 µL), H_2O_2 (100 µL) and PBS (700 µL)		50 – 100 µL of mixture	Based on endpoints: - Lipid peroxidation: 1 hour in a shaking waterbath at 37°C; - DNA damage: 24 hours - TNF-α: 12 hours
Liu et al., 2005	Human pulmonary arterial endothelial cells	Filters was soaked in DMSO for 30 min then the supernatant was collected by centrifugation and filtration	Diluted with cell medium from the stock extracts (with the conc. of DMSO lower than 0.5%)	30 min	10, 20, 30, 40, 50 and 60 µg/mL	4 or 24 hours
Karlsson et al., 2006	Human lung epithelial cells A549 Human macrophages	PM in filters was suspended in Milli-Q water by hand shake (2min) and sonication (5min). Then PM collected by freeze-dry	The particles were suspended in Milli-Q water by vortex (5min) and sonication (15min)	7 min	Epithelial cells: 70µg/mL; Macrophages: 100µg/mL	Epithelial cells: 4 hours Macrophages: 18 hours
Kubátová et al., 2006	Murine macrophage RAW 264.7 cells Bronchial epithelial BEAS-2B cells	Fractionated by hot pressurized water	The aqueous extracts were nitrogen-dried, redissolved in DMSO (0.25 wt. %) and then HBSS.	Not specified	50µg/mL, 100µg/mL and 200 µg/mL	12 hours

Author	Type of cells	PM extraction/suspension	PM solution preparation before exposure	Extraction time	Exposure concentration	Exposure time
Danielsen et al., 2011	Human lung epithelial cells A549 Human mononuclear cells THP-1	Suspended in Milli-Q water, separated by lyophilizing (Particles with tar: extracted with ethanol, evaporated to remove ethanol).	PM suspension was prepared by sonication (8min) in cell media	Not specified	- LDH activity: 0, 2.5, 25, and 100 μg/mL - ROS production: 1.56, 3.13, 6.25, 12.5, 25, and 50 μg/mL - DNA damage: 0, 2.5, 25, and 100 μg/mL - Gene expression: 0, 2.5, 25, and 100 μg/mL	Based on endpoints: - LDH activity: 24 hours - ROS production: measured continuously 3 hours after exposure - DNA damage, gene expression: 3 hours
Jalava et al., 2010, 2012	Mouse macrophage cells RAW264.7	Extracted with methanol in ultrasonic bath, evaporated all methanol	Ultrasonically resuspended in pyrogen free water with 0.3% DMSO	30min	15, 50, 150 and 300 μg/mL	24 hours
Bølling et al., 2012	Co-culture of two human cell lines, A549 pneumocytes and THP-1 monocytes	Methanol was used to dissolve PM and then evaporated. PM was suspended at 1mg/mL in cell culture medium by sonication	Extracts were vortexed for 30 second	30 min	40 μg/cm^2	12 hours and 40 hours

In vitro toxicity of bushfire emissions and woodsmoke

In vitro toxicity of bushfire/open biomass burning emissions

Franzi et al. (2011) tested the effects on mouse macrophage cells, RAW 264.7 of coarse PM (diameter of 2.1–10.2 μm) collected during a severe bushfire in California, USA; and compared the results with the toxicity of urban ambient air $PM_{2.1\text{-}10.2}$. The toxicity was evaluated using the cell viability and the activation of NF-κB. The urban ambient air PM was collected in the city of Fresno during usual ambient air quality conditions without the effects of bushfire emissions. This study found that the bushfire PM extract was five times more toxic compared with an ambient PM extract not impacted by bushfire smoke. The finding in this study was comparable with the result of Wegesser et al. (2009), who tested the toxicity of this bushfire PM in mice. The similar results obtained in these two studies, one *in vitro* and the other *in vivo*, strongly suggests that PM derived from bushfires has toxicity, at least in murine models.

Similar results were observed by Pavagadhi et al. (2013) who investigated the toxicity of $PM_{2.5}$ impacted by bushfire smoke in Singapore with samples collected during periods with and without biomass burning using the human epithelial lung cell line A549. A greater biological response was observed in cells treated with $PM_{2.5}$, specifically decreased cell viability, increased apoptosis and GSH reduction, during the biomass burning period compared with the non-exposure period (Pavagadhi et al., 2013).

Nakayama Wong and colleagues (2011) examined the effects of $PM_{2.1}$ in bushfire-affected area on human airway epithelial cells comparing results with urban ambient samples. This study analysed the expression of a variety of inflammatory genes in epithelial cells. These genes are involved in chronic obstructive pulmonary disease and asthma symptoms in humans (Nakayama Wong et al., 2011). When grouping these genes into 3 processes: xenobiotic metabolism (CYP1A1 and CYP1B1), reactive oxidative stress (HMOX1, PTGS2, DUOX1 and DUOX2) and inflammation (GAPDH, CCL20, GM-CSF, IL-1α, IL-1β, IL-8 and TNFAIP3), the study reported differences in the up-regulation of gene expression when the cells were exposed to both ambient air $PM_{2.1}$ and bushfire $PM_{2.1}$. The ambient PM induced predominantly inflammatory and oxidative stress genes, while xenobiotic and oxidative stress gene responses were mainly generated when cells were exposed to wildfire PM. Differences in the composition of PAHs and trace metals in PM, were suggested to explain differences in gene response to the two types of PM tested (Nakayama Wong et al., 2011).

Verma et al. (2009) assessed the toxicological effects of bushfire $PM_{2.5}$ by measuring the generation of reactive oxygen species (ROS) in rat alveolar macrophage cells while measuring

urban ambient air during a bushfire event. This study separated the collected $PM_{2.5}$ into two periods: during a bushfire event and after the bushfire. When comparing the toxicity of $PM_{2.5}$ collected in these two periods using equal doses for cell exposure, higher concentrations of ROS were generated from $PM_{2.5}$ collected in the period after the bushfire event when there would be less bushfire PM generated. However, this finding was contrary to a result which was also found in this study using dithiothreitol (DTT) in a cell-free system. Verma et al. (2009) measured higher DTT consumption in the extract from $PM_{2.5}$ collected during the bushfire period compared with after the bushfire event, indicating higher concentration of ROS in the bushfire PM. Again the authors suggested that these apparently contradictory results were due to the effects of different components of PM, with the DTT assay suggested to be affected by polar compounds, e.g. oxygenated PAHs, while ROS in cells was mainly affected by the presence of transition metals, e.g. Fe, Ni, Cr, and Pb. Furthermore, the measured ROS was generated by the cellular metabolism process while DTT measured the redox activity of PM components (Verma et al., 2009). Both the studies of Verma et al. (2009) and Nakayama Wong (2011) could not conclude whether PM derived from bushfire smoke was more toxic than ambient PM, however they were able to demonstrate differences in toxicological endpoints.

The effects of different sizes of bushfire PM on the generation of free radicals, one type of the ROS, causing oxidative stress in cells were investigated by Leonard et al. (2007). PM classified into 3 size fractions (ultrafine: 0.042–0.24µm, fine: 0.42–2.4µm and coarse: 4.2–24µm) was collected during prescribed burns with the sampling location in close proximity to the fires (Leonard et al., 2007). Mouse RAW 264.7 cells were exposed to suspensions of the three size fractions and the results showed significant increases in hydrogen peroxide (H_2O_2) generation and lipid peroxidation in cells treated by bushfire ultrafine and fine PM compared with the controls with no PM. No similar increases in these parameters were found for the coarse PM fraction suggesting that the generation of ROS is related to the size of PM generated by the fire and potentially its composition. A similar effect of PM size fractions on the ROS generation was also found by Guan et al. (2016), where the cells exposed to finer PM generated more ROS compared with coarser size fractions. Free radical generation during fires and their association with PM was suggested as a possible mechanism of "acute lung injury" from bushfire smoke (Leonard et al., 2007).

Jalava et al. (2006) also examined the toxicity on RAW 264.7 cells of four size fractions of PM including $PM_{2.5\text{-}10}$, $PM_{1\text{-}2.5}$, $PM_{0.2\text{-}1}$ and $PM_{0.2}$ under three different ambient conditions related to bushfires (bushfire event, mixed – both usual ambient and bushfire affected period and seasonal average ambient air quality episodes). This study found that the toxicity effect and endpoint of

different size fractions of PM varied. The smallest size fraction induced the highest apoptotic activity, while the largest fraction showed the least activity. However, when considering other toxicity markers including NO production, cytokines (TNF-α and IL-6) production and cell viability, the larger size fractions ($PM_{2.5-10}$ and $PM_{1-2.5}$) were found to be more cytotoxic than the smaller fractions ($PM_{0.2-1}$ and $PM_{0.2}$) (Jalava et al., 2006).

The discrepancy in toxicity of different size fractions of PM between the studies of Leonard et al. (2007) and Jalava et al. (2006) may be due to the difference in type of PM tested. Leonard et al. (2007) analysed newly generated PM from prescribed burns, while Jalava et al. (2006) investigated PM after long-range transport. Bushfire emission sources in the study by Jalava et al. (2006) were from Finland's neighbouring countries with resultant increases in $PM_{2.5}$ concentrations in ambient air, particularly $PM_{0.2-1}$ mass concentrations, during bushfire and mixed episodes. $PM_{0.2-1}$ was identified as the size fraction that was mostly associated with long-range transport of bushfire aerosols (Jalava et al., 2006). When comparing the toxicity of $PM_{0.2-1}$ across the different sampling periods, it was found that with the same PM dose, the particulates collected during bushfire and mixed episodes were less toxic, as assessed using cytokines production assay, than those collected during the seasonal average period. The photodegradation of PAHs in PM after long-range transport was considered to be a possible explanation for this phenomenon, since concentrations of PAHs in this size fraction were lower in the bushfire and mixed episodes compared with concentrations in the seasonal average $PM_{0.2-1}$ (Jalava et al., 2006). Photodegradation is also a possible explanation for the findings of Jalava et al. (2006) who found the locally produced coarser particulates induced more toxicological effects than the finer particles transported from more remote emission sources.

Alves et al. (2014) compared the cytotoxicity and genotoxicity of PM_{10} generated from biomass burning collected in a Brazilian city in the Amazon region. The viability of A549 cells was significantly reduced when they were exposed to PM_{10} collected during an intense fire period (more than 180 burns occurring) compared with PM collected in a lower intensity fire period (5 burns). At the genetic level, exposure to the intense biomass burning PM_{10} also caused a significantly higher frequency of micronuclei, an indicator of DNA damage, supporting the notion that PM_{10} collected in periods of intense biomass burning induces greater toxicological effects on human lung cells than PM_{10} derived from less intense burning periods (Alves et al., 2014).

In vitro toxicity of PM derived from woodsmoke

The *in vitro* toxicity of woodsmoke has been studied by several researchers to examine the effects of emissions generated from residential wood combustion systems (Bølling et al., 2012; Danielsen et al., 2011; Jalava et al., 2012, 2010; Karlsson et al., 2006; Kubátová et al., 2006; Leonard et al.,

2000; Liu et al., 2005). Due to the nature of woodsmoke, which is generated from burning wood in a burning device, samples of woodsmoke have been collected from laboratory experiments. As a result, the toxicity of woodsmoke in different combustion phases and conditions has been examined in many studies.

Liu et al. (2005) examined cell apoptosis and oxidation stress released by woodsmoke PM in human pulmonary artery endothelial cells (HPAECs). Extracts of woodsmoke PM generated from burning dry wood dust at 500°C were added to the cells and then intracellular ROS and GSH levels, cell viability, apoptosis cells and DNA fragmentation were assessed. Woodsmoke PM extracts were found to be cytotoxic with the cell viability decreased to 38% of the control at the extract concentration of 40μg.mL^{-1}. HPAECs exposed to woodsmoke released more intracellular ROS and less GSH level compared with the control, which might demonstrate that woodsmoke induced intracellular oxidative stress. This study also found that the cell apoptosis was accelerated and the DNA fragmentation of cells was enhanced when HPAECs exposed to woodsmoke (Liu et al., 2005), demonstrating a range of toxicological effects of PM emitted from combustion of wood at this temperature.

Jalava et al. (2010) investigated the biological responses of woodsmoke $PM_{1\text{-}0.2}$ and $PM_{0.2}$ emitted in smouldering combustion and normal combustion conditions (with flue gas temperature of around 160°C and 250°C, respectively) in a conventional masonry heater. The smouldering combustion condition was produced by eliminating the air supply and overloading the heater with smaller sized wood logs compared with normal combustion conditions. RAW 264.7 cells were exposed to PM extracts and the toxicity was assessed by the cell viability, cell apoptosis and generation of cytokines IL-6, TNF-α and MIP -2. Different sizes of PM were observed to have different toxicological effects depending on the size fraction where $PM_{0.2}$ produced less TNF-α but higher MIP-2 than the $PM_{1\text{-}0.2}$. PM from smouldering combustion was found to be more toxic than normal combustion conditions with larger MIP-2 response, slightly higher TNF-α production and lower cell viability response (Jalava et al., 2010).

In another study on woodsmoke, Jalava et al. (2012) examined the toxicity of PM_1 emitted from seven types of heating systems, varied by different types of fuel used (logwood, pellet and wood chip), technology applied (old and new) and types of stove (boiler, stove and tiled stove). Using the same cell type, testing procedure and toxicity endpoints as the study by Jalava et al. (2010), the study found that PM emitted from the old technology logwood boiler caused the highest cytotoxicity, and the emissions from old technology combustion generated more MIP-2 than the modern device (Jalava et al., 2012).

A comparison of the toxicity of woodsmoke $PM_{4.2}$ generated from two different combustion conditions was conducted by Danielsen et al. (2011): high temperatures (100 – 120°C) with high oxygen and much lower temperatures (below 60°C) in a low oxygen condition. A549 and THP-1 cells were exposed to suspension of PM collected from the two combustion conditions and the toxicity of PM was determined by measuring LDH release and ROS generation. For both types of cells, the PM from low oxygen combustion generated more ROS than the high oxygen combustion derived PM. LDH release did not increase significantly after exposure to either of PM suspensions (Danielsen et al., 2011). The study also found that the woodsmoke PM was more toxic than the rural ambient air PM, with significant increases in ROS production, DNA damage and oxidative gene expression in cells exposed to woodsmoke PM compared with rural ambient air PM (Danielsen et al, 2011).

In a study comparing the toxicity of different types of PM generated by human activities, Karlsson et al. (2006) examined PMs emitted from burning wood in old-type and modern wood boilers and from burning pellets. A549 cells were exposed to PM suspensions and the toxicity was assessed through DNA damage and generation of cytokines IL-6, IL-8 and TNF-α. The woodsmoke PM had been discovered to have lower potential inflammatory effects compared with traffic PM. Among three types of woodsmoke PM investigated in this study, PM from burning wood and pellets had similar effects on DNA damage, while the PM from burning wood in modern boiler had the highest IL-6 generation and PM from burning pellets had the highest IL-8 induction. Karlsson et al. (2006) concluded that the combustion efficiency of boilers did not determine the toxicity of woodsmoke PM generated.

Bølling et al. (2012) investigated the toxicity of woodsmoke $PM_{0.1\text{-}2.5}$ and $PM_{2.5\text{-}10}$ generated from different combustion phases in a cast iron stove. The combustion of biomass is often divided in three phases of ignition, flaming and smouldering (Koppmann et al., 2006). These combustion phases generate different chemical components which may have different adverse impacts (Alves et al., 2010b; Vicente et al., 2012). Bølling et al. (2012) collected samples of $PM_{0.1\text{-}2.5}$ and $PM_{2.5\text{-}10}$ in smoke from burning a mixture of birch and fir separately in the start-up phase (ignition), the burn-out phase (flaming and smouldering) and the whole combustion process (comprising both of the two phases). The combustion temperatures of these experiments were measured around 500-800°C (Bølling et al., 2012). A co-culture of A549 and THP-1 cells was exposed to the PM extract to test LDH release and cell viability. No significant differences in LDH release or the number of viable cells were found from the PM arising from the three combustion phases. This study also compared the toxicity of PM generated in these combustion experiments with PM (with geometric diameter of carbon particulate measured at 31±7 nm) from burning birch in high-temperature

combustion conditions (700-1000°C) in a conventional stove. A higher concentration of LDH release and reduction in cell viability resulted from exposure to the medium-temperature (500-800°C) combustion PM indicating that the PM from lower temperatures in a cast iron stove was more toxic than particulates collected during the high-temperature burning process (Bølling et al., 2012). However, due to the differences the experimental set-up, such as fuel type and stove used, it is not clear whether the difference in toxicity of derived PM was caused by combustion temperature or other combustion conditions.

In general, most studies on woodsmoke toxicity found the PM generated under incomplete combustion conditions (e.g. smouldering, low oxygen) or with older technology were more toxic than PM emitted from more complete combustion conditions or newer technology.

Toxicity of whole woodsmoke

Leonard et al. (2000) investigated the toxicity on RAW 264.7 cells of whole woodsmoke generated from thermolyzing bark in a furnace at 400°C. Woodsmoke generated at different periods of time after ignition (from 0 minute to 20 minutes) was bubbled through saline for 1 minute and then the bubbled solutions were added to cells. The toxicity of woodsmoke to cells was assessed based on DNA damage, lipid peroxidation level and generation of the cytokine TNF-α. The study found that woodsmoke caused DNA damage and the smoke collected at latter periods (15 minutes and 20 minutes after ignition) had more significant effects on DNA damage compared with smoke collected at the beginning of the burns. The lipid peroxidation levels and the generation of TNF-α of cells exposed to woodsmoke were also found increased compared with the control by 2.9-fold and 2.1-fold, respectively (Leonard et al., 2000).

Discussion

In vitro toxicity of PM derived from bushfire/biomass burning emissions

The results of *in vitro* toxicological testing of the effects of bushfire/biomass burning PM varied substantially between studies due to the differences in the cell types, PM extraction methods, exposure concentrations and times of exposure. However, most of the studies have shown that particulates derived from bushfire/biomass burning have an adverse impact on cells. Fine particulates ($PM_{2.5}$) from primary bushfire smoke were generally found to elicit greater biological responses than the coarse particulates ($PM_{4.2\text{-}24}$) (Leonard et al., 2007). Some studies found that PM from smoke was more toxic than ambient PM (Franzi et al., 2011; Pavagadhi et al., 2013),

while other studies have presented results which suggest that mechanisms underlying the toxicity differ between bushfire PM and ambient PM (Nakayama Wong et al., 2011; Verma et al., 2009).

Woodsmoke has been tested for toxicity of PM arising from different combustion phases and conditions using *in vitro* methods. The toxicity of woodsmoke PM appears to change as a consequence of combustion conditions, including parameters such as temperature, oxygen concentrations and technology of boiler/heater using (Bølling Anette Kocbach et al., 2012; Danielsen et al., 2011; Jalava et al., 2012, 2010). The chemical composition of PM from woodsmoke under the different combustion conditions (Rau, 1989) appears to be the likely cause of the differences in toxicity. From the results obtained in woodsmoke studies, it can be expected that the toxicity of bushfire PM may also change under different combustion conditions, however to date, no studies have addressed this issue for bushfire smoke. Wood burning could be used to understand the response to bushfires, however, because of the differences in fuel types, in terms of composition, moisture and the amount of fuel being burned in wood burning and bushfires, the results of woodsmoke PM toxicological studies may not adequately represent the toxicity of bushfire smoke. Studies on the effects of combustion conditions on the toxicity of bushfires should be conducted to enhance our understanding on the toxicity of bushfire emissions.

Furthermore, each vegetation type has a specific fuel structure and content and therefore is likely to generate different chemical compounds and in differing concentrations in smoke when being burned (McMeeking et al., 2009; Possell & Bell, 2013). Some woodsmoke studies investigated the effects of chemical composition of PM on *in vitro* biological responses. Jalava et al. (2012) observed a positive correlation between inflammatory response of RAW 264.7 and level of PAH compounds in woodsmoke PM, while Verma et al. (2009) found a significant association between ROS generated by mouse macrophages with water-soluble transition metals. The differences in chemical components generated from combustion of different vegetation types may cause different toxicological effects that have not been addressed in toxicological studies on bushfire/biomass burning and thus further investigation on toxicity of bushfire emissions from different fuel types is required.

Most of the literature on *in vitro* toxicity of bushfire and open biomass burning smoke covered in this review focused on the PM sourced from bushfire after ageing of the particulates during long-distance transport. The biological effects of PM generated at, or near, bushfires in the field have not been investigated thoroughly with only the study by Leonard et al. (2007) collecting PM in the field during prescribed burns. The toxicity of PM after long-distance transport might change due to the chemical transformation of unstable components, e.g. organic compounds (Jalava et al., 2006). Therefore, the observed toxicity of bushfire/open biomass burning PM in the majority of

studies may not reflect the effects on people who are in close proximity to the smoke. Individuals exposed at close range would include the firefighters and communities living close to natural bushfires or those residing in areas in which prescribed burns are routinely conducted.

Apart from PM, bushfire and open biomass burning emissions consist of a variety of other pollutants that may also negatively impact human health. Some of the hazards of these pollutants have been investigated and confirmed in studies of air-sheds, however few studies have investigated the *in vitro* toxicity of these pollutants when sourced from bushfires, and only a single study was found which examined the toxicity of whole woodsmoke (Leonard et al., 2000). This paucity of data may be due to the difficulties in sample collection in remote sites and the episodic nature of bushfire events which makes it difficult to assess the toxicological effects of the gaseous pollutants. Even though the toxicity of many of the individual pollutants is generally well established, the toxicity of combined gaseous pollutants is difficult to assess (Naeher et al., 2007).

In vitro methods of toxicity testing

The most commonly reported method used in testing the *in vitro* biological effects of PM from bushfire/open biomass burning or woodsmoke was to extract or to suspend PM in a solution to which the test cells are then exposed. After incubation in the culture medium containing the PM, the cells or cell supernatant are then analyzed to determine toxicological responses either by measuring cytotoxic responses or by determining other cellular responses. However, as previously noted, there are substantial differences in the experimental design and methods of these studies including the types of cells tested, extraction solvents, extraction times, doses and times of cell exposure. These differences may account for variation in the results of the toxicological studies. For example, when comparing two durations of exposure (12 hours and 40 hours, Bølling et al. (2012) found that the production of IL-6, IL-8 and LDH release increased with exposure time while TNF-α concentration decreased. Franzi et al. (2011) tested the viability of RAW 264.7 cells after exposed to the same dose of bushfire PM (10 µg) for 0.5, 1, 2 4, 6 and 24 hours and found that the number of dead cells was highest after 0.5 hour of exposure. After 24 hours the number of dead cells was similar between cultures treated by wildfire PM and PBS (10 µL) as a control. This result might be due to the higher proliferation rate of surviving cells after treatment by bushfire PM compared with the control (Franzi et al., 2011). Furthermore, the use of different toxicological endpoints among studies is also a challenge when comparing the results from different studies. The above variations are not specific to bushfire/biomass burning *in vitro* toxicological studies, but are also problems for *in vitro* studies of air pollutants in general.

To enable direct comparisons to be undertaken between studies, as well as to be able to reflect physiologically relevant conditions, it is necessary to develop standard protocols for *in vitro* toxicological testing of bushfire PM, similar to some existing protocols for nanoparticles (The International Organization for Standardization, 2010, 2016). These protocols should recommend which solvent should be used for extraction to better imitate physiological conditions. The dose and time of exposure should also be suggested based on the calculation of particulate loads inhaled into the respiratory system; this would provide results that better reflect a human exposure scenario. Types of filters used for PM collection and types of cell used for exposure should also be recommended in the protocols. The majority of studies included in this review used glass fibre and Teflon filters to collect PM for toxicity testing. However, Karlsson et al.(2006) found that the fibre from glass fibre filters accounted for up to 25% of mass of PM extracted from filters and therefore contributed to the effects on outcomes. This issue should be taken into account when recommending a suitable type of filter. When choosing types of cell used for *in vitro* toxicology studies of air pollutants, mouse macrophages RAW 264.7 and human epithelial cells A549, which have been applied in the majority of studies, are possible candidates.

As discussed previously, toxicity of whole bushfire smoke should be assessed because assessing the toxicity of bushfire smoke using extracted material may present limitations in terms of our understanding of the potential health impacts of bushfire smoke exposure. Leonard et al. (2000) evaluated the toxicity of the whole woodsmoke by bubbling the smoke into saline over 1 minute. However, the efficiency of this method depends on the solubility of the components of woodsmoke. This method only assesses the toxicity of soluble components and may underestimate the toxicity of smoke as a complex mixture made up of many compounds. Although the technique of direct exposure has not been employed by studies assessing bushfire toxicity, the toxicity of complex bushfire emissions could be assessed with *in vitro* techniques that have been used to test the hazards of cigarette smoke or airborne pollutants (Aufderheide & Scheffler, 2011; Bakand et al., 2006; Nara et al., 2013). In these studies, cells were exposed directly to smoke by drawing smoke through an incubation chamber so that it contacts with cells at the air-liquid interface. Bakand et al. (2006) used a horizontal diffusion chamber system to expose cells to NO_2; while Aufderheide & Scheffler (2011) and Nara et al. (2013) applied a device to expose cells to cigarette smoke. This direct exposure method imitates the *in vivo* exposure conditions better than the indirect method (solubilizing the gas in the culture medium), especially in the case of poorly soluble gases (Aufderheide, 2005). This method is also suitable for testing and evaluating the combined effects of gaseous and particulate phases in complex mixtures (Knebel et al., 2002).

Conclusions

This review focused on the *in vitro* toxicological effects of bushfire/open biomass burning emissions. There is a paucity of studies investigating the toxicity of bushfire/open biomass burning emission. Furthermore, the application of a wide range of experimental design and methods creates difficulties in comparing results from different studies. It is recommended that researchers standardise protocols to enable comparison between studies of different vegetation types and combustion conditions as well as toxicological outcomes to provide a clearer picture of the potential for health impacts from specific types of bushfires. The protocols should recommend types of filters used for PM collection, PM extraction method, types of cells used for exposure, exposure time and dose and toxicity endpoints evaluated. Furthermore, there is a need to evaluate whole smoke of bushfire to consider the biological responses following exposure to this complex mixture. It is also recommended that further work should be undertaken on the toxicity of newly generated bushfire PM compared with aged smoke to better understand the potential for health impacts in those exposed to bushfire smoke.

Update of recent published works

Since the review paper was published (Dong et al., 2017) further studies on *in vitro* toxicity of PM from biomass burning have been published (Deering-Rice et al., 2018; Kasurinen et al., 2018; Marchetti et al., 2019). Deering-Rice et al. (2018) introduced woodsmoke PM (size fractions 0.43–10μm) extracts to several types of human cells including embryonic kidney (HEK-293), A549, BEAS-2B cells and examined the transient receptor potential V3 (TRPV3) activation using a calcium flux assay. This study found that the activation of TRPV3 was dependent on the chemical composition of the PM and this contributed to the pneumotoxicity of particulates. Kasurinen et al. (2018) compared the differences between cell models using monocultures (A549 or THP-1) and co-cultures of these two cell types after exposure to PM_1 from wood burning. The study found that all PM samples had a negative impact on cells and there was a diversity of cellular responses (inflammatory cytokines and chemokines production, ROS production, cytotoxicity, genotoxic) which were affected by the exposed cell type and composition of PM. Another recently published study by Marchetti et al. (2019) examined the cell viability, cytokine production, oxidative stress responses and DNA damage of A549 cells exposed to PM_{10} in smoke from burning pellets, charcoal and wood. These authors also found that the cellular responses were related to the chemical composition of PM_{10} and different types of biomass may have different toxicological pathways. The lack of any recent studies on *in vitro* toxicity of PM from vegetation fires once again emphasises the urgent need for more studies on this neglected but important consideration of the impacts of wildfire smoke.

Chapter 3. EMISSIONS OF INORGANIC GASES

(This chapter is an article manuscript titled "Emissions of CO_2, CO, SO_2, NO and NO_2 from laboratory-based fires of five common vegetation types in Western Australia" which has been submitted to the Journal of Geophysical Research - Atmospheres)

Abstract

This study investigated the emission factors (EFs) for CO_2, CO, SO_2, NO and NO_2 from laboratory-based fires of vegetation from five typical vegetation types of Western Australia including three grasslands (Spinifex represented by *Triodia basedowii*, Kimberley grass represented by *Sehima nervosum* and *Heteropogon contortus*, and an invasive grass represented by *Ehrharta calycina* (Veldt grass)), Banksia woodland and Jarrah forest in different combustion conditions. Combustion conditions were altered by controlling the vegetation moisture content (<10%, 12-16% and 20-25%) and air flow rate (0, 1.25 and 2.94 $m.s^{-1}$). Burns of woodland (Banksia) and forest (Jarrah) had significantly higher EFs for CO, resulting in lower modified combustion efficiency (MCE) than those of tropical grass (Spinifex). Temperate grass (Veldt) fires had significantly lower EF_{CO2} and higher EF_{CO} than those of the tropical grass fires. EFs for SO_2, NO and NO_2 were variable between different vegetation types, indicating variation in nitrogen and sulphur content of the fuels. Moisture content did not strongly influence the MCE and EFs, but flow rate had a significant effect. The results for MCE, EFs for CO and CO_2 were similar to values reported from field measurements for similar ecosystems in Australia, indicating the applicability of these laboratory-based results. However, emission factors produced in this study are different to the profiles generated from vegetation fires in other parts of the world. To improve prediction of bushfire emissions and impacts in Australia, EFs of all fire-prone Australian vegetation types should be determined, particularly for those vegetation types in close proximity to densely populated urban areas where smoke pollution might pose a health risk.

Keywords: bushfires, vegetation fires, inorganic gases, CO_2, CO, SO_2, NO_x

Introduction

Emissions from bushfires, also called vegetation fires, including wildfires and prescribed burns, (planned fires with the purpose of reducing the combustible vegetation load) have been increasingly studied in recent decades due to an increase in frequency of these events and the significant amount of pollutants emitted to the atmosphere (Andreae and Merlet, 2001; Koppmann et al., 2006; Vicente et al., 2011). Bushfire smoke consists of many compounds, emitted in two phases: gaseous and particulate. Of the gases emitted, carbon dioxide (CO_2), carbon monoxide (CO), nitrogen oxides (NO_x) and sulphur dioxide (SO_2) are the most common combustion products

and have been measured in most of the studies investigating emissions of inorganic gases from vegetation fires.

CO_2 is the main product of biomass burning due to the high carbon content of biomass. CO is a by-product of incomplete combustion and may be present in high concentrations in bushfire smoke, depending on combustion conditions, such as fuel moisture, burning temperature and stage of the fire (Koppmann et al., 2006; Reisen and Tiganis, 2007). NO_x is a mixture of nitrogen oxides, with nitric oxide (NO) and nitrogen dioxide (NO_2) the main components (US Environmental Protection Agency – USEPA, 1999), and can react with free radicals to form ozone in the troposphere, which is an important air pollution problem and the primary component of smog (USEPA, 1999). NO is the most abundant form of NO_x emission from vegetation combustion (Andreae and Merlet, 2001). Biomass burning has been estimated to contribute to about 40% of CO and 20% of NO_x in total global emissions (Langmann et al., 2009). SO_2 is also generated from burning biomass (Andreae and Merlet, 2001). The reaction of SO_2 and other atmospheric compounds can lead to the formation of small particles, such that SO_2 is considered a major precursor of ambient $PM_{2.5}$ (European Environment Agency - EEA, 2014). The emissions of NO_x and SO_2 from bushfires have been found to be dependent on the chemical composition of fuel (Burling et al., 2010; Lacaux et al., 1996).

Emissions of bushfires can be estimated by multiplying fuel load, burned area, combustion efficiency and emission factors for pollutants of interest (Possell et al., 2015). Emission factor (EF) for pollutants is defined as the amount of pollutant generated from the burning of a mass unit of dry fuel. Therefore, it is an important metric for studies investigating emissions from fires.

Bushfire emissions are highly depended on vegetation composition which is significantly different between geographical regions (De Vos et al., 2009). The distinct vegetation composition of different areas raises the necessity of EFs for pollutants emitted from fires of local vegetation types for better emission prediction. Australia is a fire-prone country with an estimated contribution of about 8% to total global carbon emission from biomass burning (Paton-Walsh et al., 2010). In recent years, more attention has been paid to emissions from bushfires in Australia with a number of studies undertaken. Most of these examined the emissions from northern Australian savanna fires which account for a high proportion of bushfires in this country (Desservettaz et al., 2017; Paton-Walsh et al., 2010; Shirai et al., 2003; Smith et al., 2014; Wang et al., 2017a, 2017b). Other studies have investigated fires of grassland and forests in the south-eastern part of the continent where many metropolitan cities are located (Guérette et al., 2018; Paton-Walsh et al., 2014, 2005; Possell et al., 2015). Western Australia (WA) is the largest state of Australia, accounting for one third of the country's area and can experience bushfires at any time of the year due to its large

latitudinal variation (Bryant, 2008). However, research on emissions from bushfires in WA is limited (De Vos et al., 2009; Reisen and Brown, 2009; Reisen et al., 2006b). Of those that have been undertaken they have focused on the concentration of air pollutants in bushfire smoke to which firefighters were exposed, and did not produce data on emission factors for the different pollutants.

With the above context, this study was conducted to investigate the chemical composition of smoke from burning some typical vegetation types in Western Australia (WA) (including three grasslands (two tropical and one temperate), one woodland and one forest) in the laboratory with the aim of producing emission factors for the main pollutants: inorganic gases (CO_2, CO, NO, NO_2 and SO_2), volatile and semi-volatile compounds, and particulate matters from WA vegetation fires. This study also examined the effects of combustion conditions (controlled by flow rate and fuel moisture content) on the emission profiles of pollutants. This chapter will discuss the results for inorganic gases only. Data on other pollutants will be presented in other chapters of this thesis.

Methodology

Combustion experimental set-up

An oval-shaped ceramic chamber was used for combustion of vegetation samples (Figure 1). The chamber had a small door near the bottom where air could be drawn through. At the top of the chamber, a system of 6" stainless steel duct with a total length of 10 metres was connected to the outlet of the chamber to collect the emitted smoke. Smoke was drawn through the duct using an exhaust fan which could create different wind speeds in the chamber. The sampling area was located at the end of the duct system, before the exhaust fan, and consisted of five sampling ports of varying diameters to collect and measure different components of the smoke including aldehydes, particulate matter, inorganic gases, temperature, and velocity of smoke flow and VOCs, in order (Figure 3.1). The design of the horizontal duct system, varying in height levels, not only facilitated the sampling and measurement process, but also contributed to the effective mixing of smoke along the duct. The smoke was well mixed and confirmed by the relatively constant $PM_{2.5}$ mass collected at different positions on a cross-section of PM sampling point (Table A1.1, Appendix 1).

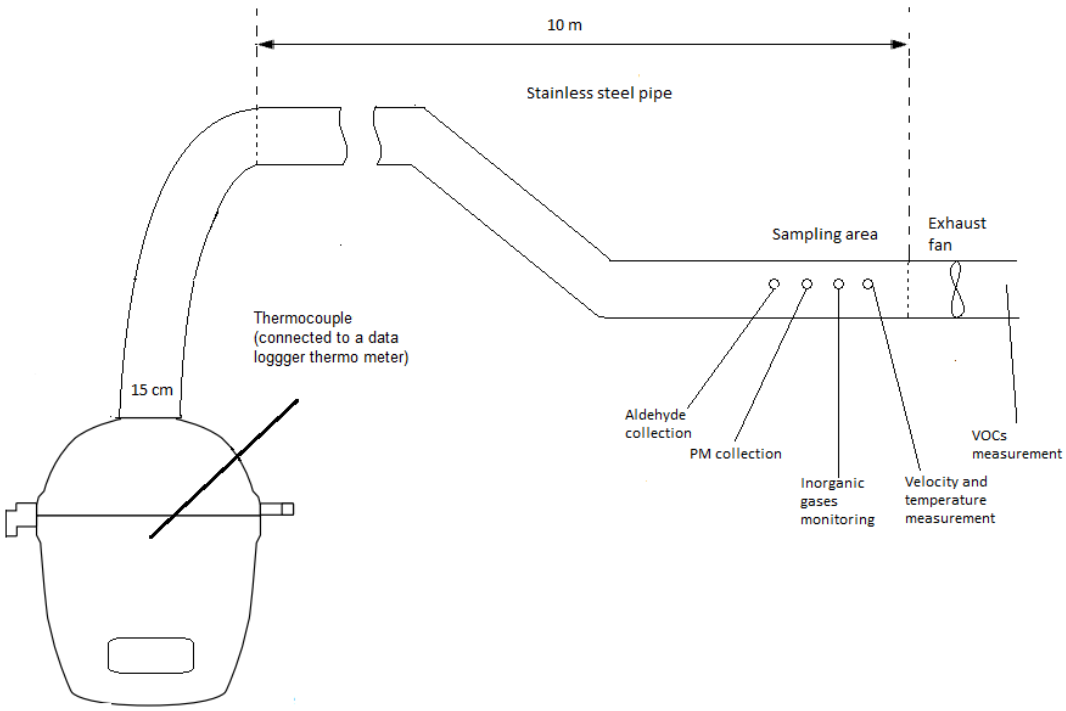


Figure 3-1. Schematic diagram of combustion experiment set-up

A thermocouple was installed in the combustion chamber to measure the temperature change inside the chamber throughout the combustion process. A thermal anemometer (TSI Model TA430) was installed to measure the temperature of the smoke at sampling points and the velocity of smoke flow inside the duct. Vegetation samples were placed in a stainless steel wire box (dimensions 30×30×30 cm) on a stainless steel frame inside the chamber. The purpose of placing vegetation in the wire box was to minimise the turbulence of vegetation caused by changes in air flow rate, which might influence the combustion process of the small amounts of vegetation used. The vegetation burn was ignited for 10 seconds using a propane torch via the door near the bottom of the chamber (Figure 3.1).

Experimental combustion conditions

Air flow rate and vegetation moisture content were used as factors to create different combustion conditions. Vegetation samples were prepared at three moisture contents: "dry" – <10%; "moist" – 12 to 16% and "wet" – 20 to 25% (see section on Vegetation sample preparation). The range of moisture levels of the vegetation was chosen to mimic the range of conditions that could be found in both wildfires and prescribed burns (Bush Fire & Environmental Protection Branch, 2007; Fire Management Branch, 2011; Marsden-Smedley, 2009).

Three air flow rates were used in the experiments (no flow, low and high flow rate). For the "no flow" condition the exhaust fan was not used and oxygen was supplied for the burn from fire-induced convective movement of air into the combustion chamber (Figure 3.1). At low and high

flow conditions, the fan was operated at speeds that created the flow rates of 1.3 $m.s^{-1}$ (equivalent to 4.5 $km.h^{-1}$) and 2.9 $m.s^{-1}$ (equivalent to 10.6 $km.h^{-1}$) respectively in the chamber. These air flow rates are within the limit wind speeds recommended for prescribed burn in different vegetation types in Australia (Bush Fire & Environmental Protection Branch, 2007; Marsden-Smedley, 2009).

Types of vegetation

Five common vegetation types, including three grasslands, a woodland and a forest from WA were chosen based on their prominence in wildfires and prescribed fires recorded by agencies in charge of bushfire fighting in WA (Bryant, 2008). Samples for each vegetation type were collected based on the abundance of species in each vegetation type (Gibson et al., 1994). Samples used for combustion consisted of up to five common species mixed in the same ratio of weight to represent each vegetation type. Due to the large geographical distribution of grasslands which account for around 85% of WA land area (WA EPA, 2006) and the regular fires in these systems, three types of grassland were included in this study.

Spinifex, dominated by the genus *Triodia*, is a hummock grassland distributed extensively in arid areas of Australia covering an area of nearly 1.4 million km^2, accounting for 18% of the total area of Australia. Spinifex is also the largest major vegetation group of WA (Department of Environment and Water Resources, 2007). *Triodia basedowii*, the most dominant species of *Triodia*, was collected in the Gascoyne, a region in the north-west of WA, and used as representative of spinifex grassland. Another tropical grassland was from the Kimberley, a northern remote region of WA which is dominated by grasslands. Due to the dry and hot weather, grassland fires occur regularly in the Kimberley (WA EPA, 2006). Two common grass species *Sehima nervosum* and *Heteropogon contortus* were collected and used as representative of Kimberley grassland. The third grassland sampled around Perth was an invasive grass, *Ehrharta calycina* (Veldt grass) which is a perennial temperate grass from South Africa. It was accidentally introduced to Australia and has become a widespread weed, invading many types of bushland in WA (Sanford & Reed, 2006). Its presence has been shown to increase the risk of bushfires (DiTomaso et al., 2013).

Banksia woodland is a common native vegetation type on the coastal plains of WA. With a diverse number of species forming several layers of fuel and being fire-prone, this vegetation type often experiences intense bushfires (Burrows and McCaw, 1990). Five dominant species including *Banksia attenuata, Hibbertia hyperricoides, Acacia saligna, Xanthorrhoea preissii* and *Allocasuarina frasenriana* were collected to form a representative sample of Banksia woodland.

Jarrah forest is another native vegetation type located in the south-west of WA. This type of vegetation is recorded as being subject to the largest number of fires, accounting for around 28% of total vegetation fires recorded by the WA Department of Environment and Conservation in the period 1999 to 2003 (Bryant, 2008). Five dominant species from the jarrah forest were collected including *Eucalyptus marginata, Corymbia calophylla, Hakea undulata, Xanthorrhoea preissii* and *Lepidosperma drummondii*.

Vegetation collection and vegetation sample preparation

Above-ground samples of the plants of grasses and leaves and twigs with diameters of less than 5mm of vegetation species representing the Banksia woodland and Jarrah forest were cut from trees in the field. These were transferred to the laboratory and stored at 4°C until processing (cut into small pieces of 20-30 cm in length and dry). Vegetation was dried gradually at 40°C in an oven until the target moisture content was achieved. Each vegetation type was prepared with three moisture content levels (<10%, 12–16% and 20–25%).

To determine moisture content, sub-samples of each species were weighed using balance (Explorer Ohaus) and then were put in an oven (Modul Temp) at 80ºC for 24h. After being dried for 24h, sub-samples were re-weighed and the moisture content of each species was calculated using the following formula:

$$MC_i = \frac{m_w - m_d}{m_w} * 100\%$$

Where: MC_i: moisture content of species i; m_w: weight before drying (g); m_d: weight after drying at 80ºC for 24h (g).

The moisture content of each vegetation type including different species were calculated from the moisture content of each species using the following formula:

$$MC = \frac{\sum_1^n m_i * MC_i}{\sum_1^n m_i} * 100\%$$

Where: MC: overall moisture content of the vegetation sample (n=1 to 5) (%); MC_i: moisture content of species i in this type of vegetation; m_i: weight of species i in the vegetation sample (g)

All vegetation samples were burned within 2 weeks of preparation after storage in plastic bags at 4ºC. The moisture content of samples changed very little during storage (0–0.4%) (see Table A1.2).

Combustion experiments

Three replicate 25 g samples of each type of vegetation were burned under each combustion condition. Veldt grass did not ignite and combust at the two highest moisture contents (moist and wet) and since the Kimberley grass species were collected in dry condition, emissions from the grasses was restricted to dry grass.

After every burn, the combustion chamber was cleaned using a brush and the whole system was cleaned thoroughly by using the extraction force of the exhaust fan for at least 30 minutes. Burns (termed "blank burn") were conducted with the application of the propane torch only (without a vegetation sample in the chamber) to take into account the possibility of pollutants remaining in the system and affecting pollutant concentrations in subsequent burns. These blank burns were conducted before the 1st and after every 9th experimental burn. In blank burns, only CO_2 and NO concentrations increased due to the combustion of propane and N_2 in the air (accounting on average for about 6% and 1% of the sampled values, respectively) and the increased values were subtracted from the vegetation burns.

CO_2, CO, SO_2, NO_2 and NO measurement

The concentrations of CO_2, CO, SO_2, NO_2 and NO in the smoke were measured using a real-time multi-gas monitor MultiRAE Lite Pumped (Honeywell, USA). CO_2 was measured by a non-dispersive infrared (NDIR) sensor (RAE Systems Inc.). Other remaining gases were measured using electrochemical sensors (RAE Systems Inc.). The use of electrochemical sensors enabled measurement of multiple gases simultaneously, but may have underestimated the gas concentrations due to potential negative cross-interference between sensors (i.e. NO_2 and SO_2). Nevertheless, the results for these gases have been presented and compared with values reported in the literature to see whether they were comparable. The working ranges of the MultiRAE were 0 to 50,000ppm (CO_2), 0 to 2,000ppm (CO), 0 to 20ppm (SO_2), 0 to 20ppm (NO_2) and 0 to 250ppm (NO) respectively. The MultiRAE was calibrated with ambient fresh air before each experiment using a pre-set procedure in the instrument. The instrument was also calibrated using reference gases periodically as recommended by the manufacturer.

Measurements of inorganic gases inside the duct during the burning process were automatically recorded every 5 seconds throughout the combustion process, starting as the vegetation was ignited and continuing for 6 minutes after ignition. The concentrations of gases generated by the burns were calculated by deducting the concentrations in ambient air (as measured inside the duct prior to ignition) from the readings recorded during the burn.

There were instances where the concentrations of some gases exceeded the working ranges of the instrumentation; this mostly occurred in the burns with the no fan condition applied. The distribution of concentration peaks of these gases with sampling time in cases that did not exceed the instrumentation's working ranges followed a sixth degree polynomial equation with R^2>0.99 (see Figure A1.1). Therefore, the sixth-degree polynomial functions of sampling time were used for the extrapolation of . the over-range concentrations of gases.

Calculation of emission factors and modified combustion efficiency

The fire-integrated emission factors (EF) of emitted pollutants were calculated using carbon mass balance method as follows (Desservettaz et al., 2017):

$$EF_i = \frac{m_i}{m_{fuel}} = \frac{m_i}{m_C} F_C$$

Where EF_i: emission factor of pollutant i (g.kg^{-1} dry fuel); m_i: mass of pollutant i emitted (g), can be calculated using the formula: $m_i = \int_{t_s}^{t_f} A \times C_t \times v_t \times dt$ (Irfan et al., 2014) where A: area of the duct at sampling points; t_s and t_f: start and finish times of sampling period; C_t: concentration of pollutant i at time t (mg.m^{-3}); v_t: velocity of smoke at time t (m.s^{-1}); dt: interval of each record (dt = 5s).

m_C is the amount of C emitted (g), and is calculated as the sum of carbon mass emitted in CO_2, CO, hydrocarbons and particulates (McMeeking et al., 2009). Hydrocarbons and aerosol carbon were not evaluated in this study, and since CO_2 and CO are the major carbonaceous products of bushfires (>95%, Meyer et al., 2012) carbon mass emitted in CO_2 and CO was used in this calculation, which might slightly inflate the EFs (May et al., 2014).

F_C is the carbon content of fuel. Since fuel composition was not analysed in this study, we used the values reported for Australian vegetation by Hurst et al. (1994) which were 45% for grass and 48% for leaves and twigs to calculate the EFs for pollutants from burning grass and woodland/forest, respectively.

Modified combustion efficiency (MCE) is a parameter indicating the efficiency of a combustion process, with more complete combustion resulting in higher values of MCE. Combustion with an MCE over 0.9 indicates a dominant flaming phase, MCEs from 0.8 to 0.9 suggest a mixture of flaming and smouldering phases, and an MCE below 0.8 indicates combustion to be mainly in the smouldering phase (Lee et al., 2008; Sinha et al., 2003).

The MCE of each burn was calculated using the formula (Ward and Radke, 1993):

$$MCE = \frac{C_{CO_2}}{C_{CO_2} + C_{CO}}$$

Where C_{CO_2} and C_{CO}: carbon content in emitted CO_2 and CO.

In some cases in order to see more clearly the relationships between emissions of gases, number of moles of some gases (calculated by dividing the amount of an emitted gas by its molecular weight) were used instead of the emission factors.

Statistical analysis

Relationships between variables were assessed using regression analysis. The influence of vegetation type on inorganic gas emissions was evaluated using one-way analysis of variance (ANOVA) and Kruskal-Wallis tests, for normally and non-normally distributed data respectively, using SPSS ver.25 (IBM). Post-hoc Tukey and Dunn-Bonferroni tests were performed for significant ANOVA and Kruskal-Wallis results, respectively. The effects of moisture content and flow rate on emissions were investigated using permutational multivariate ANOVA (PERMANOVA) using PRIMER 6^+ (PRIMER-E). A significance value of $p<0.05$ was utilised for statistical analysis.

Results

Emission factors

Carbon dioxide and carbon monoxide

Emission factors for CO_2 across all combustion conditions were similar for Spinifex, Banksia and Jarrah (Figure 3.2a). Significant differences between the emissions factors for CO from burning the three vegetation types were observed (Figure 3.2b). The EF_{CO} from Spinifex burning was significantly lower; more than 2-fold less than those of Banksia and Jarrah, which indicated the combustion of Spinifex was more complete than that of the other vegetation types.

In contrast, the EF for CO_2 from burns of different grasslands showed a significant difference between Veldt grass and the other two types of grass. EF_{CO2} of the temperate grassland was much lower than that of the tropical grasslands (Figure 3.3a). Conversely, Veldt grass burning emitted 184 $g.kg^{-1}$ (dry fuel) of CO which was more than 2-fold higher than those from Spinifex and Kimberley grass burning (Figure 3.3b).

Sulphur dioxide

Significant differences were also observed between SO_2 emissions from the different vegetation types. Banksia yielded the highest EF_{SO2}, followed by Jarrah and Spinifex. Post-hoc testing showed that grass had significantly lower EF_{SO2} compared with the woodland and forest (Figure 3.2c).

Among the three types of grasslands, Spinifex had the highest emission of SO_2, followed by Veldt grass. Kimberley grass burns generated a very small EF_{SO2}, the difference being statistically significant between Spinifex and Kimberley grass (Figure 3.3c).

Nitrogen oxides

NO was the most abundant nitrogen oxide generated. The average EFs for NO in all combustion conditions were similar for the three vegetation types of Spinifex, Banksia and Jarrah (Figure 3.2d). Meanwhile, EF_{NO} for the grasslands were significantly different, with Spinifex burns generating the highest emissions of NO, followed by Veldt grass and then Kimberley grass (Figure 3.3d).

Across all vegetation types NO_2 was emitted in smaller amounts compared with NO. Spinifex had the highest NO_2 emission, followed by Banksia. Jarrah had the lowest EF_{NO2} which was around half that of Spinifex (Figure 3.2e). For the three grassland types, the NO_2 emission results were the reverse of those found for $EF_{NO.}$ Kimberley grasses emitted the highest $EF_{NO2,}$ followed by the Veldt grass and then Spinifex (Figure 3.3e).

Since NO and NO_2 emitted from combustion are rapidly interconverted depending on combustion temperature (Paul et al., 2008), NO_x is often reported for both NO and NO_2 (Akagi et al., 2011). EFs for NO_x were significantly different between three types of grasslands but not for NO_x emissions when comparing vegetation types (Figure 3.2f).

EFs under different combustion conditions

The variation of EFs for inorganic gases under different combustion conditions controlled by fuel moisture content and air flow rate is presented in Figure 3.4. Fuel moisture content had a significant effect on the CO_2 emission factors of the Spinifex and Banksia, but not for the Jarrah. EF_{CO2} from burning wet Spinifex was higher than that from dry and moist grass, whilst lesser amounts of CO_2 were observed from moist and wet Banksia burns compared with dry woodland vegetation (Figure 3.4a). Similarly, EF_{CO} was also influenced by fuel moisture content, with significant difference between wet and dry/moist Spinifex burns, and between moist and dry Banksia burns (Figure 3.4b).

Fuel moisture content had some effect on the emission factors of SO_2 with a significant increase in EF_{SO2} observed from burning Jarrah under wetter conditions. EF_{SO2} from both Spinifex and Banksia burning did not change significantly with different moisture contents (Figure 3.4c). The EF_{NO2} from burning Spinifex was also not impacted by fuel moisture content, however the EFs for NO_2 were influenced by moisture content in Banksia and Jarrah with less NO_2 emitted from burns of moister vegetation (Figure 3.4d). For NO, the fuel moisture content had a significant effect only on the emissions from Banksia, with the lowest EF_{NO} generated from burning moist Banksia (Figure 3.4e).

The flow rate showed strong effects on emissions of CO_2, CO and SO_2 across all vegetation types. While EFs for CO_2 significantly decreased with the increase in the air flow rate, EFs for CO and SO_2 increased (Figure 3.4a, b, c). Emissions of nitrogen oxides seemed to be less affected by air flow rate, with significant changes only observed from burning Banksia for EF_{NO2} and Spinifex for EF_{NO} (Figure 3.4e, f).

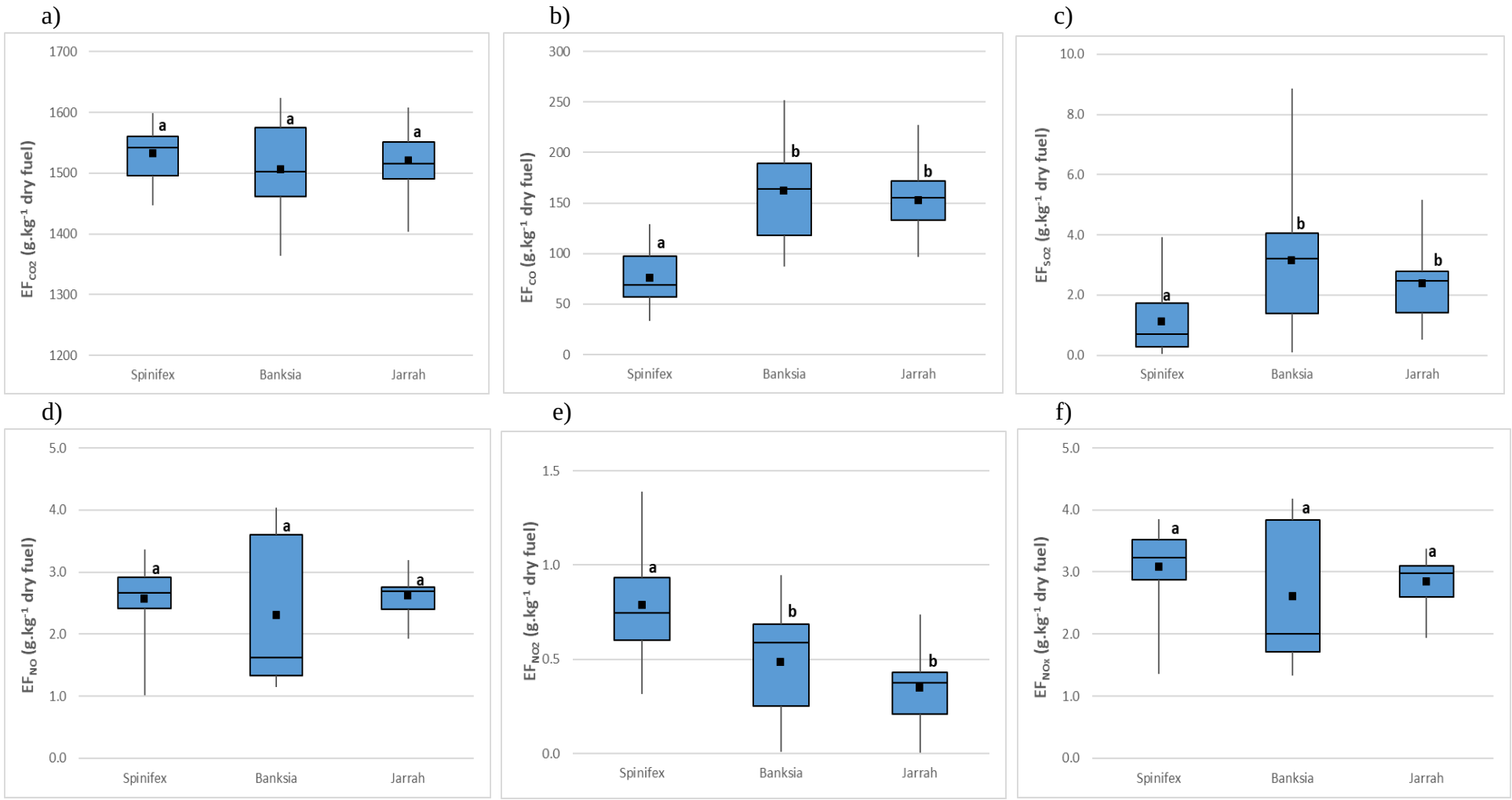


Figure 3-2. Box plots showing the EFs for inorganic gases (a - CO_2, b - CO, c - SO_2, d - NO, e - NO_2 and f - NO_x) emitted from burning Spinifex, Banksia and Jarrah in all combustion conditions. Black squares in the box plots show the mean values (n=27). Significant differences between types of vegetation are presented by the letters a, b and c at top of the box plots. The differences were evaluated using one-way ANOVA tests (for CO_2, CO, and NO_2) and Kruskal-Wallis test (for SO_2, NO and NO_x) with P<0.05

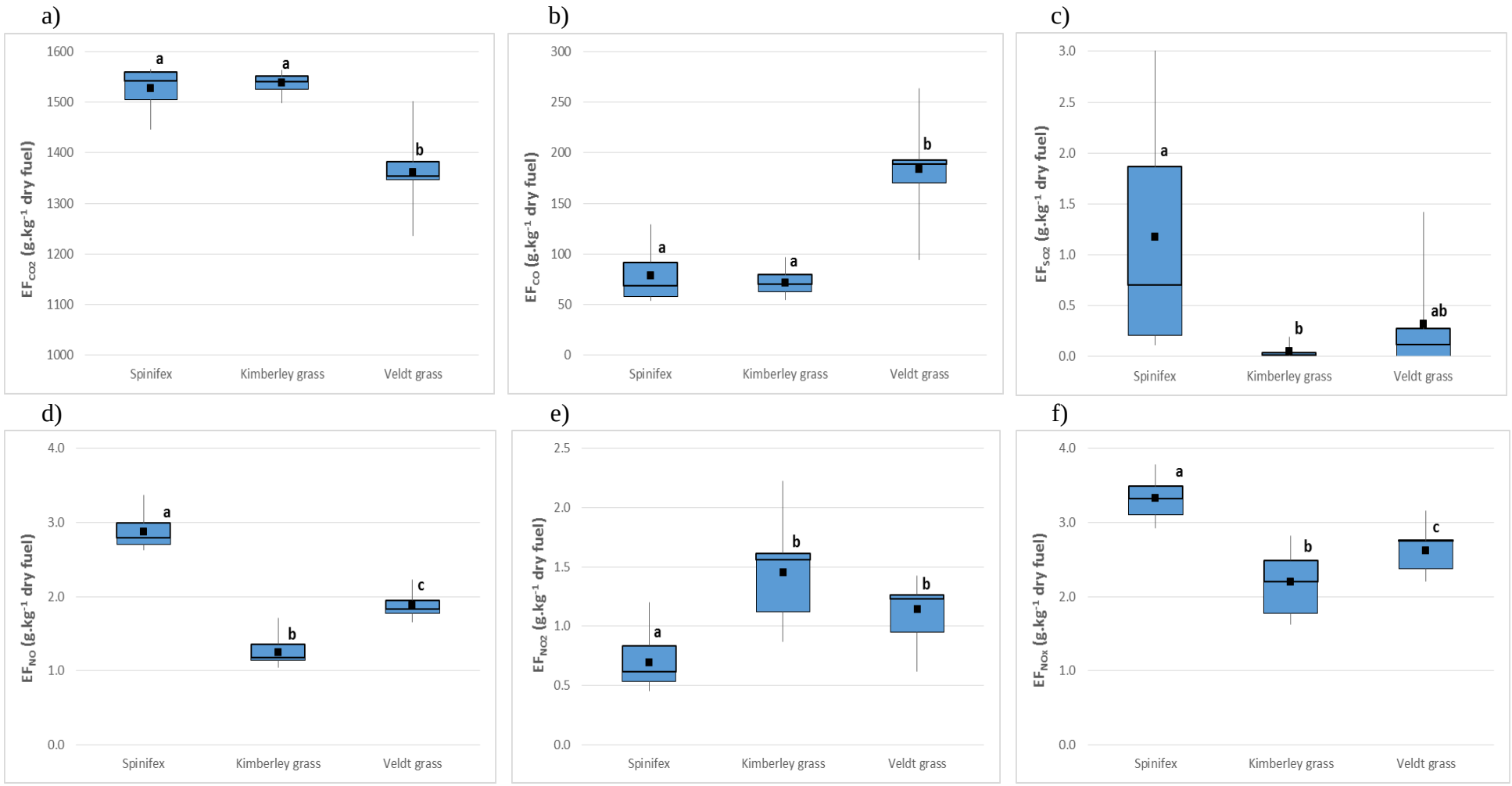
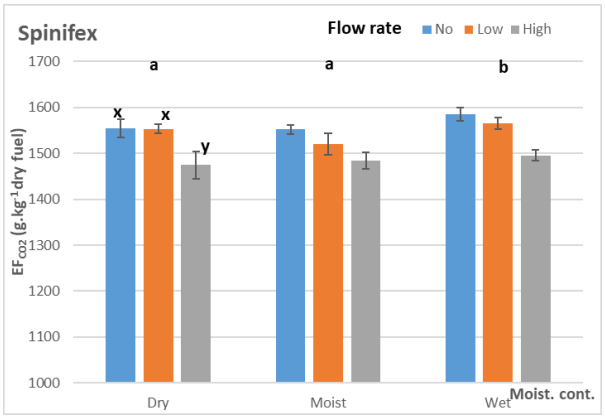
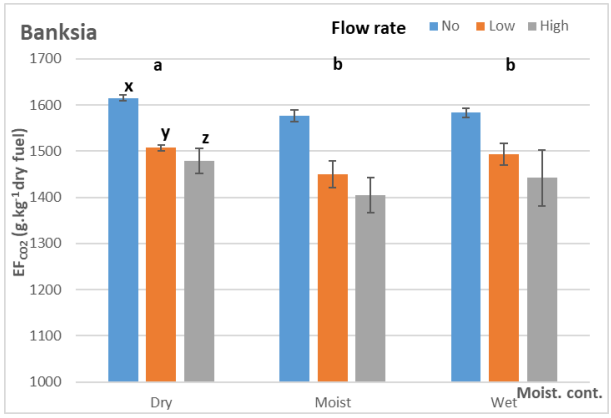
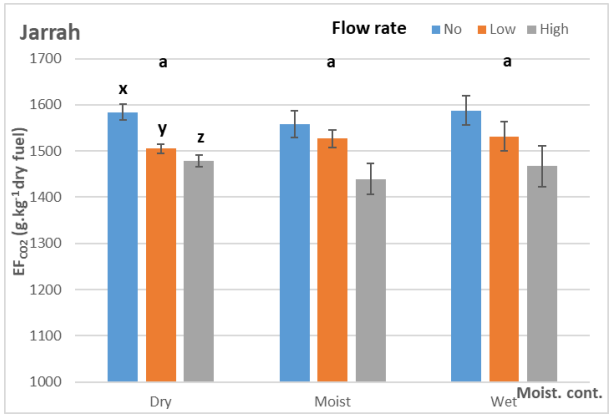


Figure 3-3. Box plots showing the EFs for inorganic gases (a - CO_2, b - CO, c - SO_2, d - NO, e - NO_2 and f - NO_x) emitted from burning Spinifex, Kimberley grass and Veldt grass in dry condition. Black squares in the box plots show the mean values (n=9). Significant differences between kinds of grasses are presented by the letters a, b and c at the top of the box plots. The differences were evaluated using Kruskal-Wallis tests (for CO_2, CO, SO_2 and NO_2) and one-way ANOVA test (for NO and NOx) with P<0.05

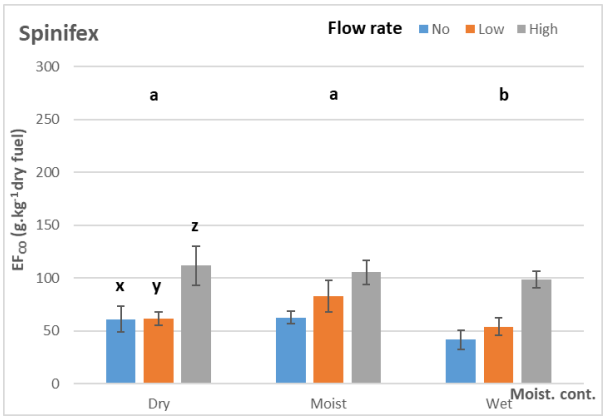
a)

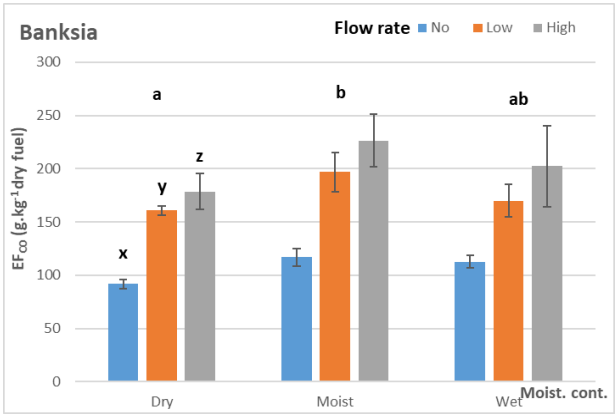

Spinifex
Flow rate No Low High
1700
1600
1500
1400
1300
1200
1100
1000
EFCO2 (g.kg-1dry fuel)
a a b
x x y
Dry Moist Wet
Moist. cont.

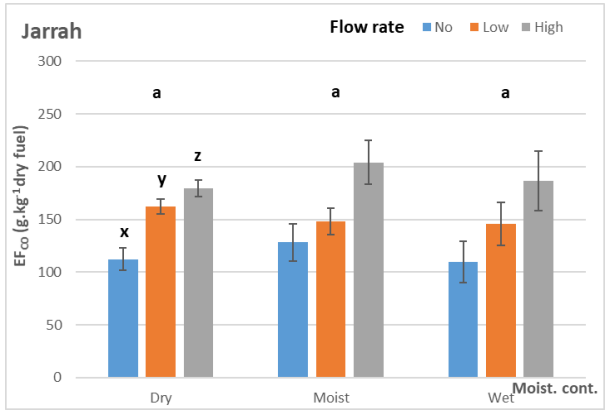

Banksia
Flow rate No Low High
1700
1600
1500
1400
1300
1200
1100
1000
EFCO2 (g.kg-1dry fuel)
a b b
x y z
Dry Moist Wet
Moist. cont.


Jarrah
Flow rate No Low High
1700
1600
1500
1400
1300
1200
1100
1000
EFCO2 (g.kg-1dry fuel)
a a a
x y z
Dry Moist Wet
Moist. cont.

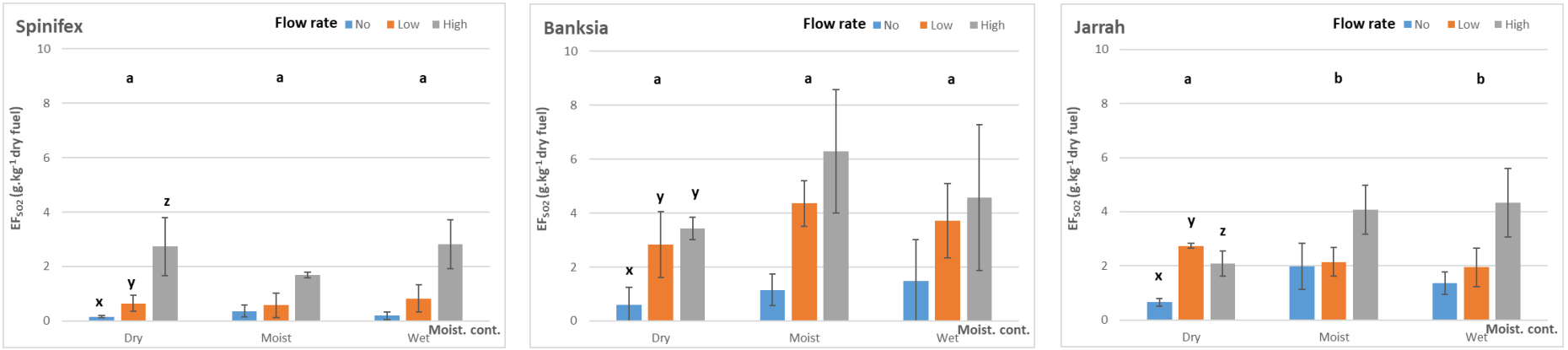
b)


Spinifex
Flow rate No Low High
300
250
200
150
100
50
0
EFCO (g.kg-1dry fuel)
a a b
x y z
Dry Moist Wet
Moist. cont.

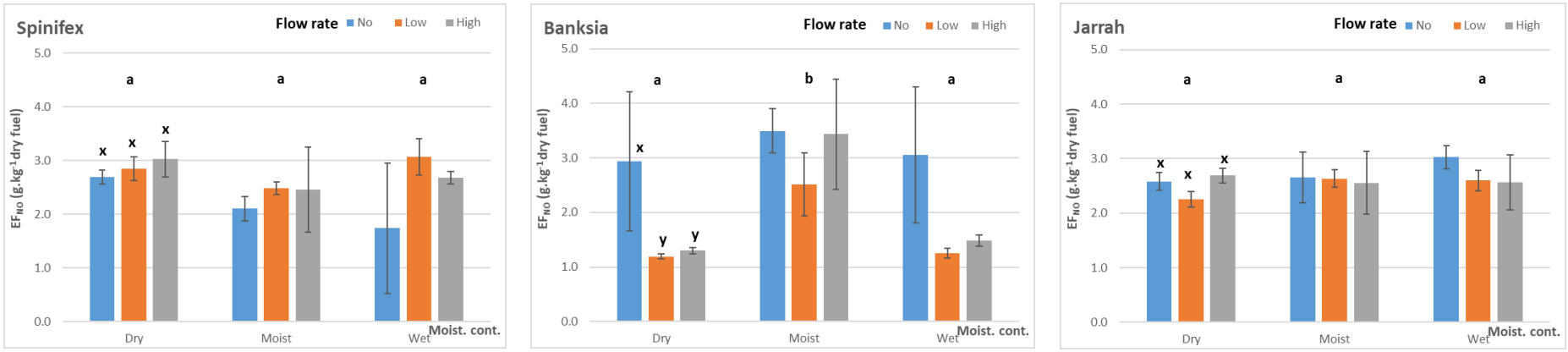

Banksia
Flow rate No Low High
300
250
200
150
100
50
0
EFCO (g.kg-1dry fuel)
a b ab
x y z
Dry Moist Wet
Moist. cont.


Jarrah
Flow rate No Low High
300
250
200
150
100
50
0
EFCO (g.kg-1dry fuel)
a a a
x y z
Dry Moist Wet
Moist. cont.

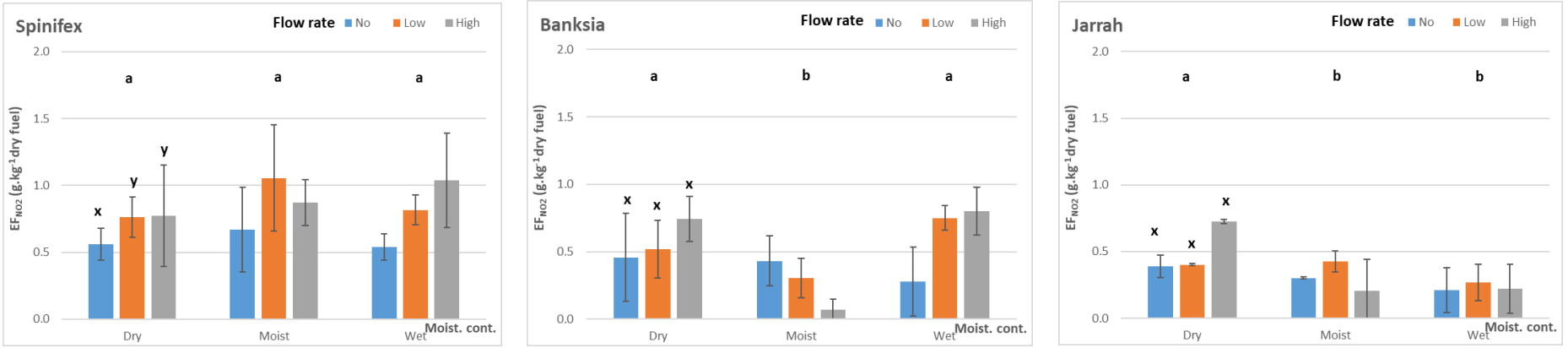
c)


Spinifex
Flow rate No Low High
EF$_{SO2}$ (g.kg^{-1}dry fuel)
10
8
6
4
2
0
a a a
x y z
Dry Moist Wet Moist. cont.
Banksia
Flow rate No Low High
EF$_{SO2}$ (g.kg^{-1}dry fuel)
a a a
x y y
Dry Moist Wet Moist. cont.
Jarrah
Flow rate No Low High
EF$_{SO2}$ (g.kg^{-1}dry fuel)
a b b
x y z
Dry Moist Wet Moist. cont.

d)


Spinifex
Flow rate No Low High
EF$_{NO}$ (g.kg^{-1}dry fuel)
5.0
4.0
3.0
2.0
1.0
0.0
a a a
x x x
Dry Moist Wet Moist. cont.
Banksia
Flow rate No Low High
EF$_{NO}$ (g.kg^{-1}dry fuel)
a b a
x y y
Dry Moist Wet Moist. cont.
Jarrah
Flow rate No Low High
EF$_{NO}$ (g.kg^{-1}dry fuel)
a a a
x x x
Dry Moist Wet Moist. cont.

e)



f)

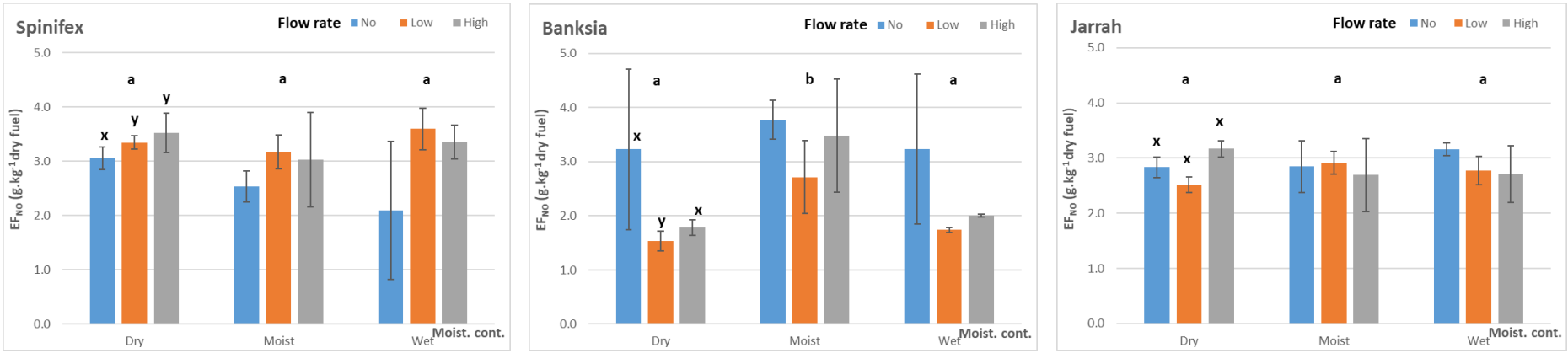


Figure 3-4. Variation of EFs for inorganic gases (a - CO_2, b - CO, c - SO_2, d - NO, e - NO_2 and f - NO_x) from burning vegetation in different moisture contents under different air flow rates (n=27). Letters a, b, c at the top of each bar group represent the significant difference between different fuel moisture contents. Letters x, y, z at the top of each bar represent the significant difference between different flow rates. Influences of moisture content and flow rate were assessed using PERMANOVA with $p<0.05$

Modified combustion efficiency (MCE)

The fire-integrated MCE (for the whole burn in 6 minutes sampling) from burning vegetation ranged from 0.75 to 0.97, depending on vegetation type and combustion conditions.

Of the different types of vegetation, Spinifex had the highest average MCE, suggesting that combustion was mainly in the flaming phase and this pattern was significantly different to the combustion of Banksia and Jarrah which consisted of two phases (Figure 3.5a). Amongst the grass types, Spinifex and Kimberley grass combustion had similar MCE values, whilst the Veldt grass had a significantly lower MCE compared with other kinds of grass, suggesting that this grass had less of the flaming and more of the smouldering phase (Figure 3.5b).

a) b)

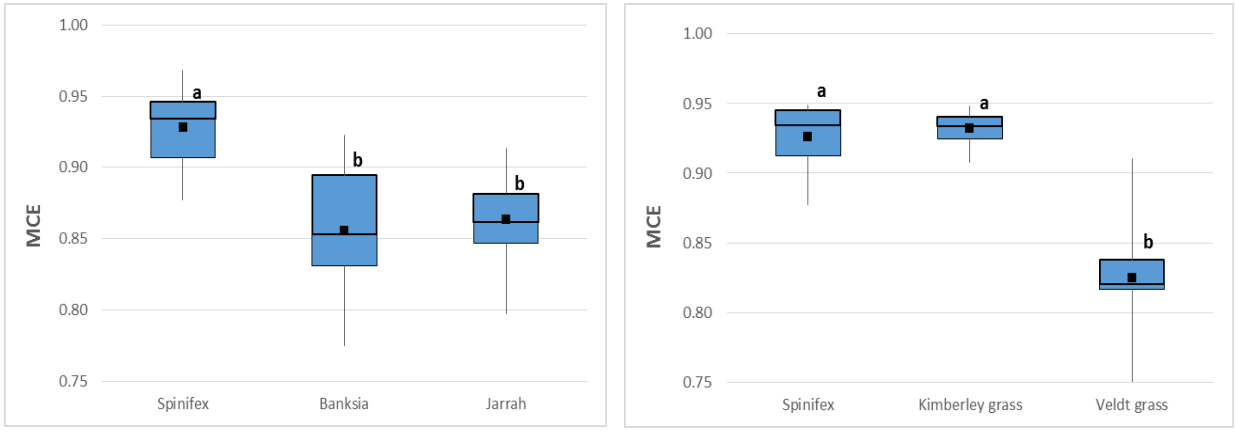
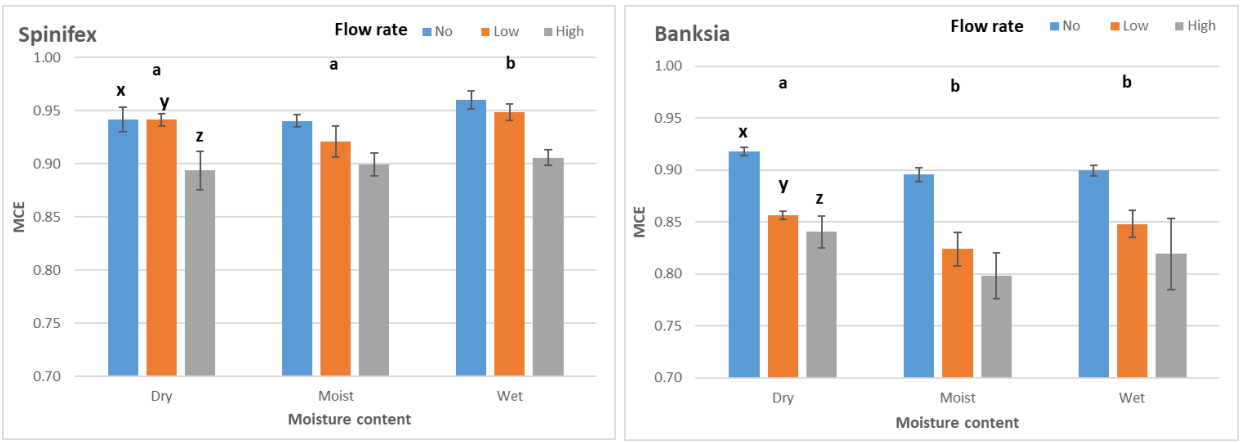


Figure 3-5. Box plots showing the MCE of vegetation burn. a) Different types of vegetation (n=27); b) Different kinds of grass (n=9). Black squares in the box plots show the mean values. Significant differences between kinds of grasses are presented by the letters at the top of the box plots. The differences were evaluated using one-way ANOVA test (for different types of vegetation) and Kruskal-Wallis test (for different kinds of grasses) with P<0.05

Fuel moisture content had impacts on the MCE from burning Spinifex and Banksia but no effect on MCE of Jarrah (Figure 3.6). Burns of wet Spinifex had significantly higher MCE than those of dry and moist grass, whilst burns of Banksia in moist and wet conditions had lower MCE than that of dry Banksia (Figure 3.6a, b). Compared with fuel moisture content, flow rate had a stronger influence on the MCE and increased flow rates significantly reduced the MCE of burns across all vegetation types (Figure 3.6). For Spinifex, MCE in the high flow rate significantly decreased compared with the no and low flow rate conditions, and there was little difference in MCE at the two lowest flow rates (Figure 3.6a). However, the effects of flow rate on MCE of Banksia and Jarrah combustion were more pronounced with a steeper decrease in MCE in the low and high flow rate conditions (Figure 3.6b, c).

a) b)



c)

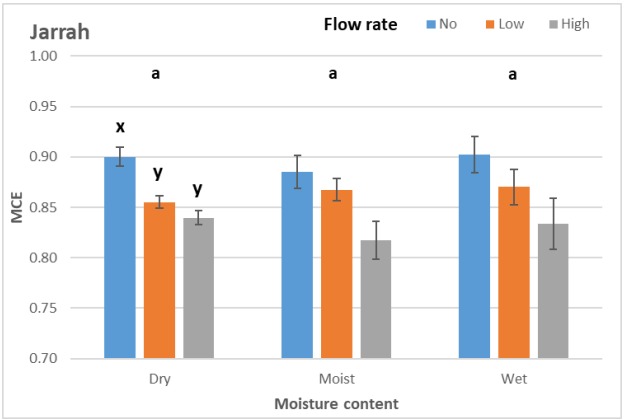


Figure 3-6. Variation of mean MCE of burns of vegetation (a - Spinifex, b - Banksia, c - Jarrah) in different moisture contents under different air flow rates (n=9). Letters a, b, c at the top of each bar group represent the significant difference between different fuel moisture contents. Letters x, y, z at the top of each bar represent the significant difference between different flow rates. Influences of moisture content and flow rate were assessed using PERMANOVA with p<0.05.

Relationship between MCE and emissions of inorganic gases

No relationship with MCE was observed for emission factors for NO, NO_2 and NO_x. A negative relationship between MCE and emissions factor for SO_2 was observed. Across all vegetation types a significant relationship was observed ($R^2 = 0.43$) between MCE and EF_{SO2} (Figure A1.2). Strong correlations were found between the number of moles of CO_2 and the number of moles of NO_x emitted from burns of individual vegetation types (except for Banksia - Figure A1.3).

Discussion

Influence of combustion conditions on EFs and MCE

Fuel moisture content influenced the combustion of different vegetation types in different ways. It was expected that the increase in vegetation moisture content would prolong the smouldering

phase, resulting in higher emissions of CO and a decrease in combustion efficiency (Chen et al., 2010; Tawfiq et al., 2015). However, this expected trend was only observed for burns of Banksia. Fires of Spinifex showed the reverse trend with a significantly higher MCE from burns of wet grass compared with those of drier grass. Spinifex is a highly flammable species since its leaves contain flammable resin (Central Land Council, 2013). It is possible that the higher amount of flammable resin in wet Spinifex compared with dry and moist vegetation might enhance the combustion efficiency since the resin might be lost during the drying process. There were no significant changes in MCE for burns of Jarrah with different moisture contents. A similar observation was reported by May (2017) in a study investigating the effects of moisture content on energy release and emissions of pyrophytic vegetation in a laboratory setting where a similar MCE was observed when burning dry (1–2%) and wet (10–15%) eucalyptus which is similar to a representative species of Jarrah in this study. This study and the study by May (2017) used similar methods to prepare vegetation in different moisture contents. The process used an oven to dry vegetation gradually until the desired levels were achieved, instead of drying vegetation to constant weight then adding water or allowing the dry sample to absorb water vapour until reaching the desired moisture contents (Chen et al., 2010; Smith et al., 2013). The slow drying method might better reflect the natural drying process of vegetation in the field and retain some combustible lipids in the vegetation, resulting in wet vegetation combusts as well as the dry samples (May, 2017).

On the other hand, the increase in air flow rate significantly decreased the EF_{CO2} and increased the EF_{CO}, resulting in a decrease of MCE. This trend was contrary to initial expectations that the higher flow rate would mean more oxygen supplied thereby enhancing combustion efficiency. The direction of air supply significantly influenced combustion efficiency in vegetation fires (Surawski et al., 2015). Surawski et al. (2015) conducted a laboratory-based study investigating combustion of wildland fuels in different fire spread modes (heading – fire propagates with the wind, backing – fire against the wind, and flanking – fire perpendicular to the wind). That study found that CO emission in heading fires was about 2-fold that of the other two remaining fire spread modes. MCE rapidly decreased and smouldering was the longest combustion phase in heading fires (Surawski et al., 2015). The design of this present study (ignition and air supply using the same door at the bottom of the chamber) might result in heading fires in which the intense force of the high air flow rate (in the context of the small amount of vegetation burned) quickly cooled down the fire and hence eliminated the flaming phase, causing the lower values of MCE and higher emission of CO. The average highest temperature recorded in the burning chamber decreased from around 200ºC

in no flow rate conditions, to 130ºC in low flow rate conditions to 70ºC in high flow rate conditions.

Relationship between MCE and EFs for inorganic gases

The inverse correlation between MCE and EF_{SO2} observed in this study was similar to the findings of some field- and laboratory-based studies (Burling et al., 2010; Sinha et al., 2003). Even though the emission of SO_2 has been found to be associated with the flaming phase (Lobert et al., 1991), Sinha et al. (2003) reported a reasonably strong negative relationship with R^2=0.56 in emissions from African savanna fires. Burling et al. (2010) also observed a negative correlation (R^2=0.55) between EF_{SO2} and MCE in laboratory measurements of wildland fuels. The natural variability of fires and differences in sulphur content in fuel might be responsible for these observations (Burling et al., 2010; Sinha et al., 2003).

Strong correlations between emission factors of CO_2 and NO_x in this study were supported by the finding of Lacaux et al. (1996) who also found a strong linear relationship between the concentrations of NO_x and CO_2 emitted from African savanna fires. CO_2 is the main product of vegetation burning and higher values of CO_2 are related to the combustion of more fuel. Since the emissions of NO_x are dependent on the chemical composition of the fuel it was not surprising to find a significant positive relationship between NO_x and CO_2 emissions. Similar results in this study confirm that the formation of NO_x in bushfires is highly correlated with CO_2 emissions and thus mainly occurs in the flaming phase (Lacaux et al., 1996).

Emission factors of different vegetation types in a global context

A comparison of EFs for inorganic gases observed in this study and other studies is presented in Table A1.3. There was only one laboratory-based data set for tropical grass fires, that of Chen et al. (2007), available for comparing with the data collected in this study. Although the EF_{CO2} was similar, the EFs for CO from tropical grasses in this study were 3-fold higher than the value reported for Dambo grass in the US. This result lead to a higher MCE in the study by Chen et al. (2007) compared with the present study (0.98 vs. 0.95). Comparisons with field-based data from Australian savanna show that the EFs for CO_2 and CO found in this study were slightly lower than those reported by Shirai et al. (2003) and Smith et al. (2014) but in the range reported by Desservettaz et al. (2017). The EFs for SO_2 for all kinds of grass were lower than the reported values for savanna with factors of more than two in other vegetation types (Akagi et al., 2011; Sinha et al., 2003). The EFs for nitrogen oxides from burning grasses in this study were also different to those reported in other studies (Table A1.3). From the results of this work, it can be

seen that there were significant differences in MCE, EFs for CO_2 and CO between temperate grass (Veldt) and tropical grass (Spinifex and Kimberley). Explanations for these differences could be many and varied but the most likely is that temperate and tropical grasses may have different chemical compositions, in addition to differences in growing season which could influence the carbon content and combustibility of the different species.

When EFs reported for Banksia are compared with laboratory-based data produced by Burling et al. (2010) and McMeeking et al. (2009) for coastal plain fuels and chaparral in the US, it appears that the MCE, EF_{CO2} and EF_{CO} values of Banksia are very similar to values reported for chaparral, however, EFs for NO_x and SO_2 of Banksia were 2 to 3 times higher than those of chaparral, respectively (Table A1.3).

For forest vegetation, the EF for CO of Jarrah was higher than laboratory measurements reported for Amazon forest species and boreal forest (McMeeking et al., 2009; Soares Neto et al., 2011). This might be due to the significant difference in fuel compostion between vegetation types. MCE, EFs for CO_2 and CO recorded in this study were similar to the values obtained from field measurements for other Australian temperate forests (Guérette et al., 2018; Paton-Walsh et al., 2014) and US temperate forest (Liu et al., 2017) while EFs for SO_2 and NO_x from Jarrah were 4 to 6 times higher than those reported for US temperate forest (Liu et al., 2017) (Table A1.3).

Despite some MCE values being similar to other vegetation types in other parts of the word, emission profiles of inorganic gases of Australian vegetation types were different for substances where emissions are highly dependent on fuel composition such as SO_2 and NO_x (Burling et al., 2010). Therefore the use of EFs from fires in one vegetation type and region may not be suitable for predicting emissions in other parts of the country. It is suggested that a more accurate understanding of bushfire emissions in Australia can only be achieved by sampling a greater range of vegetation types across different parts of the country.

Conclusion

This study has produced a data set on emission factors for typical vegetation types in Western Australia based on controlled experimental burns of representative vegetation. The agreement in MCE, EF_{CO2} and EF_{CO} between this study and other field-based studies on emissions from fires of similar ecosystems in Australia suggests that the EFs can be applied for predictive modelling purposes.

This study found that fuel moisture content and air flow rate had significant influences on modified combustion efficiency and the emission factors for CO_2 and CO. EFs for other gases which are

highly dependent on the relative fuel chemical content, including SO_2, NO and NO_2, were less influenced by the combustion conditions. However due to significant differences between laboratory and field conditions (e.g. amount of fuel burned, fuel composition), further studies focusing on generating emissions factors for these gases from field-based measurements are recommended in order to benchmark the laboratory-based data obtained in this study.

Chapter 4. EMISSIONS OF SEMI-VOLATILE AND VOLATILE ORGANIC COMPOUNDS

4.1. Introduction

Bushfire smoke contains numerous trace volatile and semi-volatile compounds which could have adverse impacts on human health depending on the degree of exposure and concentration of smoke. These include carbonyls (aldehydes and ketones) and volatile organic compounds (VOCs).

Formaldehyde is categorised as a carcinogenic substance (Group I) to humans by the International Agency for Research on Cancer (IARC, 2012) and also causes irritation to eyes and upper respiratory tract, asthma and eczema (WHO, 2010). Exposure to high concentrations of formaldehyde has been shown to cause nasal tumours in experimental rats (Leikauf and Katz, 2005). In a study of firefighters' exposure during prescribed burns in Australia, Reisen et al. (2006) reported that 28% of monitored firefighters were exposed to concentrations of formaldehyde higher than the proposed occupational exposure standard (0.3 $g.m^{-3}$). Formaldehyde emitted from bushfires also has a significant influence on the OH^- balance and ozone production in the atmosphere due to the process of photolysis (Radojevic, 2003; Sinha et al., 2003). Acetaldehyde has been found to cause cancer in rats following inhalation exposure and is categorised as a possible human carcinogen due to its ability to interfere with DNA synthesis and repair (IARC, 2010). Other aldehydes are not considered to be carcinogenic but nonetheless have adverse impacts on human health if exposure occurs at high concentrations. For example, acrolein can irritate the mucous membranes, the airways and the skin (Faroon et al., 2008).

VOCs emitted from vegetation fires can also react with nitrogen oxides in the atmosphere to form tropospheric ozone, an important greenhouse gas and precursor of smog (Shirai et al., 2003; USEPA, 1999a). Benzene, a major VOC detected in bushfire smoke (Ferek et al., 1998; Reisen et al., 2006a), is a genotoxic compound and has been associated with increased risk of acute myeloid leukaemia and cancer in children (Li et al., 2015; WHO, 2010).

In recent years, several studies have been conducted which have reported emission factors for some aldehydes and VOCs in bushfire smoke in Australia (Paton-Walsh et al., 2014, 2010; Possell and Bell, 2013b; Shirai et al., 2003; Smith et al., 2014). Shirai et al. (2003) measured and reported the mixing ratios and emission factors for some VOCs from northern Australian savanna fires using aircraft measurements. Studies by Paton-Walsh et al. (2010, 2014) and Smith et al. (2014) produced emission factors for formaldehyde from ground-based measurements at fires in field

settings. Possell and Bell (2013) reported emission factors of some VOCs from experimental burns of eucalyptus foliage. The main focus of these studies was the measurement of emissions from savanna fires in the northern part and savanna/forest fires in the south-eastern regions of Australia. Reisen and Brown (2009) and De Vos et al. (2006) measured the exposure of WA firefighters to formaldehydes and VOCs during bushfire fighting, however these studies did not calculate the emission factors for these substances.

Few studies have examined emissions of semi-volatile and volatile compounds from burns of different vegetation types under varying combustion conditions which may affect the emission characteristics (Ciccioli et al., 2001; Radojevic, 2003). In this context, this chapter investigates the emission profile of carbonyl and VOC compounds associated with different WA vegetation types under different combustion conditions.

4.2. Methodology and study design

4.2.1. Experimental set-up, preparation of vegetation samples and combustion experiments

The experimental set-up and design of the combustion experiments were described in Chapter 3. Different carbonyl compounds and VOCs were collected or measured simultaneously through several ports at the sampling area during the combustion process.

For carbonyl (and particulate matters – chapter 5) sampling, fifty grams (50 g) of vegetation was burnt each time in triplicate for each vegetation type under each combustion condition. Air samples from blank burns without vegetation were also collected to examine the potential accumulation of contaminants in the duct system. Blank burns were conducted after every nine vegetation burns.

4.2.2. Carbonyl sample collection and analysis

a) Collection

Carbonyls in smoke were collected in sorbent tubes containing silica gel pre-coated by 2,4-Dinitrophenylhydrazine (DNPH) (SKC Cat. No. 226-119) using an active sampling pump (SKC Aircheck®52) set at flow rate of maximum 1.0 $L.min^{-1}$ for a period of 6 minutes throughout the whole burning process, following the instructions of the manufacturer of sorbent tubes (SKC, 2004). The sorbent tubes were cooled to ambient temperature and the two ends were broken immediately prior to sample collection. In order to avoid the effects of sunlight on the collected carbonyls, the sorbent tubes were wrapped in aluminium foil following the manufacturer's recommendation during sampling and transport processes (SKC, 2004). After sampling, the

sorbent tubes were transported to the laboratory in a portable cooling box and then stored at <4°C until being desorbed (within 2 weeks after sampling), prior to chemical analysis.

The flow rates of the pump before and after sampling were measured using an SKC pump calibrator. The flow rate used for calculating the concentration of carbonyls was the average value of pre- and post-sampling flow rates. The difference in the flow rates before and after sampling for all sample collection was less than 10%, which is within the recommended value in the standard method TO-11A (USEPA, 1999b).

The concentrations of carbonyl compounds in blank burns were less than 3.0% of the concentrations recorded in the burnt vegetation samples (Table A2.1, Appendix 2). Emissions from blank burns were subtracted from samples to correct for the possibility of pollutants remaining in the combustion apparatus.

b) Carbonyls analysis

Carbonyl derivatives desorption and analysis

Carbonyl compounds in smoke were adsorbed by 2,4-DNPH in the sorbent tube to form DNPH-carbonyl derivatives. These derivatives were then de-adsorbed in the laboratory and measured to quantify the amount of carbonyls adsorbed. A pilot test was conducted to check whether breakthrough occurred and it found that there were no breakthroughs for most compounds when burning 50 g of vegetation and collecting samples in 6 minutes, with the exception of acrolein (Table A2.2). Sampling acrolein using DNPH tubes is not recommended by the tube manufacturer due to the instability of DNPH-acrolein derivative (SKC Operating Instructions – Sorbent Sample Tubes). Therefore, data on acrolein was not considered in the analysis and will not be discussed in this chapter.

Carbonyl samples were desorbed following the protocol of TO-11A (USEPA, 1999b) with some modifications for the use of sorbent tube. Following sampling, the sorbent tubes were broken and the silica gels contained in the tubes were transferred into 15 mL glass vials which had been cleaned thoroughly and rinsed with acetonitrile (Fisher Chemical, HPLC grade). Five (5) mL of acetonitrile was added to the vials to desorb the DNPH-carbonyl derivatives. The purity of acetonitrile was tested for carbonyl contamination as instructed in TO-11A (USEPA, 1999b). An average amount of 1.47 $ng.L^{-1}$ of formaldehyde was found in the reagent, which is within the limit recommended by TO-11A. No other carbonyl contaminants were detected (Table A2.3).

The vials were stored in a refrigerator at 4ºC before being analysed using high performance liquid chromatography-ultraviolet detector (HPLC-UV) to quantify derivatives of DNPH and seven carbonyl compounds including formaldehyde (CH_2O), acetaldehyde (CH_3CHO), acrolein

(CH_2=CHCHO), acetone (CH_3COCH_3), propionaldehyde (CH_3CH_2CHO), benzaldehyde (C_6H_5CHO) and butyraldehyde ($CH_3CH_2CH_2CHO$). Before the HPLC analysis, solvent in vials was divided into 2 aliquots of 2 mL and transferred into 2 mL vials; one was used in HPLC analysis and one was stored at 4ºC for confirmatory analysis if necessary (USEPA, 1999b).

A mixed standard of carbonyl-DNPH derivatives was purchased from Sigma-Aldrich (Cat. No. 47649-U). A Phenomenex Luna Omega 1.6um C18 100A (100 x 2.1 mm) column was used and the UV detector was operated at 360 nm. The results obtained from HPLC-UV were concentrations of derivatives of DNPH and carbonyl compounds. Therefore, in order to calculate the concentrations of carbonyls in solution, the ratio of molecular weights between carbonyl compounds and their relative DNPH derivatives was taken into account using the following formula (USEPA, 1999b):

$$C_{al} = C_{de} \times \frac{MW_{al}}{MW_{de}}$$

Where:C_{al}: Concentration of carbonyl compound (mg.L^{-1}); C_{de}: Concentration of relative DNPH derivative (mg.L^{-1}); MW_{al}: Molecular weight of carbonyl; MW_{de}: Molecular weight of relative DNPH derivative.

Quality assurance/Quality control (QA/QC)

Calibration standard curves for all compounds had strong linear responses with the R^2 in the range of 0.998–0.999. Duplicate measurements were conducted for every 10th sample and the coefficients of variation were in the range of 0.43–4.43%. Limits of detection (LoD) were calculated as 3 times of the standard deviation of blank sampling tubes. In cases when the standard deviations could not be identified (e.g. concentration was lower than the method detection limit), method detection limits were used (Table A2.3).

4.2.3. Calculation of modified combustion efficiency (MCE) and emission factors (EFs) for carbonyls

To examine the effect of combustion efficiency on the carbonyl emissions, relationships between modified combustion efficiency (MCE) and EFs for carbonyls were investigated. The MCE was calculated using the ratio of excess carbon content emitted in the forms of carbon dioxide (CO_2) and carbon monoxide (CO) (Chapter 3).

EFs for carbonyls in each burn were calculated using the carbon mass balance method using the following formula (Desservettaz et al., 2017):

$$EF_i = \frac{m_i}{m_{burned}} = \frac{m_i}{m_C} F_C$$

Where EF_i: Emission factor for compound i (g.kg^{-1} dry fuel); m_i: amount of i emitted, calculated using formula: $m_i = C_i \times v \times A \times t$ (Wardoyo et al., 2006), where C: Average concentration of compound i in that burn (mg.m^{-3}); v: Average velocity of smoke at sampling area during sampling time (m.s^{-1}) (see Chapter 5, section 5.2.5); A: Area of the duct at sampling points (A = 0.018 m^2); t: Sampling time (s), t = 360 s. m_{burned}: Amount of dry vegetation (0% of moisture content) burned (g); m_C: amount of C emitted (g), calculated as the sum of carbon mass emitted in CO_2, CO since these gases account for more than 95% of carbon amount emitted from biomass burning (Meyer et al., 2012). F_C: the carbon content of fuel. We used the values reported for Australian grass (0.45) and leaves and twigs (0.48) by Hurst et al. (1994).

The emissions (concentration and EF) of total carbonyls were calculated as the sum of emissions of six individual compounds.

4.2.4. VOCs measurement

A portable Gas Chromatograph/Mass Spectrometry (GC/MS) instrumentation HAPSITE ER (Inficon, USA) was used to identify VOCs in vegetation smoke. The sampling probe of the HAPSITE was located at the end of the pipe system, facing directly into the flow of smoke. The instrument was set up to draw in the smoke which was then transferred using nitrogen as a carrier gas, through a narrow-bore fuse silica column 15 metres long to the detector. A thin layer of material known as stationary phase which can selectively attract VOCs coats the inside of this column. When smoke passes through the column, the chemicals in the stationary phase react with different VOCs in the smoke at different rates and the VOCs, which have the fastest reaction rate, will exit the column first. Following this principle, VOCs are separated from one another, and then are identified by the Mass Spectrometer (Inficon, 2008). A mixture of internal standards is added to the sample inlet flow to verify the performance and sensitivity of the instrument. VOCs in smoke were identified using the chemical inventories AMDIS and NIST associated with the HAPSITE software (Inficon, 2008). The concentrations of some VOCs such as benzene, toluene, ethyl benzene and xylene can be calculated and quantified by the associated software based on the areas of these chemicals' peaks in the mass spectra, however, in this study concentrations of VOCs in the vegetation fire smoke were too low, thus precluding quantification (De Vos et al., 2009). In order to compare the emission of VOCs produced during the combustion of different vegetation types, the areas of the peaks appearing in the mass spectra were utilised instead of concentrations.

Owing to an equipment malfunction during the burns of Kimberley grass, no VOC data for this type of vegetation were collected.

4.2.5. Statistical analysis

Values that were lower than the detection limits were replaced by half the detection limit for statistical analysis (USEPA, 2000). The influence of vegetation type on the carbonyls emitted was evaluated using one-way analysis of variance (ANOVA) analysis with a post-hoc Tukey test for normally distributed data and using Kruskal-Wallis test with Dunn's post-hoc test for data without a normal distribution (SPSS ver.25, IBM). The effects of moisture content and flow rate on carbonyl emissions were investigated using permutational multivariate ANOVA (PERMANOVA) (PRIMER 6^+, PRIMER-E). The correlations between MCE and EFs for carbonyls were examined using regression analysis (SPSS ver.25, IBM). The significance value of $p<0.05$ was utilised for statistical interpretation.

4.3. Results

4.3.1. Emission of carbonyl compounds

a) Composition of carbonyl emission in smoke from burning vegetation

Distributions of emission (in both concentration and EF) of individual carbonyl compounds (presented as % of emission of total carbonyls) are shown in Figure 4.1 and Table A2.4. For all vegetation types, formaldehyde and acetaldehyde were the most abundant compounds emitted from vegetation fires, accounting for 71 to 96% of emissions of total carbonyls investigated (Table A2.4). Acetone was the third most abundant compound for all vegetation types, with the exception of Kimberley grass. Carbonyl emissions from Kimberley grass had a significant proportion of butyraldehyde (16%), the third most abundant carbonyl from burns of this vegetation type (Figure 4.1).

EFs for most of the carbonyls (except for butyraldehyde) emitted from vegetation fires were significantly correlated with one another (Table A2.5). Pairs of compounds with the strongest correlations were formaldehyde – acetaldehyde, formaldehyde – propionaldehyde, acetaldehyde – propionaldehyde and acetone – propionaldehyde with R^2 coefficients of 0.93, 0.89, 0.96, and 0.81, respectively.

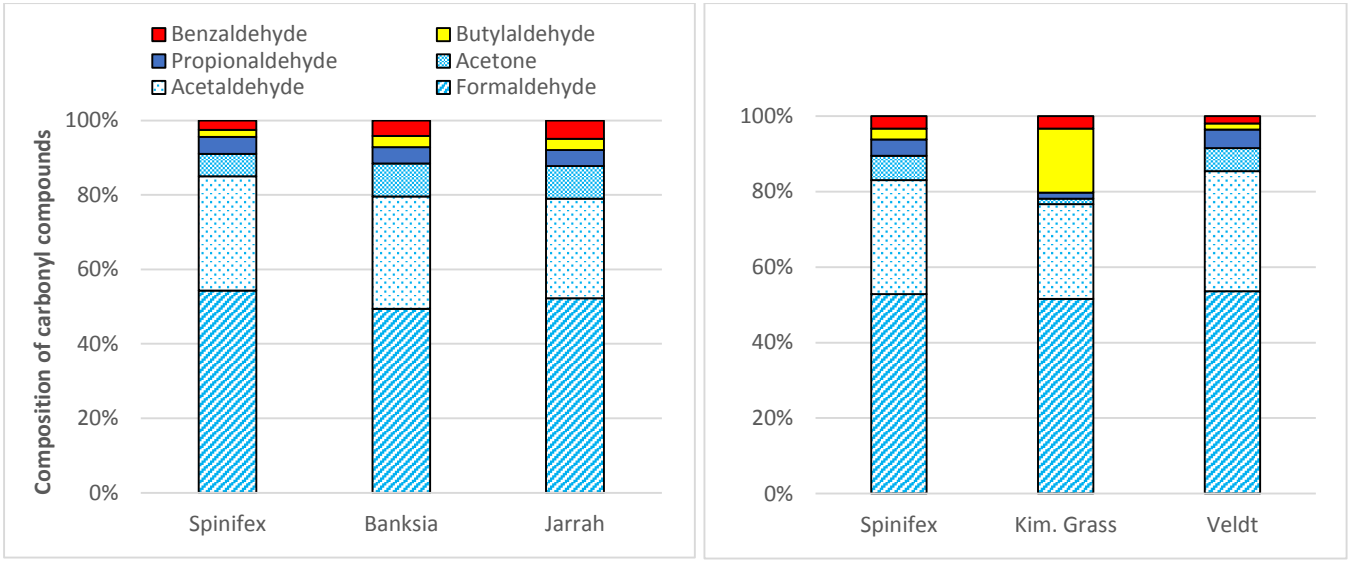


Figure 4-1. Bar graphs showing the proportions of emission of individual carbonyls in emission of total carbonyls from burning three vegetation types across all combustion conditions (n=27, left); and three types of dry grasses in three levels of flow rates (n=9, right)

b) Emission factors for aldehydes from different vegetation types

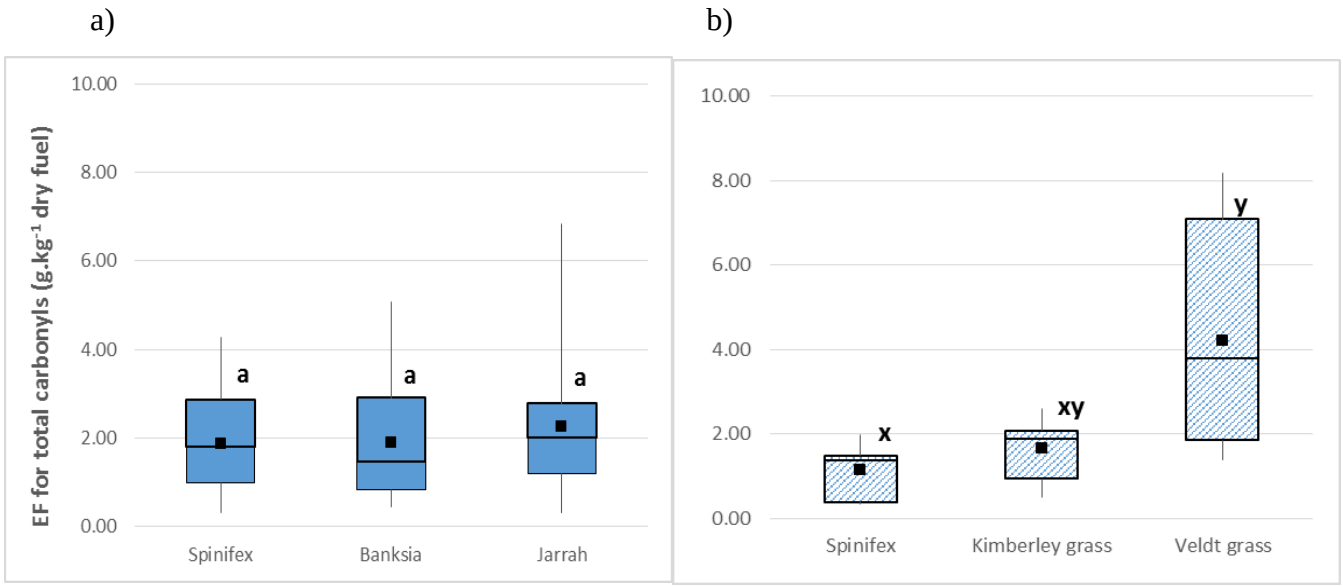


Figure 4-2. Box plots showing the emission factors for total carbonyls emitted from burning a) different types of vegetation (n=27); and b) different types of grassland (n=9) across all combustion conditions. Mean values are presented as the black squares in the box plots. Statistical difference are presented using letters a, b (between vegetation types) and x, y (between grasslands). The differences were investigated using Kruskal-Wallis tests (for different vegetation types) and one-way ANOVA (for different grasslands) with significance value p<0.05

Among the three vegetation types, Jarrah burns emitted the highest amount of carbonyls per unit of fuel burned (2.26 $g.kg^{-1}$ (dry fuel)), followed by Banksia (1.91 $g.kg^{-1}$) and then Spinifex (1.86 $g.kg^{-1}$). The total carbonyl emission was not significantly different between grassland, woodland and forest (Figure 4.2) and was similar for most individual compounds (Figure 4.3). There were significant differences ($p<0.05$) in EFs for the remaining two compounds (butyraldehyde and

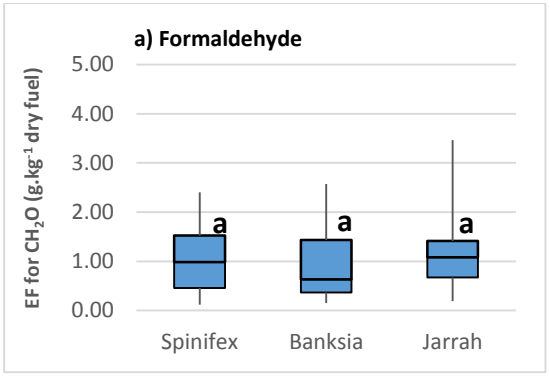
benzaldehyde) between grassland and woodland/forest. Banksia and Jarrah had similar EFs for all individual compounds (Figure 4.3).

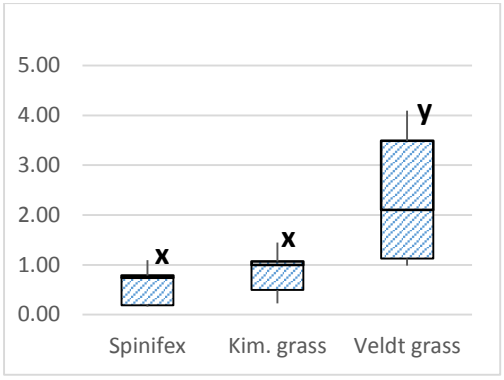
In contrast, the EFs were very different between tropical and temperate grasslands. Aldehyde emission from the Veldt grass (4.20 $g.kg^{-1}$) was significantly higher than that from other types of grassland (1.14 and 1.67 $g.kg^{-1}$ for Spinifex and Kimberley grasses, respectively) (Figure 4.2). For individual compounds, Veldt grass also had the highest EFs for most compounds except for butyraldehyde where the highest emissions were from Kimberley grass burns (Figure 4.3). Emissions from burns of Spinifex and Kimberley grass were also similar for most compounds with the exception of butyraldehyde. EFs for the two most abundant compounds, formaldehyde and acetaldehyde, from the burns of the two tropical grasslands were significantly different from those of temperate grassland (Figure 4.3).

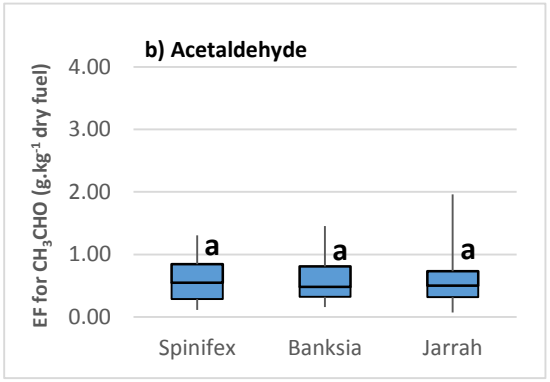
c) Effects of combustion conditions on emissions

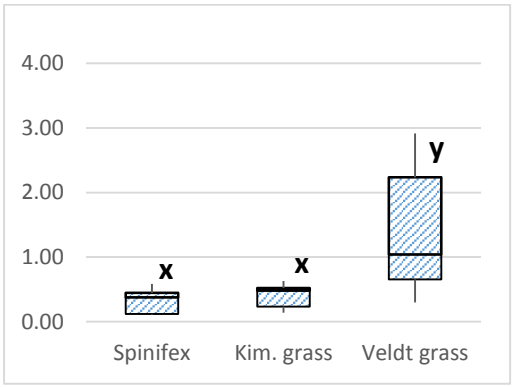
Variation of EFs for total and individual carbonyl compounds from burns of vegetation in different moisture contents under different air flow rates are presented in Figure 4.4. Burning Spinifex with higher moisture content appeared to generate higher emissions of carbonyls. A similar trend was also observed in Jarrah burns for most compounds, except for acetone and benzaldehyde (Figure 4.4d, g). Wetter Banksia emitted higher amounts of carbonyls compared with dry Banksia, however the highest EFs for formaldehyde, acetaldehyde, butyraldehyde and benzaldehyde from Banksia burns were observed at the intermediate moisture content. Among the six compounds, acetone was the least affected by fuel moisture content (Figure 4.4d).

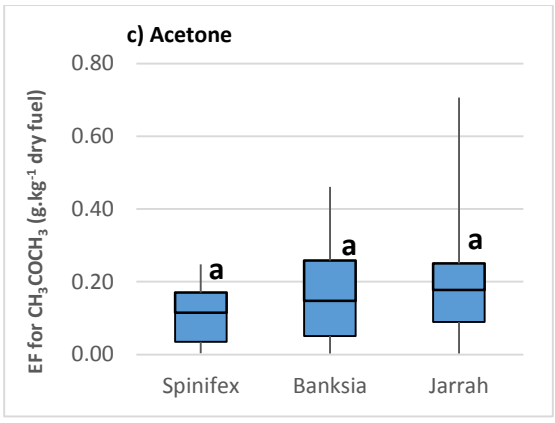
Flow rate had an influence on emissions of both total and individual carbonyls across all vegetation types (Figure 4.4). Higher EFs for most individual carbonyls were found when burning vegetation at higher flow rates, with the exception of benzaldehyde from Jarrah burns (Figure 4.4g). Between vegetation types, EFs for carbonyls from Banksia burns seemed to be more strongly affected by flow rate with significant increases observed with increasing flow rate. Meanwhile, EFs from Spinifex and Jarrah burns were only significantly different between no flow and low/high flow conditions. Burning these two types of vegetation at low and high flow rates generated similar EFs for carbonyls (Figure 4.4).

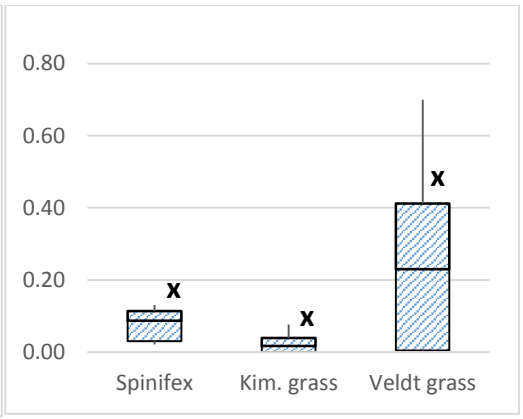

a) Formaldehyde
EF for CH_2O ($g.kg^{-1}$ dry fuel)
5.00
4.00
3.00
2.00
1.00
0.00
a
a
a
Spinifex
Banksia
Jarrah

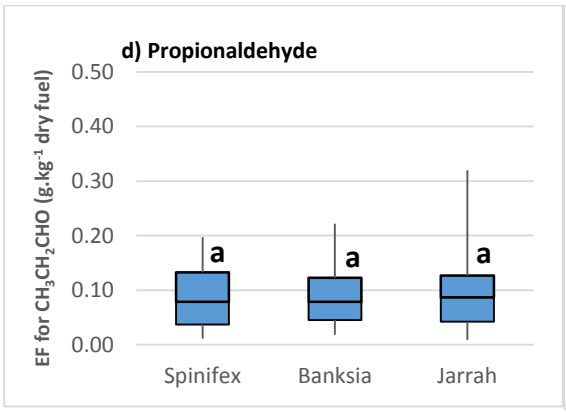

5.00
4.00
3.00
2.00
1.00
0.00
x
x
y
Spinifex
Kim. grass
Veldt grass

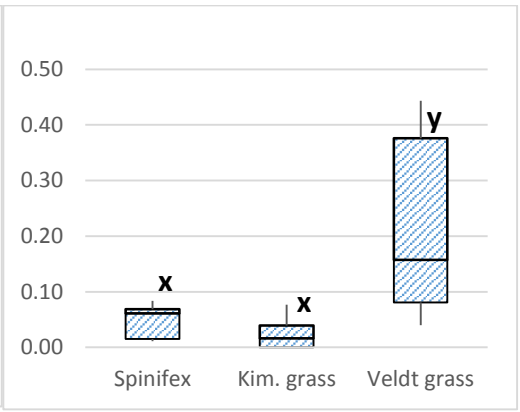

b) Acetaldehyde
EF for CH_3CHO ($g.kg^{-1}$ dry fuel)
4.00
3.00
2.00
1.00
0.00
a
a
a
Spinifex
Banksia
Jarrah


4.00
3.00
2.00
1.00
0.00
x
x
y
Spinifex
Kim. grass
Veldt grass


c) Acetone
EF for CH_3COCH_3 ($g.kg^{-1}$ dry fuel)
0.80
0.60
0.40
0.20
0.00
a
a
a
Spinifex
Banksia
Jarrah


0.80
0.60
0.40
0.20
0.00
x
x
x
Spinifex
Kim. grass
Veldt grass


d) Propionaldehyde
EF for CH_3CH_2CHO ($g.kg^{-1}$ dry fuel)
0.50
0.40
0.30
0.20
0.10
0.00
a
a
a
Spinifex
Banksia
Jarrah


0.50
0.40
0.30
0.20
0.10
0.00
x
x
y
Spinifex
Kim. grass
Veldt grass

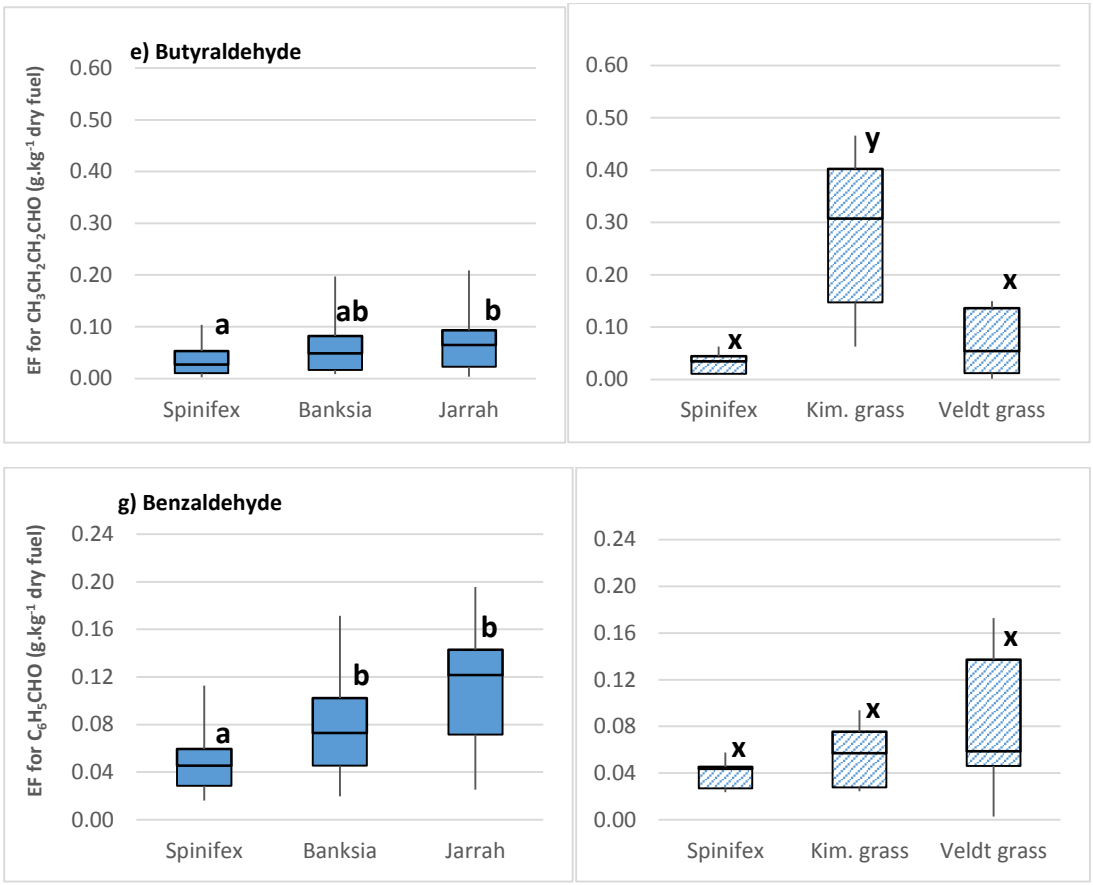
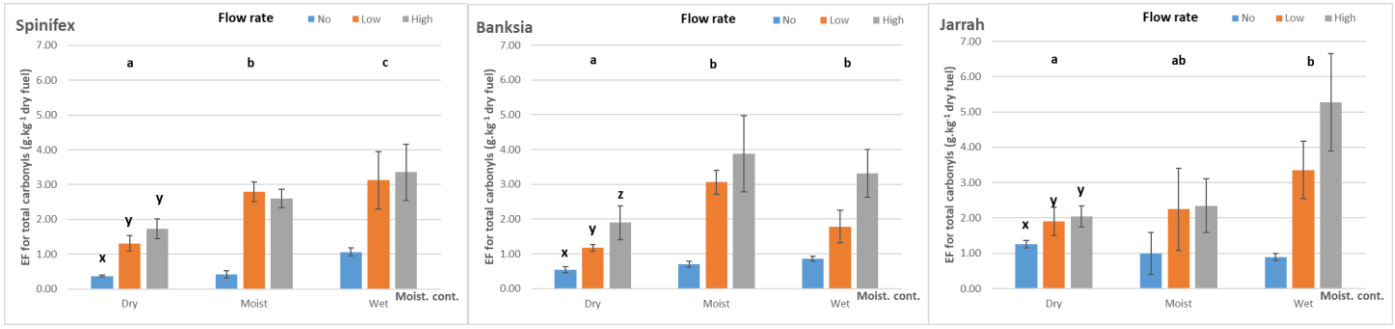
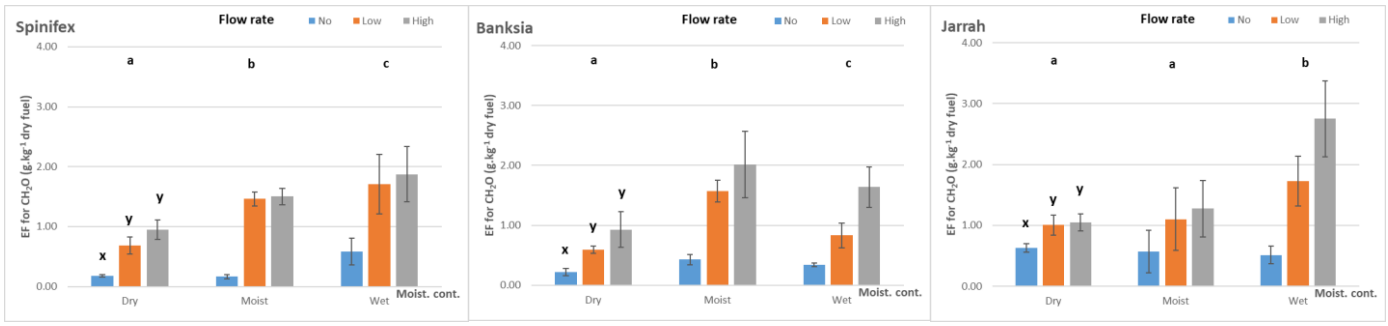


Figure 4-3. Box plots showing the emission factors for individual carbonyl compounds (a - formaldehyde, b - acetaldehyde, c - acetone, d - propionaldehyde, e - buryladehyde, f - benzaldehyde) emitted from burning different types of vegetation (solid plots, n=27); and different types of grassland (crossed plots, n=9) across all combustion conditions. Statistical differences are presented using letters a, b (between vegetation types) and x, y (between grasslands). The differences were investigated using Kruskal-Wallis tests (for different vegetation types) and one-way ANOVA (for different grasslands) with significance value $p<0.05$

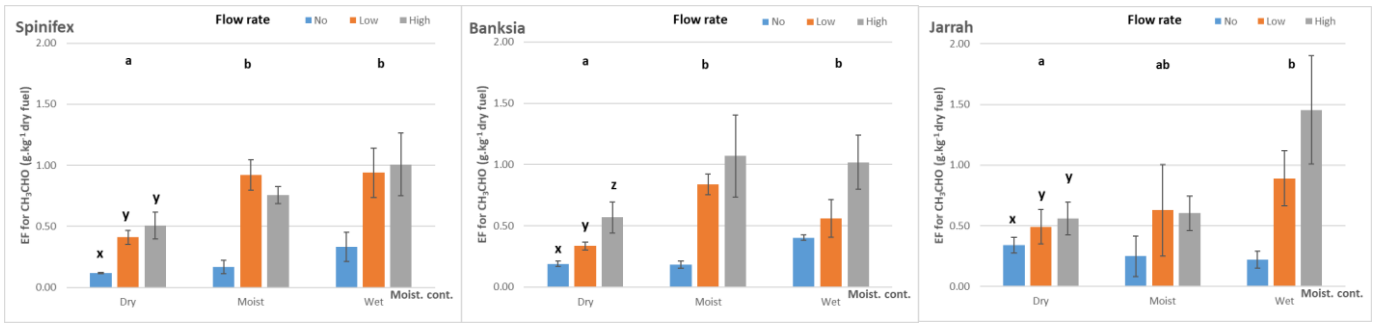
a) EF for total carbonyls



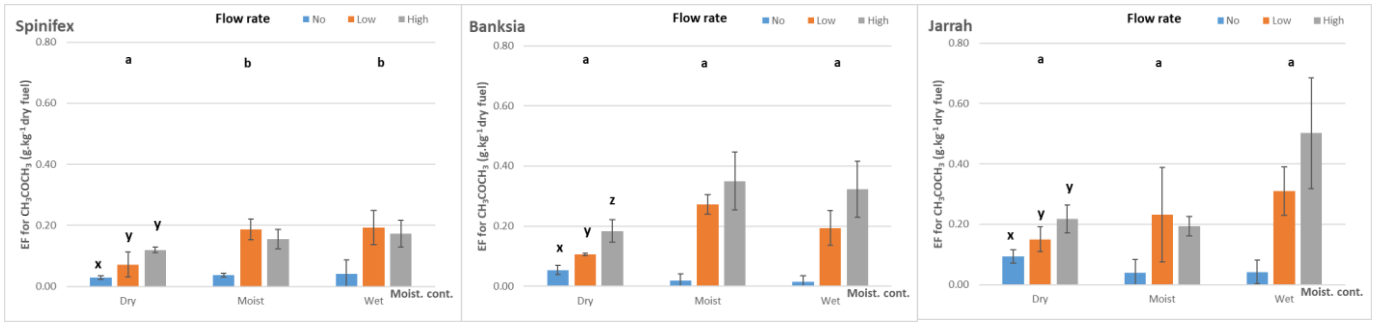
b) EF for formaldehyde



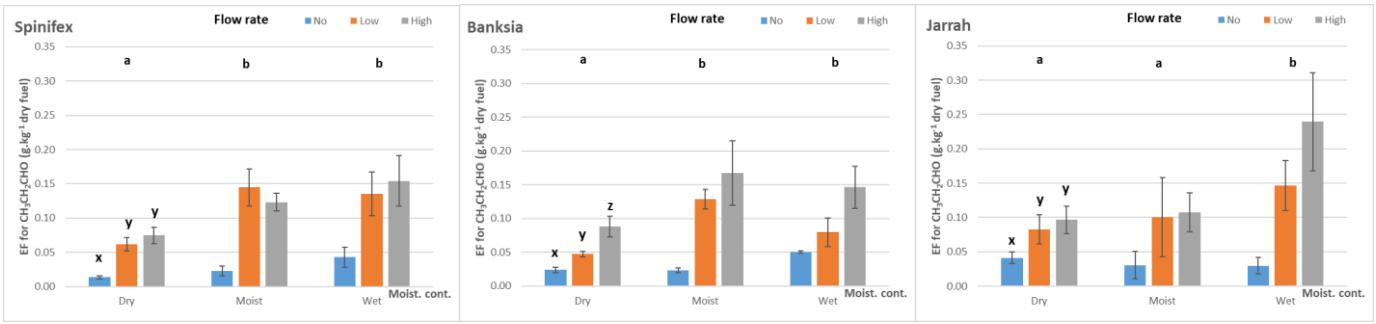
c) EF for acetaldehyde



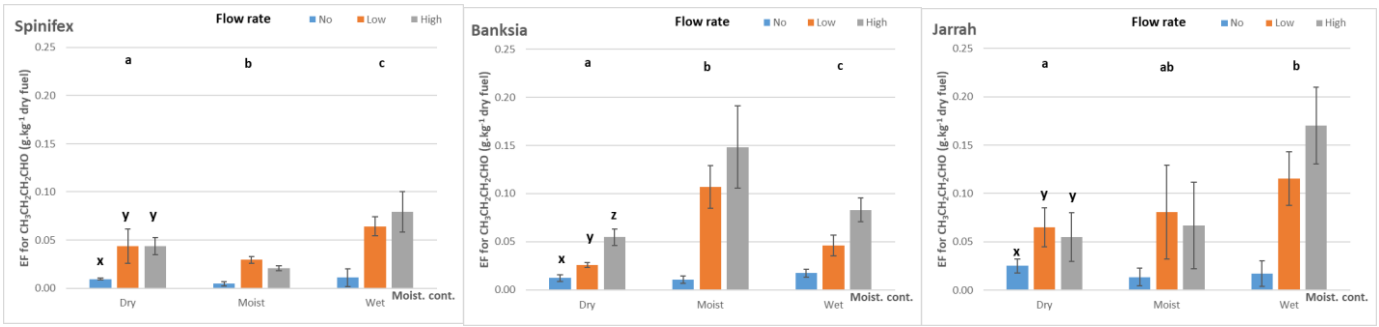
d) EF for acetone



e) EF for propionaldehyde



f) EF for butyltaldehyde



g) EF for benzaldehyde

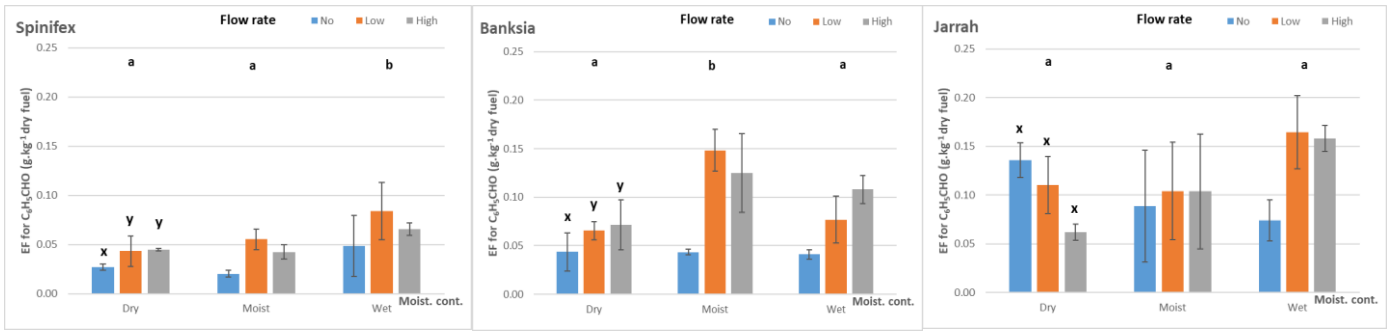


Figure 4-4. Variation of EFs for total and individual carbonyls from burning vegetation in different moisture contents under different air flow rates (n=27). Letters a, b, c at the top of each bar group represent the significant difference between different fuel moisture contents. Letters x, y, z at the top of each bar represent the significant difference between different flow rates. Influences of moisture content and flow rate were assessed using PERMANOVA with p<0.05

d) Correlations between EFs and MCE

The EFs for most carbonyls, with the exception of butyraldehyde, were significantly inversely correlated with the MCE across all vegetation types (Figure A2.1). Acetone emissions had the strongest correlation with MCE (R^2=0.51), followed by propionaldehyde and benzaldehyde (R^2=0.38). A weak non-significant correlation between butyraldehyde and MCE was observed which was caused by the extreme emission of this compound from Kimberley grass compared with other types of vegetation (Figure 4.3). If values from burns of Kimberley grass are excluded, a significant inverse correlation between butyraldehyde and MCE was also observed for burns of the remaining four vegetation types (R^2=0.43) (Figure A2.1).

The R^2 values of negative correlations between the EFs for formaldehyde, acetaldehyde, acetone, propionaldehyde, benzaldehyde and MCE of Banksia burns were higher than 0.8, indicating a very strong influence of combustion efficiency on the emissions of these semi-volatile gases (Table A2.6). Aldehyde emissions from Kimberley grass and Veldt grass combustion also had strong relationships with MCE with the R^2 varying from 0.70 to 0.88. Burns of Spinifex and Jarrah showed much lower correlations between the amount of carbonyls emitted and combustion efficiency (Table A2.6).

4.3.2. Emissions of VOCs

A range of VOCs were detected in the vegetation smoke with 15 compounds identified, including benzene, toluene, ethylbenzene, xylene, styrene, furfural, benzaldehyde, benzonitrile, isopropylbenzene, phenol, benzofuran, m-cymene, indene, p-cymene and naphthalene. Benzene, toluene, styrene and indene were the VOCs most frequently detected in emissions of the vegetation fires. Jarrah yielded the largest number of identifiable VOCs compared with other types of vegetation and generated some VOCs that were not detected in the burns of other vegetation types, such as isopropylbenzene, m-cymene and p-cymene (Table A2.7).

The emissions (in terms of area of peak) of benzene, toluene, ethylbenzene and xylenes (BTEX) which are common VOCs measured in emissions from biomass burning from combustion of different vegetation types are compared in Figure 4.5. Veldt grass and Jarrah emitted larger amounts of BTEX compared with Banksia and Spinifex. Veldt grass combustion generated the highest average emissions of benzene, toluene and ethylbenzene, followed by the Jarrah burning. Conversely, Jarrah yielded the highest average peak area of xylene, followed by the Veldt grass (Figure 4.5).

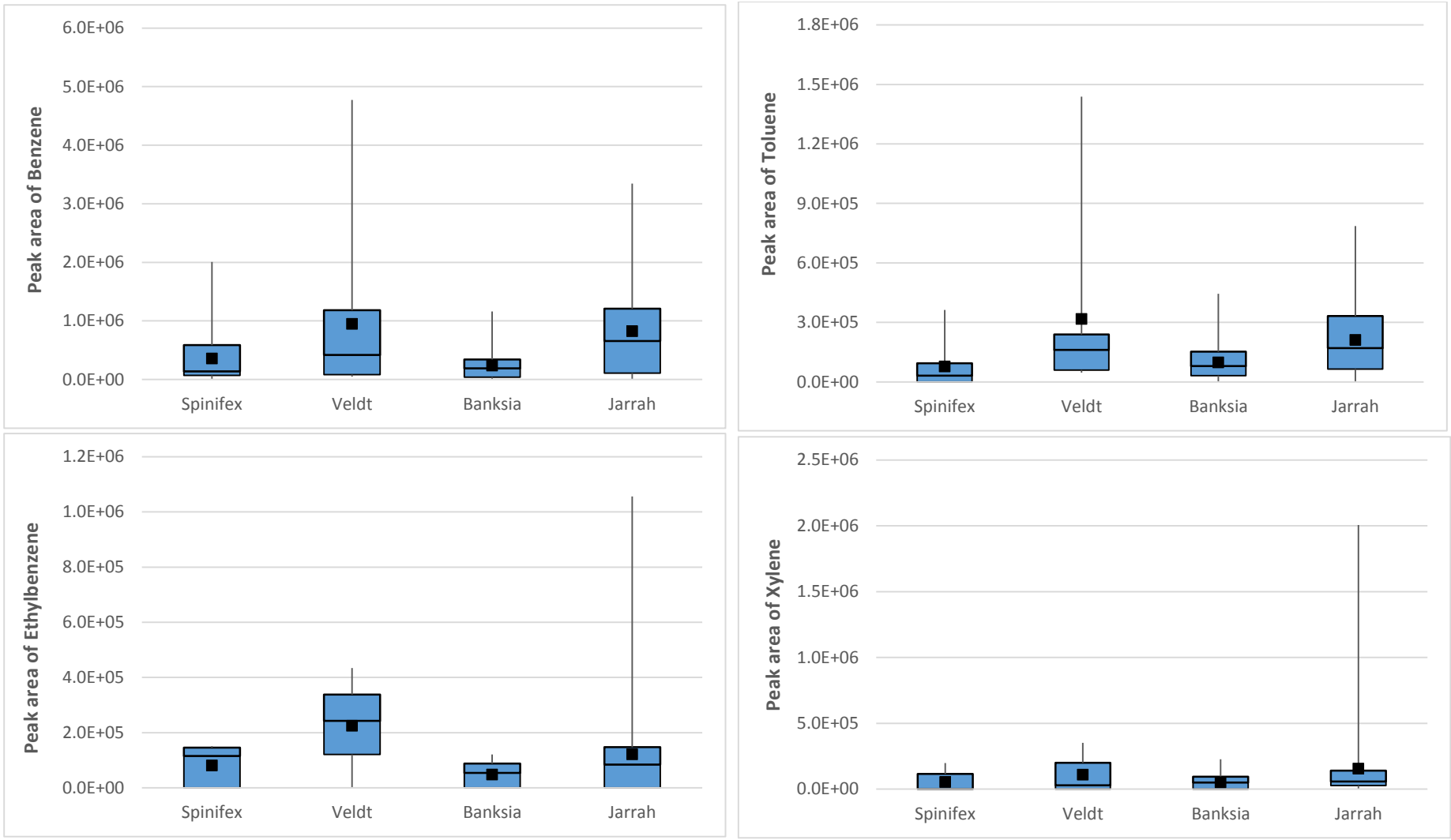


Figure 4-5. Box plots showing peak areas of BTEX from vegetation burning across all combustion conditions. Black squares in the box plots show the mean peak areas of BTEX (n=27 for Spinifex, Banksia and Jarrah; n=9 for Veldt grass)

4.4. Discussion

4.4.1. Carbonyl emissions

Abundance of carbonyls

The abundance of formaldehyde and acetaldehyde emissions observed in this study was consistent with results of other studies on bushfire smoke (Christian et al., 2003; Reisen et al., 2006b; Vicente et al., 2011). Christian et al. (2003) measured trace gas emissions from laboratory fires of savanna fuel and reported that EFs for formaldehyde and acetaldehyde were about 6-fold and 4-fold higher than that of acetone. Reisen and Brown (2009), when measuring the concentrations of pollutants to which bushfire fighters are exposed, also reported formaldehyde as the dominant aldehyde from vegetation fires, with formaldehyde and acetaldehyde measured at $g.m^{-3}$ level while other aldehydes including acrolein, and 2-furaldehyde were detected at $mg.m^{-3}$ level. Vicente et al. (2011) also reported that formaldehyde and acetaldehyde were the main aldehydes found in forest fire smoke.

Formaldehyde was more abundant in the vegetation fire smoke than acetaldehyde as observed in this work as well as in many other studies (Burling et al., 2011; Christian et al., 2003; Guérette et al., 2018; Liu et al., 2017). However, some other studies reported higher EF for acetaldehyde compared with that for formaldehyde (Hurst et al., 1994a; Vicente et al., 2012; Yokelson et al., 2008). The difference in order of abundance of these two main compounds in vegetation fire smoke might be due to differences in sampling and measurement methods between studies. The sampling methods applied by Hurst et al. (1994) and Vicente et al. (2011) involved the collection of smoke in Tedlar bags, whereas in our study and other studies carbonyl collection used 2,4-DNPH tubes, impregnated filter cassettes or glass flasks (De Vos et al., 2009; Guérette et al., 2018; Reisen and Brown, 2009). Formaldehyde is a polar organic compound and has been found to be lost using Tedlar bags (Pau et al., 1991), which may explain the discrepancy. Acetaldehyde and formaldehyde in the study by Yokelson et al. (2008) were measured using different methods (Fourier transform infrared spectroscopy FTIR for formaldehyde and proton-transfer reaction mass spectrometry PTR-MS for acetaldehyde) which might influence the comparison between EFs for these two aldehydes. Other studies which used FTIR to measure both formaldehyde and acetaldehyde reported similar relative orders of abundance to our study (Burling et al., 2011; Guérette et al., 2018).

Comparison with values reported in literature

A comparison of EFs for carbonyls from biomass burning for similar vegetation types around the world is provided in Table A2.8. Due to the lack of data on butyraldehyde and benzaldehyde emissions in the literature, only EFs for the four remaining compounds were compared. Owing to the greater abundance and potentially higher risk of human exposure to formaldehyde from bushfire smoke compared with other compounds, this discussion focuses on emissions of formaldehyde.

The EFs for formaldehyde between tropical grassland (Spinifex and Kimberley grass) and woodland/forest (Banksia and Jarrah) obtained in this study were consistent with findings of field-based studies in Australian vegetation fires in recent years (Guérette et al., 2018; Paton-Walsh et al., 2010; Smith et al., 2014). Guérette et al. (2018) measured the EFs for formaldehyde from prescribed fires in Australian temperate forest and found similar EFs compared to those from Australian savanna reported by Smith et al. (2014) and Paton-Walsh et al. (2010). However, in this study, Veldt grass had a 2-fold greater EFs for formaldehyde compared with Banksia and Jarrah. In addition to distinct fuel composition which resulted in differences in emissions, significantly lower MCE was observed in burning Veldt grass compared with those of other vegetation types (Chapter 3) and might explain the high EF for formaldehyde from this grass.

EFs for formaldehyde, acetaldehyde and acetone from Spinifex were in good agreement with values reported from African savanna burns in a laboratory-based study by Christian et al. (2003). Meanwhile, EF for formaldehyde from Banksia was 3-fold higher than that reported from chaparral in the US under laboratory burning conditions (Burling et al., 2010). EFs for formaldehyde from Jarrah forest were 5-fold higher, but the EFs for acetaldehyde and acetone were lower (1.5 and 3-fold, respectively) than those from eucalyptus burns reported in the experimental study by Yokelson et al. (2008). It is not possible to compare values obtained from Veldt grass burns due to the relative scarcity of data on emissions from temperate grass fires (Urbanski et al., 2009). The difference in EFs from vegetation fires in different parts of the world highlights the need to generate EFs from local Australian vegetation types to better predict emissions from bushfires in Australia.

When compared with data obtained in field-based studies, EFs for formaldehyde in our study were 1.4 to 2.2-fold lower than those reported for similar vegetation types (Burling et al., 2011; Liu et al., 2017; Paton-Walsh et al., 2005). The lower values in our study might be due to differences arising from laboratory-based versus field-based measurements. While measurements undertaken in controlled laboratory conditions capture all the phases of a fire, measurement in the field, especially at the ground platform, may be biased toward the smouldering phase since products

from the flaming phase are quickly transported upwards by convection (Burling et al., 2010; Paton-Walsh et al., 2014). In addition, formaldehyde emission in the field is significantly influenced by photochemical processes with decreases via photo-dissociation when reacting with OH^- and increases as a product of the oxidation of VOCs. Since VOCs are also major emissions from vegetation fires, the secondary formation of formaldehyde (by the oxidation of VOCs) in the first short period of a fire may compensate for its loss (by the reaction with OH^-) (Paton-Walsh et al., 2010), another reason for higher EFs reported from field-based measurements compared with our laboratory-based data.

Effects of combustion conditions and combustion efficiency on emissions

Carbonyl emissions from Spinifex and Jarrah increased as fuel moisture content increased, which was expected (Koppmann et al., 2006). For Banksia the burns of moist vegetation generated higher amounts of some carbonyls compared with those emitted from wet Banksia fires. This trend was not anticipated and occurred only in conditions when the rates of air flow were increased (see Figure 4.4). Higher rates of air flow might have disturbed the fuel bed, causing fluctuating combustion conditions which override the effect of fuel moisture content.

The increase of air flow was found to reduce the combustion efficiency of the burns and decrease the MCE (Chapter 3). Negative correlations between carbonyl EFs and MCE indicated that more carbonyls were emitted at lower MCE values. Therefore, higher EFs for carbonyls were observed with the high flow rate conditions as expected.

The strong inverse correlations between MCE and EFs for carbonyls for most vegetation types were similar to the results of other studies, confirming that the carbonyl emissions were associated with the smouldering phase. Guérette et al. (2018) reported a strong negative correlation between EF for formaldehyde and MCE ($R^2 = 0.79$) for temperate forest fires in Australia. The study by Christian et al. (2003) examined the emissions of African vegetation fuel in the laboratory and they also found strong negative correlations between MCE and EFs for acetone and acetaldehyde with R^2 of 0.82 and 0.87, respectively. Vicente et al. (2011) also observed a strong inverse relationship ($R^2 = 0.8$) between EF for acetaldehyde and MCE of a forest fire in Portugal.

Extrapolating EFs for other carbonyls from EF for formaldehyde

Formaldehyde in bushfire emissions is commonly measured and reported but the availability of information on other carbonyls is scarce (Table A2.8). Strong correlations between EFs for formaldehyde and other compounds including acetaldehyde, propionaldehyde and acetone across all vegetation types, observed in this study, were used to investigate the potential extrapolation of

EFs for these carbonyls from the EF for formaldehyde to estimate emissions of infrequently measured compounds. Extrapolating functions where the linear regression functions (with zero intercept) were obtained by plotting EFs for formaldehyde (X axis) and EFs for another compound (Y axis) (Figure A2.2). EFs for acetaldehyde, acetone and propionaldehyde can be extrapolated from EF for formaldehyde using multiplication factors of 0.563, 0.142 and 0.087, respectively (R^2 values of the regressions are 0.93, 0.73 and 0.89, respectively). EFs for formaldehyde and acetaldehyde (or acetone) reported simultaneously for several vegetation types in a limited number of studies were also plotted (Burling et al., 2011; Christian et al., 2003; Guérette et al., 2018; Lawson et al., 2015; Liu et al., 2017) and these data points were close to the extrapolating lines (Figure A2.2). The extrapolated values using this proposed function show a good level of agreement with measured values (coefficient of variation <17%). This consistency suggests the applicability of using these functions in extrapolating emissions of other carbonyls from that of formaldehyde across different types of vegetation fires.

4.4.2. VOC emission

Qualitative results confirmed that several VOCs were present in vegetation fire smoke. The most frequently detected included benzene and toluene, which were also identified in other studies (Barboni and Chiaramonti, 2010; de Gouw et al., 2006; Shirai et al., 2003).

The emissions of VOCs from vegetation burning are complex and depend on different factors including fuel type and combustion conditions (Barboni and Chiaramonti, 2010). Barboni and Chiaramonti (2010) reported significantly higher concentrations (1.5 to 3-fold) of BTEX in the smouldering phase compared with those emitted in the flaming phase of prescribed burns. Therefore, the higher emissions of BTEX from Veldt grass burning compared to Banksia and Spinifex was likely due to higher contribution of the smouldering phase in the combustion of Veldt grass as the MCE of Veldt grass burns was observed to be significantly lower than the combustion of other types of vegetation (Chapter 3). The more diverse composition of VOCs and the higher BTEX emissions of Jarrah compared to those of Banksia and Spinifex might be due to the high content of biogenic VOCs in eucalyptus leaves including isoprene, monoterpenes, and sesquiterpenes that can create more VOCs in emitted smoke when burnt (Maleknia et al., 2009). The averages of some BTEX across all combustion conditions showed high standard deviations (Figure 4.5) due to the markedly increases of emissions from combustion in high flow rate conditions compared to those from burns in no fan conditions which indicated the significant effect of air flow rate on the combustion and the emissions of these VOCs. However, the approach of

using peak areas of these VOCs was not scientifically robust and only provided a preliminary comparison in VOC emissions between vegetation types.

4.5. Conclusion

Several VOCs including benzene were detected in smoke from vegetation fires. Carbonyl emissions were similar for tropical grassland (Spinifex), woodland (Banksia) and forest (Jarrah) vegetation consistent with the findings reported from field-based measurements in other Australian studies. However, burns of a temperate grass (Veldt grass) generated greater carbonyl emissions than the tropical grasses (Spinifex and Kimberley grasses). Due to the scarcity of data on the emission of carbonyls from temperate grassland, further research into emissions from this type of grassland should be conducted.

Combustion conditions affected carbonyl emissions, with higher EFs observed from burns using fuel with higher moisture content and under higher air flow conditions. Negative correlations between modified combustion efficiency and emission factors for most carbonyls were observed, confirming that the emission of carbonyls is primarily associated with the smouldering phase. Even though the emission factors for carbonyls obtained in this study were lower than those reported from field based measurements in similar vegetation types, due to differences in sampling conditions and the chemical transition of carbonyl compounds, this study has demonstrated the effects of combustion conditions on carbonyl emissions. This study has also proposed functions which may be useful to predict emissions of infrequently measured carbonyls (acetaldehyde, acetone and propionaldehyde) from that of formaldehyde, a commonly measured and reported substance. Field-based studies on the emission factors for these carbonyls are recommended to validate this laboratory-based data.

Chapter 5. EMISSIONS OF PARTICULATE MATTER

(Within the content of this chapter, I was helped in analysing PAHs by Bo Strandberg – Department of Public Health and Community Medicine at the Institute of Medicine, University of Gothenburg, Sweden – who contributed about 10% of the work load)

5.1. Introduction

Particulate matter (PM) is one of the major air pollutants emitted from vegetation fires and consists of a high proportion of particulates with diameter of less than 2.5μm ($PM_{2.5}$) which can penetrate deeply into the lungs and impact the health of populations and individuals (Alves et al., 2010a; Reid et al., 2005b). Epidemiological studies have revealed that exposure to $PM_{2.5}$ is associated with cardiopulmonary morbidity and mortality, as well as exacerbation of diabetes mellitus and adverse birth outcomes (Feng et al., 2016).

The nature and degree of health effects of $PM_{2.5}$ may be influenced by its components including toxic substances such as polycyclic aromatic hydrocarbons (PAHs) and metals adsorbed onto surfaces of PM (Cavanagh et al., 2009; Dieme et al., 2012). Many PAHs are defined as mutagenic and/or carcinogenic compounds and are known to cause lung cancer (Choi et al., 2010). A study by Alves et al. (2010) found that the dominant PAHs in shrubland burning particles were alkylated compounds, benzo(a)anthracene, pyrene, phenanthrene, fluoranthene and chrysene. The higher concentrations of these PAHs were present in finer-size particulates. When studying PAH emissions from different firewood types in Australia, Zou et al. (2003) reported that most genotoxic PAHs were present in the particulate phase.

Garcia-Hurtado et al. (2014) found that the major trace metals in $PM_{2.5}$ emitted from shrub wildfire in Spain were Cu, Zn, Zr, Pb, Ti, and Ba. In a study comparing metals in aerosols in Singaporean ambient air in non-bushfire conditions and in the period affected by smoke from biomass burning, Pavagadhi et al. (2013) observed higher concentrations of metals including Al, Cr, Fe, Mn, Co, Ni, Zn, Cu, Cd and Pb in $PM_{2.5}$ during the affected period compared with those in non-bushfire conditions. Some first-row transition metals such as Fe, Ni and Cu absorbed in PM have been suggested to produce free radicals that may cause oxidative stress when accumulated in the body (Jiang et al., 2014). This phenomenon may have negative impacts on human health by causing chronic illness such as lung damage and cancer (Pham-Huy et al., 2008).

Bushfire emissions are one of the most significant sources of $PM_{2.5}$ pollution in Australia due to the fire-prone nature of the country (Environmental Protection Authority Victoria, 2018). The number of studies investigating the emissions of PM from bushfires in Australia has increased in recent years (De Vos et al., 2009; Desservettaz et al., 2017; Reisen and Brown, 2009; Wang et al.,

2017a, 2017b; Wardoyo et al., 2006). Wardoyo et al. (2006) investigated the particle number and emission factors (EF – amount of pollutant emitted from burning a unit amount of fuel) for $PM_{2.5}$ from experimental combustion of five trees species in Queensland. Studies by De Vos et al. (2009) and Reisen and Brown (2009) measured the concentrations of respirable particles which bushfire fighters were exposed to when conducting prescribed burns. No data on chemical composition of PM was produced by those studies. Wang et al. (2017a, 2017b) reported the EF for total suspended particles from forest and savanna fires in the northern and south-western areas of Australia. Desservettaz et al. (2017) measured emissions from tropical savanna fires and reported the EFs for particulates in Aitken mode (0.015–0.1µm) and accumulation mode (0.1–0.67 µm) and some chemical components of the particulates including SO_4^{2-}, NO_3^-, NH_4^+ and Cl^-. Data on emission of $PM_{2.5}$ from vegetation fires in Australia and its chemical compositions is scarce.

This chapter investigates the emission of PM_{10} and $PM_{2.5}$ and the chemical composition of $PM_{2.5}$ (water-soluble metals and PAHs) emitted from burning different vegetation types of Australia in varying conditions. In addition to the aim of providing data on $PM_{2.5}$ emissions from vegetation fires in Australia, the chapter also investigates the influences of vegetation types and combustion conditions on $PM_{2.5}$ emission.

5.2. Methodology and study design

5.2.1. Study design and experimental conditions

The details of the experimental set-up and design are outlined in Chapter 3. PM collection was conducted concurrently with the carbonyl sampling with fifty gram (50g) of vegetation was burn in each experiment (Chapter 4).

5.2.2. PM collection

Personal Modular Impactors (PMI) (SKC, Cat. No. 225-5-37) with two inlets of 2.5 µm and 10 µm connected to each other were used to collect $PM_{2.5}$ and $PM_{2.5\text{-}10}$ using active sampling pumps (SKC Aircheck®52). $PM_{2.5\text{-}10}$ was collected onto 25 mm polyvinyl chloride (PVC) filters (SKC, Cat. No. 225-5-25) and $PM_{2.5}$ was collected onto 37 mm PVC filters (SKC, Cat. No. 225-5-37-P). For each experimental burn, three filters were used to collect $PM_{2.5}$ for subsequent analysis (water-soluble metals, PAHs and toxicity testing). PVC filters were used for gravimetric and metals analysis due to their low tare weight and following the recommendations of National Institute for Occupational Safety and Health (NIOSH) 3700 (National Institute for Occupational Safety and Health, 2003). Polytetra-fluoroethylene (PTFE) membrane filters (37 mm, Zefluor®, Pall Laboratory) were used for PAH analysis as recommended in NIOSH 5506 (NIOSH, 1998). The

PM samplers were located inside the duct, facing the smoke flowing through the duct. Pumps were set at a flow rate of 3.0 $L.min^{-1}$ as recommended by the PMI manufacturer. Where the concentrations of particulate in smoke were too high, clogging the filters, causing the pump to stop, the pump flow rate was adjusted to 1.0 or 1.5 $L.min^{-1}$ to ensure the pumps ran smoothly throughout the sampling process. Temperatures were measured at PM sampling sites. The highest temperatures of smoke measured at the PM sampling area were 40ºC, 51ºC and 57ºC for no, low and high flow conditions, respectively. The average residual $PM_{2.5}$ mass in the system (identified by blank burns) was 7.0 µg (accounting for 1% of the sampled values) and this residue was deducted from $PM_{2.5}$ masses collected from vegetation fires.

Gravimetric analysis

The masses of PM_{10} and $PM_{2.5}$ were determined by the differences in weight between pre-sample and post-sample filters using a microbalance (Mettler Toledo XP6 Excellence Plus). Before being weighed, the filters were conditioned in a desiccator in a conditioning room for at least 48 hours to obtain stable humidity and temperature conditions, following the European Study of Cohorts for Air Pollution Effects (ESCAPE) protocol (EU-multicenter study RUPIOH, 2009). A static electricity remover (Stablo-ex, Shimadzu) was used to treat the filters before weighing in order to eliminate static electricity which might affect their weights (EU-multicenter study RUPIOH, 2009).

Concentrations of PM_{10} and $PM_{2.5}$ were calculated using the formula:

$$C = \frac{m_{PM}}{V} = \frac{m_{PM}}{F \times t}$$

Where C: concentration of PM generated ($mg.m^{-3}$); m_{PM}: mass of PM collected (µg); V: volume of collected smoke (L); F: flow rate of pump ($L.min^{-1}$); t: sampling time (min).

Determination of the optical properties of $PM_{2.5}$

The optical reflectance of PM collected onto filters was measured using a Smoke Stain Reflectometer (Diffusion System Ltd, EEL Model 430), following the standard operational protocol RUPIOH SOP 4 (ESCAPE, 2008). The darkness of filters containing PM was expressed as the difference in reflectance when compared with a control filter. The control filter was chosen as the median reflectance value from among five randomly chosen new filters. The reflectance of a filter was obtained by averaging the reflectance values measured at five different points on the filter's surface. The calibration was repeated using the control filter after measuring every 25 sampled filters (ESCAPE, 2008).

The absorption coefficient was calculated using the formula (ESCAPE, 2008):

$$a = \frac{A}{2V} \times \ln(\frac{R_F}{R_S})$$

Where: a: absorption coefficient of the sample filter (m^{-1}); A: area of PM stain on the sample filter (m^2). $A = \pi(\frac{d}{2})^2$where d is the diameter of the smoke stain round on filter. In this study, d = 33mm so the value of A is 855.3 x 10^{-6} m^2; V: volume of air sampled (m^3); R_F: the average reflectance of the blank burns as percentage of R_0. R_0 is the reflectance of the clean control filter (=100); R_S: the reflectance of the sample filter as percentage of R_0.

Mass absorption coefficient σ ($m^2.g^{-1}$), which can represent the amount of elemental carbon (EC) in particulates (Chen et al., 2007; Gramsch et al., 2004; Reid et al., 2005b),was then calculated by dividing the absorption coefficient α (m^{-1}) by the $PM_{2.5}$ concentration ($mg.m^{-3}$) where:

$$\sigma = \frac{\alpha}{C} \times 1000$$

After the mass of PM and smoke stain reflectance of filters had been measured, they were stored at –20°C prior to chemical analysis.

5.2.3. $PM_{2.5}$-bound water-soluble metal analysis

PVC filters used to collect $PM_{2.5}$ were placed into 15 mL polypropylene tubes and 5 mL of ultrapure water 18.2MΩ.cm (Millipore) was added to each tube. The water-soluble metals in PM were extracted by ultrasonication (Branson 2410) for 2×30 minutes at 37ºC (Akhtar et al., 2014; Heal et al., 2005; Pavagadhi et al., 2013). After extraction, the extracts were centrifuged at 8,000 rpm for 10 minutes to remove particles and filter debris from extracts. Thereafter 2 aliquots of 2 mL of centrifuged extract were diluted five times with ultrapure water and acidified to 2% using HNO_3 (Thermo Fisher Scientific). One aliquot was used for water-soluble metal analysis, while the second was stored at <4ºC for later analysis if necessary.

Thirteen water-soluble metals including alkali, alkaline-earth, transition and other metals (Na, K, Ca, Ni, Fe, Cu, Cd, Cr, Pb, Zn, Mg, Mn and Al) were analysed. K and Na were measured using inductively coupled plasma optical emission spectrometry (iCAP™ 7600 duo ICP-OES, Thermo Fisher) while the remaining metals were measured using inductively coupled plasma mass spectrometry (iCAP™ RQ ICP-MS, Thermo Fisher).

To examine the recovery rate of the method, 50 µL of standard solution 100 ppm (ICP-MS-E Verification standard of High Purity Standards) was spiked to blank filters. The spiked filters were extracted following the above extraction procedure. The percentage of recovery of this method ranged from 86% to 113% for different metals. Calibration standard curves for all metals had

strong linear responses with the R^2 values (0.9996–1.000). Duplicate measurements were conducted for every 10th sample and the coefficient of variation was 0.4 to 4.4%. A summary of QA/QC parameters for analysis of water-soluble metals in $PM_{2.5}$ is shown in Table A3.1 in Appendix 3.

5.2.4. $PM_{2.5}$ - bound PAH analysis

PAH analysis was undertaken for one sample (filter with the highest mass of $PM_{2.5}$ collected to ensure PAH detection) for every vegetation type collected in each combustion condition. $PM_{2.5}$ collected onto PTFE filters was sent for PAH analysis at the University of Gothenburg, Sweden, following a protocol described by Jorgensen et al. (2013). Filters were extracted in dichloromethane for 10 minutes using an ultrasonic extractor (Sonica, Soltec, Italy). Extracts were then cleaned via a 2 cm column of silica gel with sodium sulfate on top. Prior to analysis, the extract solutions were concentrated to the final volume of 20 to 30 µL using a pure nitrogen stream. An internal standard mixture containing 16 deuterated USEPA PAHs (Dr. Ehrenstorfer, Augsburg, Germany) was added to samples before extraction and PAH standards before analysis.

The concentrations of 16 USEPA PAHs (Table A3.2) were then measured in the extracts using high resolution gas chromatography/low-resolution mass spectrometry (HRGC/LRMS, Agilent Technologies, Inc., Santa Clara, Calif.). Electron impact ionisation and selected ion monitoring mode was applied for the MS, and the GC column was a 60m × 0.32 mm I.D. non-polar capillary column (J&W DB-5, Agilent). Helium was used as gas with a flow of 1 $mL.min^{-1}$. GC injector temperature was set to 230ºC. The GC oven was temperature programmed of 50ºC and hold 3 minutes, $10ºC.min^{-1}$ to 180ºC and hold 5 minutes, $3ºC\ min^{-1}$ to 300ºC and hold 20 minutes.

Urban dust with known-concentrations of PAHs (Standard Reference Material – SRM 1649a, purchased from the National Institute of Standards and Technology, Gaithersburg, MD, USA) was used as the quality control (QC) sample. Measured concentrations of 12 PAHs in SRM deviated less than 15% compared with certified concentrations (with the exception of BaA for which the deviation was 20%). QA/QC parameters of PAHs analysis are presented in Table A3.2.

5.2.5. Calculation of emission factors (EFs)

EFs for $PM_{2.5}$ in each burn were calculated using the following formula (Alves et al., 2011; Wardoyo et al., 2006):

$$EF_{PM2.5} = \frac{m_{PM2.5}}{m_{burned}} = \frac{C_{PM2.5} \times v \times A \times t}{m_C} \times F_C$$

Where: $EF_{PM2.5}$: emission factor of $PM_{2.5}$ ($g.kg^{-1}$ dry fuel); $m_{PM2.5}$: amount of $PM_{2.5}$ collected, calculated as $C_{PM2.5} \times v \times A \times t$; $C_{PM2.5}$: average concentration $PM_{2.5}$ ($mg.m^{-3}$); v: average velocity of smoke at sampling area during sampling time ($m.s^{-1}$); A: area of the duct at sampling point (A = 0.018 m^2); t: sampling time (s), t = 360 s; m_{burned}: amount of dry vegetation burned (g), expressed as the ratio of amount of carbon emitted m_C (g) over the carbon content of the vegetation F_C (48% for the forest and woodland, 45% for the grasses) (Hurst et al., 1994a).

The average velocity of smoke was obtained by averaging the recorded velocities (every 5 seconds) during the whole sampling period. The PMI heads used to collect PM were located inside the duct, thereby reducing the duct area, and thus increasing the velocity of smoke. However, the velocity of smoke was measured at a point after the PM sampling point. A trial was conducted and the average ratio of velocity between two points (PM sampling point and velocity recording point) was 1.28 for conditions using the fan. This ratio was used to estimate the average velocity of smoke flow at the PM sampling point in the calculation. For the no flow condition, because the velocity of smoke flow was based on the natural dispersion of smoke and was therefore small (around $0.3 m.s^{-1}$), the difference in velocity at these two sampling points was negligible and assumed to be equal to the recorded velocity.

Average EFs for water-soluble metals and PAHs in $PM_{2.5}$ were calculated based on the average EFs for $PM_{2.5}$ following the formula: $EF_i = EF_{PM_{2.5}} \times C_i$; where EF_i: emission factor for pollutant i (PAHs or water-soluble metals) in $PM_{2.5}$ ($mg.kg^{-1}$ dry fuel); $EF_{PM_{2.5}}$: emission factor for $PM_{2.5}$ ($g.kg^{-1}$ dry fuel); C_i: average concentration of pollutant i in $PM_{2.5}$ ($mg.g^{-1}$).

5.2.6. Statistical analysis

Metals and PAH concentrations that were below the limit of detection (LoD) were replaced with a value of half the respective LoD (USEPA, 2000). One-way analysis of variance (ANOVA) and Kruskal-Wallis analysis (SPSS ver.25, IBM) were used to analyse the effects of vegetation type on the emission characteristics of $PM_{2.5}$, in cases of normally and non-normally distributed data, respectively. Appropriate post-hoc tests were also conducted (Tukey test for ANOVA and Dunn-Bonferroni test for Kruskal-Wallis). The influences of combustion conditions (fuel moisture and flow rate) on $PM_{2.5}$ emissions were evaluated using permutational multivariate ANOVA (PERMANOVA, PRIMER 6^+). Regression analysis was used to assess the correlations between variables (SPSS ver.25, IBM). A significance value of $p<0.05$ was used for all statistical analysis.

5.3. Results

5.3.1. Physical characteristics of PM

Ratio of $PM_{2.5}$ and PM_{10}

The majority of PM_{10} emitted from vegetation burning was in the smaller size fraction, namely $PM_{2.5}$. A linear regression (with zero intercept) between the concentrations of PM_{10} and $PM_{2.5}$ generated from vegetation fires (n=99) indicated that $PM_{2.5}$ accounted for 98.7% of the PM_{10}. The $PM_{2.5}/PM_{10}$ was consistent between different vegetation types which averagely varied from 97.5 to 98.4%, with the exception of Spinifex which had a significantly lower ratio of $PM_{2.5}$ to PM_{10} (94.7%) compared with other vegetation types. The mean concentrations of PM_{10}, $PM_{2.5}$ and ratio of $PM_{2.5}/PM_{10}$ are provided in Table A3.3. For all three types of vegetation, the $PM_{2.5}/PM_{10}$ were positively correlated with MCE (the R^2 of 0.31, 0.43 and 0.62, respectively for Spinifex, Banksia and Jarrah). Given that $PM_{2.5}$ was the major component of the PM_{10} emitted from vegetation burns, only $PM_{2.5}$ will be discussed in the following sections.

Mass absorption coefficient of $PM_{2.5}$

Mass absorption coefficients of $PM_{2.5}$ from three vegetation types of Spinifex, Banksia and Jarrah were significantly different. Spinifex derived $PM_{2.5}$ had the highest mass absorption coefficient, followed by Jarrah and then Banksia (Figure 5.1). Among the three types of grass, burning dry Spinifex and Kimberley generated $PM_{2.5}$ having similar absorption coefficients which were significantly higher than those from Veldt grass burns (Figure 5.1). A weak relationship between MCE and the mass absorption value of $PM_{2.5}$ emitted from vegetation combustion was observed (R^2=0.17, p=0.02).

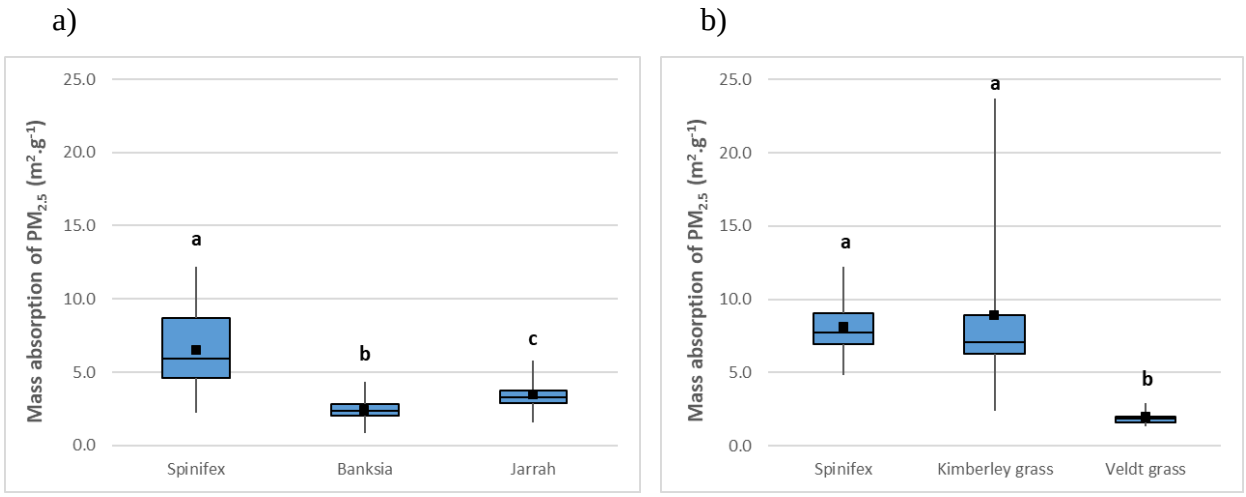


Figure 5-1. Box plots show the mass absorption coefficient of $PM_{2.5}$ emitted from burning a) different types of vegetation in all combustion conditions (n=27) and b) different types of grass in dry condition (n=9). Black squares in the box plots show the mean values. Significant differences between vegetation/grass

types are presented by the letters a, b at top of the box plots. The differences were evaluated using one-way ANOVA tests with P<0.05

5.3.2. Emission factors for $PM_{2.5}$

Jarrah yielded the highest emission of $PM_{2.5}$, followed by Banksia and Spinifex. ANOVA tests showed that the emission of $PM_{2.5}$ of Spinifex was significantly lower than the two other types of vegetation ($p<0.001$). The difference in $EF_{PM2.5}$ between Banksia and Jarrah was not statistically significant (Figure 5.2, a). Among the three types of grass, Spinifex and Kimberley grass generated similar emissions of $PM_{2.5}$, whilst the Veldt grass emitted significantly higher $EF_{PM2.5}$ which was 6 to 8-fold higher the Spinifex and Kimberley grass, respectively (Figure 5.2, b).

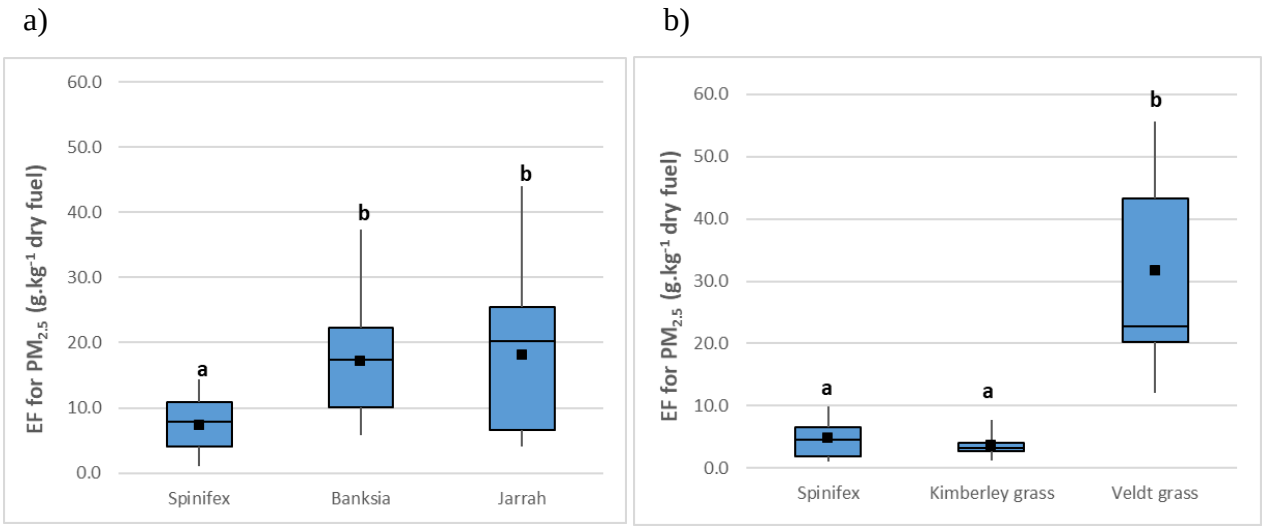


Figure 5-2. Box plots show the EFs for $PM_{2.5}$ emitted from burning a) different types of vegetation in all combustion conditions (n=27) and b) different types of grass in dry condition (n=9). Black squares in the box plots show the mean values. Significant differences between vegetation types/grass kinds are presented by the letters a, b at top of the box plots. The differences were evaluated using one-way ANOVA tests with P<0.05

Combustion conditions significantly influenced the emission factors for $PM_{2.5}$ (Figure 5.3). The EFs for $PM_{2.5}$ increased in moist and wet conditions when compared with burning dry vegetation. Vegetation burns in higher flow rates generated higher emissions of $PM_{2.5}$ when compared with those obtained from the no flow condition. PERMANOVA tests revealed that, for Banksia and Jarrah, the emissions for $PM_{2.5}$ at different flow rates were significantly different from each other, whilst the significant differences were between no flow and low/high flow conditions in the case of Spinifex burns (Figure 5.3).

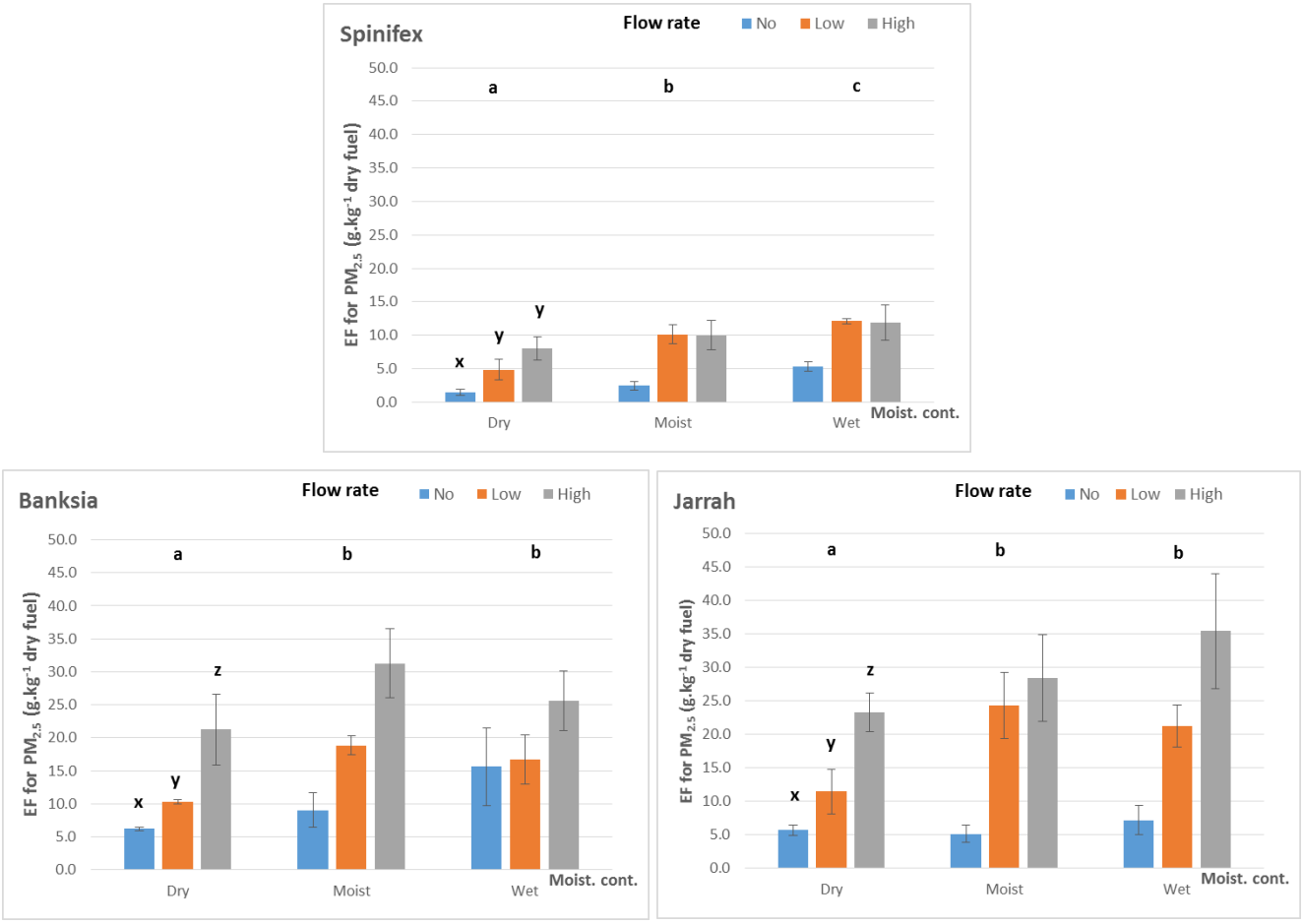
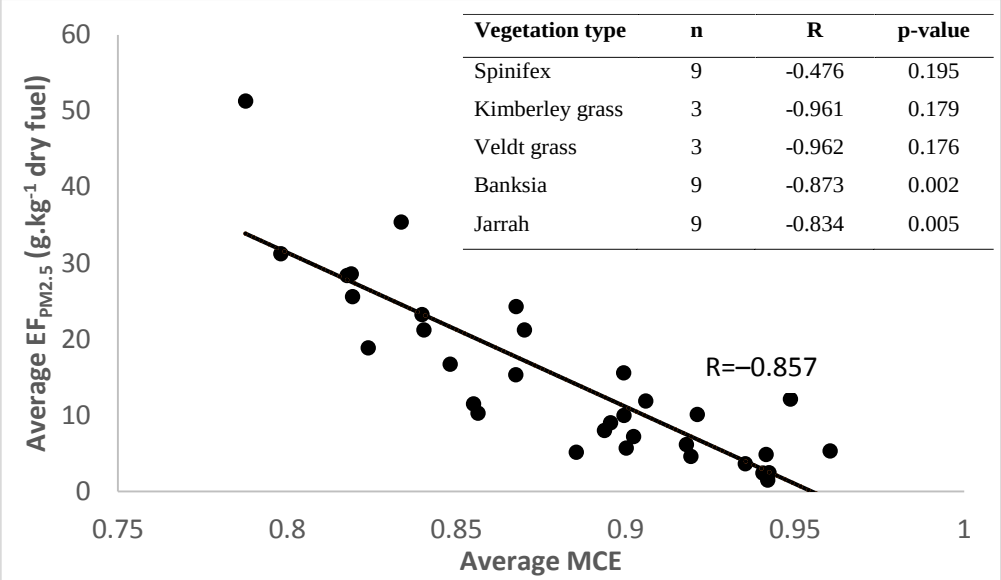


Figure 5-3. Emission factors for $PM_{2.5}$ emitted from Spinifex, Banksia and Jarrah burning in different combustion conditions (n=27). Letters a, b, c at the top of each bar group represent the significant difference between different fuel moisture contents. Letters x, y, z at the top of each bar represent the significant difference between different flow rates. Influences of moisture content and flow rate were assessed using PERMANOVA with p<0.05

A strong negative relationship was observed between MCE and $EF_{PM2.5}$ of burning vegetation (Figure 5.4). When examining the correlations between MCE and $EF_{PM2.5}$ for individual vegetation types, strong correlation coefficient values R were observed for most vegetation types, with the exception of Spinifex (Figure 5.4).



Vegetation type	n	R	p-value
Spinifex	9	-0.476	0.195
Kimberley grass	3	-0.961	0.179
Veldt grass	3	-0.962	0.176
Banksia	9	-0.873	0.002
Jarrah	9	-0.834	0.005

Figure 5-4. EF for $PM_{2.5}$ as a function of MCE. The R and p-value of correlations between $EF_{PM2.5}$-MCE for individual vegetation types are presented in the attached table

5.3.3. Chemical characteristics of $PM_{2.5}$

Concentrations of $PM_{2.5}$-bound water-soluble metals

K and Na were the dominant water-soluble metals in $PM_{2.5}$, accounting for more than 97% of the mass of the 13 metals analysed. The next most abundant metals were Ca, Mg and Zn which were present in $PM_{2.5}$ at concentrations up to 6.5 µg.mg^{-1}. Other metals, including Al, Cr, Mn, Fe, Ni, Cu and Cd were present in $PM_{2.5}$ at very low concentrations and many of them were below the analytical limit of detection (Table 5.1).

Jarrah and Banksia burning generated $PM_{2.5}$ containing similar total concentrations of metals which were about 3-fold higher than that generated from Spinifex (Table 5.1). Kruskal-Wallis tests revealed that the concentrations of individual metals were significantly different in the PM derived from the combustion of the three vegetation types, with the exception of Mg, which varied little (Table A3.4-a). $PM_{2.5}$ from Banksia and Jarrah burns had a higher composition of K, Na and Mg than that from Spinifex, whereas Spinifex burning generated a higher concentration of other metals (Ca, Zn, Al, Cr, Mn, Fe, Ni, Cu, Cd and Pb) compared with Banksia and Jarrah (Table 5.1).

$PM_{2.5}$ from burns of the dry grasses had significantly different total concentrations of water-soluble metals. Veldt grass $PM_{2.5}$ yielded the highest metal concentrations, followed by Spinifex. Kimberley grass had a very low $PM_{2.5}$-bound metals concentration, 10 to 17-fold lower than those of Spinifex and Veldt grasses, respectively (Table 5.1). The significant lower total concentration of metals of the Kimberley grass $PM_{2.5}$ was due to the very low concentrations of K and Na.

Concentrations of other metals in $PM_{2.5}$ derived from Kimberley grass burns were in comparable ranges to those of the other two types of grasses (Table 5.1 and Table A3.4).

Concentrations of metals in $PM_{2.5}$ were not significantly affected by the fuel moisture content for most vegetation types, except for Spinifex (Figure A3.1). Moist Spinifex derived $PM_{2.5}$ had a higher total metal concentration compared with that from burning dry/wet Spinifex. Air flow rate also did not significantly influence the $PM_{2.5}$-bound total metal concentrations (Figure A3.1).

The EFs for dominant individual metals and total metals from vegetation fires were calculated from EFs for $PM_{2.5}$ and mass percentage of metals in $PM_{2.5}$ are presented in Table A3.5. Jarrah had the highest EFs for K and Mg; Banksia had the highest EFs for Na and Ca; whilst Veldt grass generated the highest emission of Zn, per unit of fuel burnt, respectively. Since EF for $PM_{2.5}$ was strongly negatively correlated with MCE, it is not surprising that the emissions factors for abundant metals also had strong negative correlations with MCE ($R=-0.88$ for EF_K-MCE and $R=-0.83$ for EF_{Na}-MCE).

Table 5-1. Mean concentrations (Mean ± SD) of water-soluble metals in $PM_{2.5}$ generated from vegetation burning. The mean values were calculated by averaging values in all combustion conditions. Unit: $\mu g.mg^{-1}$ PM

Metals	Different types of vegetation (n=27)			Different types of dry grass (n=9)		
	Spinifex	Banksia	Jarrah	Spinifex	Kimberley grass	Veldt grass
K	60 ± 29	140 ± 33	150 ± 28	50 ± 19	5.3 ± 3.1	90 ± 24
Na	7.4 ± 3.3	41 ± 14	23 ± 6	7.1 ± 2.4	0.34 ± 0.20	14 ± 3.5
Ca	1.3 ± 1.6	0.78 ± 1.0	0.48 ± 0.94	2.5 ± 2.1	0.28 ± 0.24	0.056 ± 0.031
Mg	0.12 ± 0.18	0.24 ± 0.35	0.33 ± 0.67	0.17 ± 0.26	0.051 ± 0.037	0.032 ± 0.032
Zn	0.17 ± 0.26	0.11 ± 0.08	0.056 ± 0.103	0.25 ± 0.40	0.27 ± 0.15	0.59 ± 0.22
Al	0.035 ± 0.058	0.028 ± 0.095	0.017 ± 0.021	0.049 ± 0.060	0.012 ± 0.029	0.0030 ± 0.0031
Cr	0.010 ± 0.029	< 0.0001	0.002 ± 0.006	0.028 ± 0.047	0.008 ± 0.023	0.0010 ± 0.0005
Mn	0.012 ± 0.030	0.0010 ± 0.0012	0.011 ± 0.019	0.031 ± 0.047	0.0003 ± 0.0002	0.0012 ± 0.0014
Fe	0.035 ± 0.058	< 0.003	0.027 ± 0.015	0.053 ± 0.069	0.012 ± 0.014	0.0049 ± 0.0035
Ni	0.013 ± 0.036	< 0.0001	0.0017 ± 0.0042	0.036 ± 0.058	0.014 ± 0.019	0.0017 ± 0.0014
Cu	0.021 ± 0.047	0.0018 ± 0.0021	0.0083 ± 0.019	0.054 ± 0.072	0.0052 ± 0.0033	0.0024 ± 0.0043
Cd	0.015 ± 0.009	< 0.0001	< 0.0001	0.028 ± 0.048	0.0040 ± 0.0049	0.0074 ± 0.0033
Pb	0.0072 ± 0.017	< 0.0002	< 0.0002	0.018 ± 0.027	0.0010 ± 0.0010	0.0034 ± 0.0064
∑metals	69 ± 31	180 ± 39	180 ± 31	60 ± 20	6.3 ± 3.0	110 ± 25

Concentrations of PAHs in $PM_{2.5}$

Pyrene (Pyr) and fluoranthene (Flu) were the most abundant PAHs in $PM_{2.5}$ produced by vegetation burning. Pyr comprised up to about 21% of the PAH mass (16 PAHs), followed by Flu which accounted for about 17% (Table 5.2). Other major PM-bound PAHs were benzo(a)pyrene (BaP – 10%), chrysene (CHR – 9%), benzo(b)fluoranthene (BbF – 8%), and benzo(a)anthracene (BaA – 8%). When grouping PAHs based on the number of aromatic rings, 4-ring PAHs were the major group contributing more than 50% of the total mass concentrations of PAHs. Other high-molecular weight groups also accounted for significant proportions, at 27% and 14% for 5-ring and 6-ring PAHs, respectively. Low-molecular weight PAHs (2-ring and 3-ring) were not abundant (<6%).

Among the three vegetation types, Jarrah had the highest total concentration of PAHs which was 1.7 and 1.9- fold higher than those in $PM_{2.5}$ derived from Spinifex and Banksia burns, respectively. Jarrah derived $PM_{2.5}$ also had the highest concentrations of individual PAHs among the three types of vegetation (Table 5.2). $PM_{2.5}$ generated from burning Kimberley grass contained higher concentrations of PAHs, which was also 1.7 and 1.9-fold higher than those from dry Spinifex and Veldt grass, respectively (Table 5.2).

The EFs for PAHs which were calculated from EFs for $PM_{2.5}$ and mass concentrations of PAHs and are presented in Table A3.5. Due to having the highest $EF_{PM2.5}$ and concentration of PM-bound PAHs among three types of vegetation, Jarrah burning generated $PM_{2.5}$ with the highest emission factors for 16 PAHs which was 1.7-fold and 3.2-fold higher than those from Banksia and Spinifex, respectively. For the three types of grassland, although having the lowest PAH concentration in $PM_{2.5}$, Veldt grass burns yielded the highest EF for $PM_{2.5}$-bound 16 PAHs which was about 5-fold higher than those of the other grass types due to its significantly higher EFs for $PM_{2.5}$ (Table A3.5).

Table 5-2. Mean concentrations of 16 PAHs in $PM_{2.5}$ from vegetation burning. Unit ng.mg^{-1} $PM_{2.5}$

PAHs		Different vegetation types (n=27)			Different types of grass (n=9)		
		Spinifex	**Banksia**	**Jarrah**	**Spinifex**	**Kimberley grass**	**Veldt grass**
Naphthalene	**NaP**	0.57 ± 0.42	0.49 ± 0.34	0.84 ± 0.52	0.45 ± 0.39	2.3 ± 2.3	0.39 ± 0.30
Acenaphthylene	**AcPy**	0.37 ± 0.36	0.56 ± 0.35	1.3 ± 0.60	0.24 ± 0.04	0.36 ± 0.16	0.83 ± 0.58
Acenaphthene	**Acp**	0.30 ± 0.12	0.047 ± 0.019	0.78 ± 0.37	0.093 ± 0.055	0.37 ± 0.20	0.55 ± 0.21
Fluorene	**FL**	0.51 ± 0.37	0.37 ± 0.17	0.61 ± 0.19	0.17 ± 0.13	0.19 ± 0.11	0.86 ± 0.17
Phenanthrene	**PA**	39 ± 35	48 ± 23	75 ± 37	8.6 ± 1.5	14 ± 4	88 ± 22
Anthracene	**Ant**	7.9 ± 6.7	8.6 ± 4.2	11 ± 6	1.9 ± 0.4	2.4 ± 0.4	15 ± 4
Fluoranthene	**Flu**	260 ± 160	270 ± 98	480 ± 180	110 ± 55	220 ± 40	190 ± 30
Pyrene	**Pyr**	330 ± 200	330 ± 110	590 ± 190	150 ± 77	300 ± 38	230 ± 42
Benzo(a)anthracene	**BaA**	120 ± 50	120 ± 52	240 ± 94	73 ± 15	140 ± 36	59 ± 7
Chrysene	**CHR**	140 ± 75	140 ± 63	320 ± 130	77 ± 11	170 ± 46	67 ± 13
Benzo(b)fluoranthene	**BbF**	170 ± 76	130 ± 55	240 ± 100	110 ± 37	200 ± 19	57 ± 13
Benzo(k)fluoranthene	**BkF**	110 ± 35	96 ± 43	210 ± 93	100 ± 32	88 ± 4	49 ± 9
Benzo(a)pyrene	**BaP**	220 ± 91	150 ± 67	290 ± 130	160 ± 59	260 ± 13	68 ± 8
Dibenzo(a,h)anthracene	**DBA**	33 ± 15	20 ± 10	60 ± 42	33 ± 11	22 ± 4	13 ± 2
Benzo(g,h,i)perylene	**BghiP**	160 ± 66	100 ± 48	230 ± 100	120 ± 60	170 ± 1	45 ± 4
Indeno(1,2,3-cd)pyrene	**IND**	160 ± 60	110 ± 53	200 ± 91	130 ± 61	170 ± 9	50 ± 6
$\sum_{16\ PAHs}$		**1700 ± 750**	**1500 ± 570**	**2900 ± 1100**	**1100 ± 210**	**1800 ± 160**	**930 ± 140**

5.4. Discussion

5.4.1. Size and optical properties of PM

The major abundance of $PM_{2.5}$ in PM_{10} observed in this study was consistent with the findings of other studies. McMeeking et al. (2009) investigated the emissions of US vegetation fires in laboratory-based experiments and found a ratio of $PM_{2.5}$ to PM_{10} of 93.5%. Vicente et al. (2013) reported that 85 to 97% of PM_{10} emitted from wildfires in Portugal was fine particulate $PM_{2.5}$. In a paper reviewing studies of bushfires in Southeast Asia, Radojevic (2003) stated that 99% of particulates were less than 2.5μm.

It has been found that the flaming phase is characterised by the high emission of fine particulates, whilst more coarse particulates are generated in the smouldering phase (Garcia-Hurtado et al., 2014). MCE indicates the fraction between the flaming and smouldering phases of a combustion process and the greater the duration of the flaming phase, the higher the value of MCE (Lee et al., 2010). Therefore, the positive correlations between the ratio of $PM_{2.5}/PM_{10}$ and MCE observed in this study were expected.

Mass absorption coefficients of $PM_{2.5}$ have been found to be dependent on combustion phases with larger coefficients observed for $PM_{2.5}$ generated from flaming-dominant combustion compared with those from the smouldering-dominant fire (Reid et al., 2005a). Among the three types of vegetation, Spinifex is the most combustible fuel and Spinifex fires were mostly in the flaming phase with a corresponding higher absorption coefficient (Chapter 3). Veldt grass burns were mostly in the smouldering phase, hence the mass absorption coefficient of $PM_{2.5}$ emitted from burning this grass type was significantly smaller than those derived from burns of other grass types which were mostly in the flaming phase (Chapter 3)Chen et al. (2007) found a strong positive correlation between emission factors for light absorption and EC, with higher values of mass absorption coefficients corresponding with higher EC in particulates. Thus, from mass absorption coefficient values, it can be suggested that Spinifex generated $PM_{2.5}$ with the highest fraction of EC, followed by Jarrah and Banksia. Among the three grass types, Veldt grass burning derived $PM_{2.5}$ with a lower fraction of EC than those from Spinifex and Kimberley grass.

The weak correlation between MCE and mass absorption coefficients of $PM_{2.5}$ observed in this study was consistent with what was reported by Ferek et al. (1998) and McMeeking et al. (2009) where Ferek et al. (1998) observed that the emission of EC was independent of MCE and showed variability between fires. In a study examining the emissions of vegetation fires in a laboratory setting, McMeeking et al. (2009) reported a very weak correlation between EF_{EC} and MCE with R^2=0.09.

5.4.2. Emission factors for $PM_{2.5}$

Effects of vegetation types

The differences in emission factor for $PM_{2.5}$ from different Western Australian vegetation types measured in this study support the results reported in other studies on emissions from biomass burning in other parts of the world (Akagi et al., 2011; Chen et al., 2007; McMeeking et al., 2009). In a recent compilation of emissions from biomass burning, Akagi et al. (2011) reported a nearly 2-fold higher $EF_{PM2.5}$ from a temperate forest than from a tropical savanna. This result is similar to the findings of this study which had significantly higher $PM_{2.5}$ emissions from burning woody systems such as forest (Jarrah) and woodland (Banksia) compared with Spinifex dominated grassland. Different $EF_{PM2.5}$ emissions between different grass types (tropical – Kimberley and Spinifex grasses vs. temperate – Veldt grass) found in this study are also similar to the patterns observed by Chen et al. (2007) who reported a significant difference in EF for $PM_{2.5}$ from combustion of two different kinds of grass (namely Dambo grass and Montana grass). Dambo grass from the savannas of southern Africa is a sub-tropical grassland, whilst Montana grass collected in Missoulla USA is a temperate grass. The $PM_{2.5}$ emission factor of the Montana grass was 5-fold higher than that of the Dambo grass (Chen et al., 2007).

Effects of combustion conditions

The higher EFs for $PM_{2.5}$ when burning moist and wet vegetation compared with those from burning dry vegetation in this study were similar to results reported by Shen et al. (2013) who investigated the influence of fuel moisture content on the emission of total PM from residential wood burning. Shen et al. (2013) observed a 3 and 7-fold increase in total PM emission factor when burning wood with moisture content of 14% and 27%, respectively, compared with that from wood burn at moisture content of 5%. It was identified that a higher water content in fuel required more energy to be vaporized and reduced the combustion efficiency, thus yielding higher emissions of particulate matter (Shen et al., 2013). The higher EF for particulates may also be explained by the higher emission of organic constituents of PM from the pre-ignition pyrolysis process when burning wetter fuel (May et al., 2014).

The results on the influence of flow rate on the emission of $PM_{2.5}$ in this study were also similar to those of Shen et al. (2013) who observed a 4-fold increase in EFs for PM_{10} when burning wood in enhanced air conditions compared with normal air supply. Vegetation burns in higher flow conditions had lower combustion efficiency (Chapter 3) and therefore generated higher emissions of $PM_{2.5}$. In addition, the ability of the exhaust fan to draw particulates from remaining ash into

the emission flow, could also contribute to the higher $EF_{PM2.5}$ in low and high flow conditions compared with no flow burns in which the fan did not operate.

The strong negative correlation between MCE and $EF_{PM2.5}$ observed in this study was similar to findings of other studies on emissions of biomass burning. A laboratory-based study by Hosseini et al. (2013) also observed a strong negative relationship (R^2=0.8) between $EF_{PM2.5}$ and MCE. Strong positive correlations between emissions of particulates and other substances negatively associated with the MCE (i.e. organic carbon, carbon monoxide) were also reported (Alves et al., 2011; Desservettaz et al., 2017; Reisen and Brown, 2009), indirectly indicating the negative correlations between particulate emission and MCE. A negative relationship between $EF_{PM2.5}$ and MCE was also reported by McMeeking et al. (2009), however this correlation was not strong (R^2=0.39).

Comparison of EFs with other studies

EFs for $PM_{2.5}$ obtained in this study were compared with values reported for similar vegetation types in Australia and other parts of the world (Table A3.6). The majority of previously reported data on EF_{PM} were obtained from laboratory-based studies. In order to facilitate comparison with other laboratory-based studies and match their combustion conditions as closely as possible, results obtained with no assisted air flow from this study were used for comparison purposes. The $EF_{PM2.5}$ of Spinifex and Kimberley grasses were similar to that reported for Dambo grass, an African sub-tropical grassland, in an experimental study by Chen et al. (2007). The $PM_{2.5}$ emission of Veldt grass was also between the values reported for US temperate grassland/rangeland in experimental studies conducted by Chen et al. (2007) and McMeeking et al. (2009). However, the $EF_{PM2.5}$ for tropical grasses in this study were about 3 to 5-fold lower than those reported for tropical savanna from field-based studies (Desservettaz et al., 2017; Sinha et al., 2003). There are a number of differences between the studies which may account for this discrepancy including differing combustion conditions (i.e. fuel moisture content and wind speed), fuel type and fuel composition. In addition, the study by Desservettaz et al. (2017) had field sampling that was biased towards burns with low MCE. Finally, the difference in the size of particulates collected by the studies (Desservettaz et al., 2017 reported EF for $PM_{0.67}$; Sinha et al., 2003 reported EF_{PM4}) may also contribute to the differences in the EFs reported. If including results obtained with air flow conditions in this study, the $EF_{PM2.5}$ for Spinifex was comparable with the value reported for tropical savanna in the compiled data reported by Akagi et al. (2011).

The $EF_{PM2.5}$ of Banksia was similar to laboratory-based values reported for chaparral by McMeeking et al. (2009) but higher than those also reported for chaparral by Burling et al. (2011)

(field-based) and Hosseini et al.(2013) (laboratory-based). The $EF_{PM2.5}$ of Jarrah was 2-fold lower than that reported in the compilation (Akagi et al., 2011) (Table A3.6).

5.4.3. Metals and PAHs composition of $PM_{2.5}$

Water-soluble metals

Due to the large mass proportion, potassium is used as a marker of aerosols from biomass burning (Garcia-Hurtado et al., 2014; Hosseini et al., 2013; McMeeking et al., 2009). Radojevic (2003) reported that the mass percentage of K in aerosols from bushfire smoke varied from 10 to 20%. In this study, across all vegetation types, $PM_{2.5}$ from combustion of Banksia and Jarrah had higher concentrations of K, accounting for 14 to 15% of PM mass, compared with those from grass burning which had lower K concentrations of 0.5 to 9% of $PM_{2.5}$ mass (Table 5.1). Similar results were found by Chen et al. (2007) who reported elemental K accounting for nearly 24% and less than 1% of $PM_{2.5}$ mass when burning sagebrush and grass, respectively. Zhang et al. (2013) found that $PM_{2.5}$ emitted from burning leaves had a higher K concentration compared with burning wood. Banksia and Jarrah have higher proportion of leaves and therefore might generate $PM_{2.5}$ with higher mass percentages of potassium when compared with grasses.

The composition of the cytoplasm of plant tissue includes the elements K, Na, Ca, Mg and Zn (Andreae et al., 1998), therefore as might be anticipated, the proportion of these metals in vegetation fire derived $PM_{2.5}$ were elevated. These results were consistent with the findings of McMeeking et al. (2009) and Hosseini et al. (2013) who also reported that K, Na, Ca and Mg were abundant inorganic metals in $PM_{2.5}$ emissions from vegetation fires.

The variation in metals concentrations in $PM_{2.5}$ derived from burning different types of vegetation was pronounced in this study, with significant differences observed for most metals between vegetation types. This suggests that vegetation types had distinct chemical compositions. Furthermore, vegetation growing in different soil conditions may uptake different concentrations of metals from soil which is likely another reason of this observation. Combustion conditions did not significantly affect the metal composition of $PM_{2.5}$ for most vegetation types which was expected, given that the concentration of metals in particulates is known to be determined mainly by the chemical composition of vegetation (Chen et al., 2007; Christian et al., 2003; Hosseini et al., 2013). Where differences in metals concentrations were observed between different combustion conditions (namely burning moist Spinifex compared to dry and wet Spinifex) this might be due to differences in sample collection times. The grass used for preparing moist Spinifex was collected separately and consisted of seed-stems, which was different from the grass used for

preparing the dry and wet samples. This may have caused potential differences in fuel composition and hence resulted in the differences in $PM_{2.5}$-bound water-soluble metal concentrations.

PAHs

The abundance of Pyr, Flu, BaP, CHR, BbF and BaA in $PM_{2.5}$-bound PAHs observed in this study was consistent with other studies which also found a similar abundance of particle-phase PAHs emitted from biomass burning (Alves et al., 2010a; Garcia-Hurtado et al., 2014; Hosseini et al., 2013). Alves et al. (2010a) found BaA, Pyr, PA, Flu and CHR being major PAHs in wildfire smoke particles; Garcia-Hurtado et al. (2014) reported BaA, Flu and Pyr as most abundant PAHs in $PM_{2.5}$ and PM_{10} emitted from shrubland fires; Hosseini et al. (2013) observed that BaP, BaA, Pyr, CHR, BaP Flu, BkF were major PAHs bound in $PM_{2.5}$ from wildland fires. The insignificant fraction of low molecular PAHs (2 and 3 rings) was also similar to results of other studies on particle-phase PAHs since these PAHs mainly occur in the vapour phase and therefore might be lost during the processes of PM sampling and the weighing and storing of filters (Choi et al., 2010).

Ratios of individual PAHs, including PA/PA+Ant, Flu/Flu+Pyr, BghiP/BaP and IND/IND+BghiP, have been suggested to apportion possible sources of PAHs (Alves et al., 2011; Hosseini et al., 2013; Tobiszewski and Namieśnik, 2012; Vicente et al., 2012). Table A3.7 presents the ratios of PAHs obtained in this study as well as other studies on vegetation fires and other emission sources. In this study, the ratios of PAHs between different vegetation types were quite similar to each other, with the average PA/PA+Ant, Flu/Flu+Pyr, BghiP/BaP and IND/IND+BghiP ratios in the range of 0.83–0.88, 0.43–0.45, 0.67–0.71 and 0.52–0.53, respectively. The consistency in these PAH ratios in PM across different vegetation types confirms their potential to be used to represent PAH emissions from biomass burning. The PA/PA+Ant and Flu/Flu+Pyr ratios in this study were similar to those reported for forest and chaparral (Hosseini et al., 2013; Vicente et al., 2012). However, they were also close to the ratios for vehicle emissions (Rogge et al., 1993) (Table A3.7). BghiP/BaP ratio in this study was similar to value reported for chaparral but lower than those of shrubland (Alves et al., 2011; Garcia-Hurtado et al., 2014; Hosseini et al., 2013). IND/IND+BghiP of this study was slightly higher than those reported for forest and chaparral but lower than those of shrubland (Alves et al., 2011; Garcia-Hurtado et al., 2014; Hosseini et al., 2013; Vicente et al., 2012). Both ratios of BghiP/BaP and IND/IND+BghiP from vegetation fires were significantly different to those from traffic and vehicle sources (Table A3.7). The results of this study confirm previous suggestions that of the four proposed ratios, BghiP/BaP and IND/IND+BghiP may be the most useful for apportionment purposes to differentiate emissions from biomass burning and traffic/vehicular exhaust (Tobiszewski and Namieśnik, 2012; Vicente et al., 2012).

Potential health effects of $PM_{2.5}$

A number of studies have reported health effects associated with exposure to bushfire smoke derived PM (Chen et al., 2006; Johnston et al., 2007). Therefore, bushfires occurring in vegetation types that generate high emissions of $PM_{2.5}$ such as Jarrah forest, Banksia woodland or Veldt grass may increase the risk of adverse impacts on human health due to the elevated PM emission, especially to people exposed at proximate distance (e.g. firefighters or people in communities close to bushfire events) or vulnerable people (e.g. those with chronic diseases such as asthma or chronic obstructive pulmonary disease). The health effects of $PM_{2.5}$ have also been found to be associated with its chemical components including metals and PAH compounds (Feng et al., 2016; Kim et al., 2013). Metals are considered as toxic PM components since they can induce reactive oxygen species (ROS) that cause cellular inflammation or damage cells exposed to PM (Feng et al., 2016). PAHs can cause both short-term (e.g. irritation and inflammation) and long-term (e.g. cancer and DNA damage) effects in exposed animals and humans (Kim et al., 2013). With the higher concentrations of PAHs and metals, $PM_{2.5}$ generated from Jarrah forest and Banksia woodland could be more hazardous to human health when compared with those from grasslands. However, the higher concentrations of some transition metals (Fe, Cu, Mn, Ni) which can induce ROS and cause oxidative stress (Carter et al., 1997; Feng et al., 2016) in $PM_{2.5}$ from Spinifex may potentially play an important role in impacting human health. It is also noteworthy that the results obtained in this study need to be considered in context of an experimental study with fresh smoke. $PM_{2.5}$ in aged smoke may be chemically transformed and different to that which is newly generated and may therefore also differ in its human health impact (Leonard et al., 2007).

Limitations

There were a number of limitations in this study. Firstly, the use of low pump flow rate when collecting PM (in some cases, see 5.2.2) may increase the size of 50% cut-off (d_{50}) PM collected. Reducing the flow rate by 50% (3.0 to 1.5 $L.min^{-1}$) and 33% (3.0 to 1.0 $L.min^{-1}$) may increase d_{50} 1.4-fold and 1.7-fold, respectively (Trakumas and Smith, 2008). However, since $PM_{2.5}$ is the dominant proportion in PM_{10} in vegetation burning emissions (>98%) PM collected with flow rates of 1.0 and 1.5 $L.min^{-1}$ could be assumed as $PM_{2.5.}$ Secondly, isokinetic PM sampling could not be conducted in this study and this might result in the underestimation of PM emissions from burns with the fan applied. Lastly, the collection of PM from the hot smoke flux (the highest temperature at the sampling area was 57 ºC) might also cause the underestimation of PM collected

since some non-methane organic compounds might have not been condensed completely to the particulate phase (Hosseini et al., 2013).

5.5. Conclusion

This study has investigated emission profiles of PM from fires of typical vegetation types of Western Australia which have not been previously reported. $PM_{2.5}$ was the major component of PM_{10} generated from vegetation fires and had a strong negative correlation with modified combustion efficiency. The emission of $PM_{2.5}$ and its chemical composition was highly dependent on vegetation types. $PM_{2.5}$ emissions from forest and woodland were similar to each other and significantly different from grassland. Combustion of grasslands in different geographical areas also resulted in significantly different emission factors for $PM_{2.5}$.

Variation in combustion conditions influenced the emission of $PM_{2.5}$, with emission factors increased with greater fuel moisture content and higher flow rate. However, the water-soluble metal concentrations in $PM_{2.5}$ appeared to be more dependent on the type of fuel burned rather than the combustion conditions. Mass concentrations of water-soluble metals in $PM_{2.5}$ from forest and woodland were higher than those from grasslands. The EFs for $PM_{2.5}$ and its chemical components provide useful data to predict the emissions and to assess possible effects to human health from bushfires in Australia, especially at local and regional scales.

Chapter 6. TOXICITY OF PARTICULATE MATTER

(There were two people who helped me in conducting research related to this chapter, each contributed about 10% of the work load. Professor Graeme Zosky – University of Tasmania provided advice on designing the toxicological experiments, and Michael Morici – School of Medical and Health Sciences, ECU helped in running the cytokine analysis)

6.1. Introduction

The effects on human health of smoke from bushfires, especially to the human respiratory system, have been investigated in several epidemiological studies. Most studies have found that bushfire PM_{10} (particulates with diameter of less than 10 µm) has negative impacts on human health with an increase in the number of hospital admissions during bushfire episodes (Chen et al., 2006; Crabbe, 2012; Johnston et al., 2007; Morgan et al., 2010; Tham et al., 2009). For example, on days when bushfire events occurred and the ambient PM_{10} concentration exceeded 15 $\mu g.m^{-3}$, the number of people admitted to hospital in Brisbane (Australia) due to respiratory causes increased by 9 to 19 % above the baseline (Chen et al., 2006). Similarly, Johnston et al. (2007) observed a significant increase in respiratory admissions in Darwin (Australia) when the concentration of PM_{10} in ambient air increased by 10 $\mu g.m^{-3}$ as a result of bushfires. However, these studies mainly focused on the epidemiological relationships between bushfire events and cardiorespiratory morbidity and mortality, rather than the toxicological impacts of the smoke.

Whilst several studies have measured the toxicity of atmospheric pollutants in ambient air or from specific sources such as cigarette smoke (Aufderheide and Scheffler, 2011; Cavanagh et al., 2009; Cervellati et al., 2014; Guastadisegni et al., 2010), few studies have been undertaken to measure the toxicity of vegetation fire emissions. A small number of studies have compared the toxicity of ambient particulate matter in usual airshed conditions to periods affected by wildfire emissions (Franzi et al., 2011; Nakayama Wong et al., 2011; Pavagadhi et al., 2013; Wegesser et al., 2009), however, there is a paucity of data focused on the toxicity of smoke generated from vegetation fires.

The majority of PM_{10} emitted from bushfires is $PM_{2.5}$ (>98%) (Radojevic, 2003, Chapter 5). The emission of $PM_{2.5}$ in bushfire smoke, and the associated chemical composition of the particles, has been found to be affected by vegetation types (Chen et al., 2007; Christian et al., 2003; McMeeking et al., 2009, Chapter 5). The differences in composition of chemicals bound in aerosols may affect the toxicity but there have been very few studies that have addressed this issue (Dong et al., 2017).

Cell viability and cytokine production are some common assays have been conducted to test the *in vitro* toxicity of bushfire/biomass burning derived $PM_{2.5}$ (Alves et al., 2014; Danielsen et al.,

2011; Jalava et al., 2006, 2010, 2012; Liu et al., 2005). Cell viability is the most basic assay in in vitro toxicity evaluation (Pavagadhi et al., 2013). Cytokines are proteins secreted from cells and represent the response and interaction between cells (Zhang et al., 2007). Some pro-inflammatory cytokines which promote inflammation are used as marker of inflammation such as interleukin (IL) - 1, IL-6, IL-8 and tumour necrosis factor (TNF)-α (Eisenbrand et al., 2002).

This chapter presents the results related to *in vitro* toxicological experiments investigating and comparing the toxicity of $PM_{2.5}$ derived from burning different vegetation types. The association between chemicals in the $PM_{2.5}$, including water-soluble metals and polycyclic aromatic hydrocarbons (PAHs), on the response was also evaluated.

6.2. Methods and materials

6.2.1. Burning experiments, PM collection and chemical analysis

Details of vegetation sample preparation and burning experiments are described in Chapter 3. $PM_{2.5}$ collection and chemical analysis are described in Chapter 5. Since the water-soluble metals were analysed from different PVC filters to those used for toxicological testing, the concentrations of metals introduced to cells were calculated using the formula: $C = \frac{\sum C_i \times m_i}{\sum m_i}$, where: C was the concentration of a given $PM_{2.5}$-bound metal in a given pooled PM sample used for the PM toxicity test (see 6.2.3), C_i was the measured concentration of that metal in $PM_{2.5}$ on a PVC filter collected in burn i, m_i was the mass of $PM_{2.5}$ on the other PVC filter which was also collected in burn i and used for toxicological testing. PAHs were analysed from one representative filter for each vegetation type in each combustion condition and assumed to be the PAH concentrations in solution which cells were exposed to.

6.2.2. Cell line

To examine the toxicity of $PM_{2.5}$ from vegetation fires, the human alveolar epithelial cell line A549 (ATCC) was used. A549 cells were chosen as epithelial cells are exposed directly to PM when smoke is inhaled. Furthermore, this cell line has been widely used in research relating to the toxicity of air pollutants (Bølling et al., 2012; Danielsen et al., 2011; Karlsson et al., 2006; Pavagadhi et al., 2013).

6.2.3. $PM_{2.5}$ sample preparation

$PM_{2.5}$ collected onto PVC filters was extracted using methanol (Alfa Aesar™>99.8%) for the toxicological testing following the procedure reported by Bølling et al. (2012) and Jalava et al.

(2012, 2010, 2006). Because of the small amount of $PM_{2.5}$ collected in experiments, up to three filters of the same combustion condition were combined to make one PM sample for toxicity analysis. In cases when less than three filters were pooled, blank filters were added to make the total number of pooled filters of three in order to have the same potential effect of filter material on the PM extract. These filters were placed in a pre-weighed glass vial and 15 mL of methanol was added to cover all filters. The PM on filters was extracted into methanol for 3 × 5 minutes in an ultrasonic bath (Branson 2210). After the extraction, filter papers were discarded and the extract was evaporated using a pure nitrogen gas stream (99.999%) until dry. The glass vial was re-weighed to identify the amount of $PM_{2.5}$ extracted and thereafter stored at –20ºC prior to toxicity testing.

6.2.4. Cell culture and exposure

Epithelial cells A549 were cultured in Ham's F-12K (Kaighn's) medium (Gibco®, Cat No. 21127022) supplemented with 10% of Fetal Bovine Serum (FBS, Interpath Services, Victoria, Australia) and 0.5% of Penicillin-Streptomycin (Gibco®, Cat No. 15140122) at 37ºC in 5% CO_2 and were split when the confluence was over 80%. Cells were exposed to $PM_{2.5}$ at passages 6 to 8.

The dry PM samples were resuspended into Ham's media at a concentration of 1000 $\mu g.mL^{-1}$ with manual agitation for 5 minutes, followed by a sonication for 5 minutes. A549 cells were cultured in 96-well plates at a density of 200,000 $cells.mL^{-1}$ (100 $\mu L.well^{-1}$) for cell viability assay and 24-well plates at a density of 100,000 $cells.mL^{-1}$ (500 $\mu L.well^{-1}$) for cytokine assays s overnight. Then the old media was decanted and c PM suspension was added to cells at concentrations of 50 and 500 $\mu g.mL^{-1}$ for 24 hours at 37ºC in 5% CO_2. These concentrations were chosen based on a pilot study using a dose range of 15 to 500 $\mu g.mL^{-1}$ (Appendix 4). The exposure of cells to each PM suspension was undertaken in duplicate in each experiment. The results of cellular responses to each PM suspension were average values of the duplicates. The average coefficients of variation of duplicates in cell viability measurements were 5% and 7% at exposure doses of 50 $\mu g.mL^{-1}$ and 500 $\mu g.mL^{-1}$ (except for measurements with cell viability of lower than 10% at high exposure dose), respectively, and those of IL-8 production measurements were 5% and 6%, respectively.

Extracts from blank PVC filters were prepared with the same preparation steps for sampled filters as described in section 2.4, dissolved in culture media at dilution ratios equivalent to 50 and 500 $\mu g\ PM.mL^{-1}$ and then added to cells to examine the effects of blank filters on the cellular responses. Cells exposed to fresh media only were used as the control.

6.2.5. Cell viability assay

Cell viability after 24 hours of exposure was assessed using 3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) assay (Trevigen TACS® MTT – Cat No. 4890-25-K). The MTT assay was conducted following the manufacturer's instructions (Trevigen). Specifically, 10 µL of MTT reagent was added to each well and the plate was incubated at 37ºC for 2 hours until the purple formazan stained cells were visible. Detergent (100 µL) was then added to each well and the plate was incubated overnight at room temperature. Absorbance was read at 570 nm using EnSprire Multimode Plate Reader (PerkinElmer). The number of viable cells in wells was then determined based on a function of their relative absorbance. In order to take into account the impact of PM on absorbance readings, the background absorbance of PM suspension (without cells) was determined simultaneously and subtracted from the absorbance of exposed cells. The cell viability in each well was calculated using the following formula:

$$Cell\ viability\ (\%) = \frac{No.cell_{sample}}{No.cell_{control}} \times 100$$

Where $No.cell_{sample}$, $No.cell_{control}$ were the number of cells in wells exposed to samples and controls. Samples with the cell viability less than 100% were considered cytotoxic, while those with the cell viability of higher than 100% had cellular proliferation.

6.2.6. Cytokine assays

After 24 hours of exposure, the supernatant was collected, and centrifuged at 1000 ×g for 15 minutes (Eppendorf Centrifuge 5424) to remove particles and cells. The supernatant was then aliquoted and stored at –80ºC for cytokine analysis.

The production of the pro-inflammatory cytokines interleukin (IL)-8 and tumour necrosis factor (TNF)-α were measured using Human Premixed Multi-Analyte Kit following the instructions of the manufacturer (R&D Systems, Minnesota, United States). Briefly, the supernatant collected from cell exposure experiments was mixed with fluorescently labelled magnetic beads and incubated in the dark for 2 hours at room temperature. These microspheres were pre-coated with cytokine-specific antibodies which bound the respective cytokines in the supernatant during incubation. A secondary antibody labelled with biotin was then added. The beads were then treated with streptavidin-phycoerythrin (Streptavidin-PE) and the production of phycoerythrin was used as a fluorescent reporter with the BioRad-Bioplex™ 200 system used for detection.

The IL-8 production was adjusted using the relative viable cell numbers in order to see the IL -8 generation in relation to viability (Table A4.1). Due to the extremely low cell viability (0%) in

some samples and the potential adsorption of IL-8 on PM at the high exposure dose (see 6.4.1), the adjustment was only conducted for results obtained at the low exposure dose.

6.2.7. Statistical analysis

Statistical analysis was conducted using SPSS ver.25 (IBM) with the significance value $p<0.05$. Kruskal-Wallis tests were used to assess the difference in cellular responses between samples and controls, and between two doses of exposure. Relationships between concentrations of individual chemical species and cellular responses were analysed using Spearman's correlation. A principal component analysis (PCA) was conducted for the water-soluble metals and PAHs (excluding five metals Cr, Cd, Ni, Fe and Pb, which had many values below the limit of detection – Table A4.2 - Appendix 4) to identify factors that explained the majority of variance (determined by the scree plot) of chemical data. Prior to the PCA, data on concentrations of chemical species were standardised using z-scoring due to the large differences in their scales. In the preliminary analysis of PCA using a cut-off loading of |0.50|, Acp and Mg were found not to have strong loading on any factors and Zn had similar strong loadings (>0.5) on two factors. Thus these three species were omitted (Matsunaga, 2011) and the PCA was rerun with remaining variables (including 6 water-soluble metals and 15 PAHs). The relative scores of identified factors were used as substitutes for chemical species in the multiple regression analysis with the cellular responses to examine the attributions of $PM_{2.5}$-bound compounds on the *in vitro* toxicity of $PM_{2.5}$.

6.3. Results

6.3.1. Cytotoxicity

Exposure to the higher concentration of $PM_{2.5}$ was associated with decreased cell viability for most vegetation types. At exposure dose of 50 $\mu g.mL^{-1}$, cells exposed to extracts of $PM_{2.5}$ from all vegetation types showed variable cell viability compared with the control. While Spinifex and Veldt grass $PM_{2.5}$ extracts increased the number of cells growing, extracts of $PM_{2.5}$ from Banksia and Jarrah burns decreased the cell viability compared with the control. At the higher exposure dose, $PM_{2.5}$ from Spinifex burns was not cytotoxic, whilst those from burns of other vegetation types decreased the cell viability relative to those obtained at low exposure dose (Figure 6.1, Table A4.1). The cytotoxicity levels varied between vegetation types. $PM_{2.5}$ from Banksia burns reduced cell viability to 84% at 50 $\mu g.mL^{-1}$, and viability fell to 34% at the high exposure dose. The lower exposure concentration of extract from Jarrah burns had a more pronounced impact on cell viability reducing it to 50% and at the higher dose of 500 $\mu g.mL^{-1}$ this went down further to 34%.

Veldt grass derived $PM_{2.5}$ increased cell viability to 129% at the dose of 50 µg.mL^{-1} however this decreased to 103% at 500 µg.mL^{-1} (Figure 6.1, Table A4.1).

$PM_{2.5}$ from Jarrah burning seemed to have the strongest cytotoxic effect on human epithelial cells among the three vegetation types, followed by Banksia and Spinifex (Figure 6.1, left). Banksia and Jarrah PM extracts decreased the cell viability at both exposure concentrations compared with the control. In contrast, the viability of cells exposed to Spinifex burning derived PM extracts increased slightly in comparison with the control, with values of 116% and 114% at doses of 50 µg.mL^{-1} and 500 µg.mL^{-1}, respectively (Table A4.1). For dry grasses, cells exposed to particulates from Spinifex burning yielded lower cell viability at the lower exposure dose but higher cell viability at the higher exposure dose when compared with those treated with Veldt grass $PM_{2.5}$. It is notable that cell viability was not decreased below the control following exposure to PM derived from either type of grass, at either exposure dose (Figure 6.1, right).

Cells exposed to blank filter extract, prepared to be equivalent to the PM extracts at 50 µg.mL^{-1} and 500 µg.mL^{-1}, had cell viability of 118% and 105%, respectively, in a comparison with the control (Table A4.1).

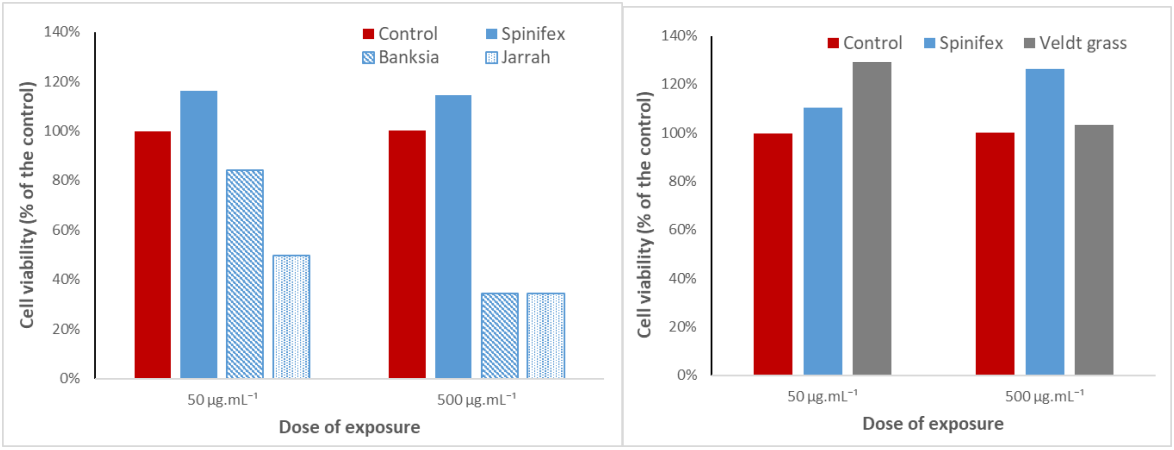


Figure 6-1. Cell viability (presented as percentage of the control) exposed to $PM_{2.5}$ from burning different vegetation types (left, n=9) and two types of dry grass (right, n=3) at two doses of exposure

6.3.2. Cytokine production

The concentrations of the cytokine TNF-α were below the limit of detection (<9 pg.mL^{-1}) in the experimental samples and controls. Cells exposed to the blank filter extract, prepared to be equivalent to the PM extracts at 50 µg.mL^{-1} and 500 µg.mL^{-1}, produced IL-8 of 104% and 103%, respectively, in a comparison with the control (Table A4.1). The effect of blank filters on cellular IL-8 production was negligible.

The IL-8 production of cells exposed to $PM_{2.5}$ varied among different vegetation types and between two doses of exposure (Figure 6.2). At a dose of 50 µg.mL^{-1}, $PM_{2.5}$ from Spinifex and Jarrah burning generated similar average concentrations of IL-8 and higher (1.2-fold) than that produced by the control. These IL-8 production levels were also higher than that of Banksia burning derived $PM_{2.5}$ (Figure 6.2). At dose of 500 µg.mL^{-1}, $PM_{2.5}$ from Banksia burns yielded the highest IL-8 production, 1.9-fold higher than that of the control (Table A4.1). Jarrah derived $PM_{2.5}$ generated a similar amount of IL-8 compared with the control at the high exposure concentration, whilst $PM_{2.5}$ of Spinifex burns caused very low cellular generation of IL-8, which was about one third of the control. The IL-8 production of cells exposed to $PM_{2.5}$ from Banksia and Jarrah were 5.6 and 2.6-fold, respectively, higher than those exposed to $PM_{2.5}$ from Spinifex burning (Figure 6.2, left). The adjusted IL-8 production of cells exposed to Jarrah $PM_{2.5}$ at the lower dose was much higher than those of other vegetation types (3.2 and 2.7-fold of Spinifex and Banksia, respectively) (Table A4.1).

Between the two grasses, the IL-8 production (and the adjusted IL-8 production) of cells exposed to $PM_{2.5}$ from Spinifex burns was higher than that arising from exposure to Veldt grass burning $PM_{2.5}$ at the low exposure dose. By contrast, at the dose of 500 µg.mL^{-1}, cells exposed to the Veldt grass $PM_{2.5}$ produced 4.3-fold higher IL-8 concentration when compared with those exposed to Spinifex burning $PM_{2.5}$ (Figure 6.2 and Table A4.1).

Production of IL-8 was increased in cells treated with $PM_{2.5}$ from Banksia and Veldt grass burning at the higher dose compared with those treated at the lower dose. Meanwhile, Spinifex and Jarrah $PM_{2.5}$ had a reverse dose response with a reduction in the measured concentrations of IL-8 produced when the cells were treated at the higher dose (Table A4.1).

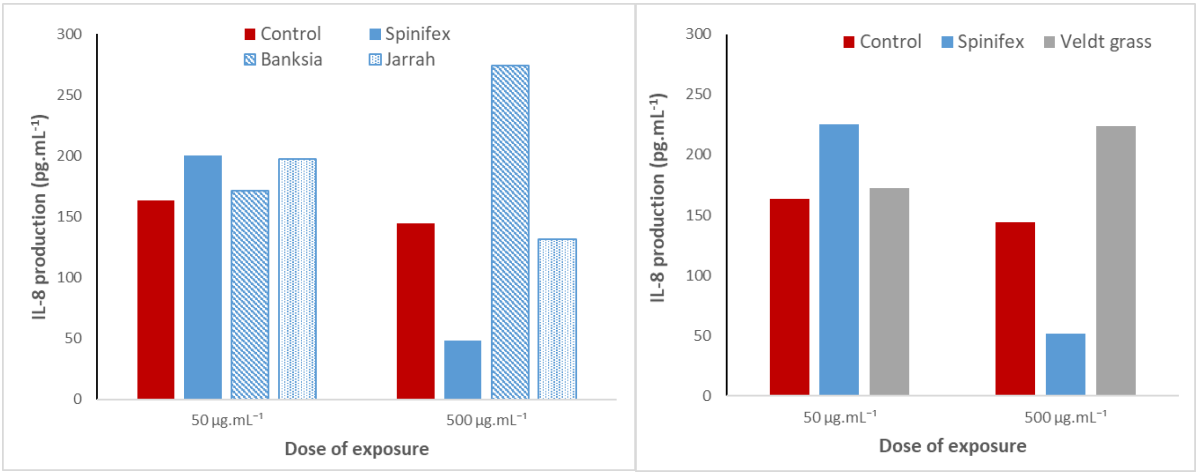


Figure 6-2. Concentration of IL-8 produced by cells exposed to $PM_{2.5}$ from burning different vegetation types (left, n=9) and two types of dry grass (right, n=3) at two doses of exposure

Correlation between cytotoxicity and cytokine production

The cell viability and cytokine production in this study were negatively associated as expected. At the higher exposure dose the correlation between cell viability and IL-8 production was significant but moderate (Spearman's coefficient ρ=0.52), whilst this correlation was weak at the lower dose (ρ=0.04).

6.3.3. Chemical concentrations of $PM_{2.5}$ and correlation with toxicity

Details on chemical composition of $PM_{2.5}$ from vegetation burns were described in Chapter 5. Among the three vegetation types, Jarrah and Banksia had similar total concentrations of metals (the sum of concentrations of individual metals) in $PM_{2.5}$, whilst metal concentration of $PM_{2.5}$ from Spinifex was 3-fold lower than those derived from forest and woodland burning. For the different grassland types, $PM_{2.5}$ from burning dry Veldt grass contained higher concentrations of metals than those found in Spinifex (Table A4.2).

Jarrah burning generated $PM_{2.5}$ containing a much higher concentration of 16 PAHs, around double, than those emitted from burning Spinifex and Banksia. BaP, a carcinogenic PAH, was also present in the highest amount in the $PM_{2.5}$ emitted from Jarrah burning. Total concentrations of PAHs in $PM_{2.5}$ derived from burning two types of dry grass were similar (Table A4.2).

Spearman's correlation coefficients between individual and total concentrations of water-soluble metals/PAHs with toxicological responses are presented in Table 6.1. The correlation analysis excluded some metals including Cr, Cd, Ni, Fe and Pb with low concentrations which were below the limit of detection in many samples. The cellular toxicological responses were more significantly correlated with the concentrations of water-soluble metals in $PM_{2.5}$ than with the concentration of PAHs. The number of viable cells at both doses were negatively correlated with the total concentration of metals. The IL-8 production at the lower dose had no relationship with total metal concentrations, however at the higher dose IL-8 was positively correlated with total metal concentrations (ρ=0.63). Some individual metals had strong correlations (ρ>0.5) with biological responses including K, Na, and Zn (Table 6.1).

Insignificant and weak correlations were observed between total PAH concentration and toxicity endpoints (Table 6.1). The production of IL-8 of cells when exposed to PM extract at a dose of 50 $\mu g.mL^{-1}$ had a significant weak positive correlation (ρ = 0.43) with the total amount of 16 PAHs in $PM_{2.5}$. For individual PAHs, significant correlations were also mainly observed between the amount of IL-8 produced at dose of 50 $\mu g.mL^{-1}$ and some high-molecular PAHs including BbF, BkF, BaP, DBA, BghiP and IND.

Table 6-1. Spearman's correlation coefficients between individual PM-bound water-soluble metals/PAHs and toxicological responses

Concentration ($\mu g.mg^{-1}$ PM)	**Number of viable cells 50 $\mu g.mL^{-1}$**	**Number of viable cells 500 $\mu g.mL^{-1}$**	**IL-8 production ($pg.mL^{-1}$) 50 $\mu g.mL^{-1}$**	**IL-8 production ($pg.mL^{-1}$) 500 $\mu g.mL^{-1}$**
K	**–0.669****	**–0.568****	–0.071	**0.524****
Na	**–0.508****	**–0.644****	**–0.393***	**0.768****
Ca	0.071	0.171	0.025	–0.335
Cu	0.017	0.171	**0.534****	**–0.546****
Zn	**0.537****	0.303	–0.007	0.094
Mg	0.109	0.049	0.083	–0.274
Mn	0.040	0.170	**0.490****	**–0.452****
Al	0.223	0.174	0.217	–0.334
$\sum_{\text{Water-soluble metals}}$	**–0.606****	**–0.598****	–0.158	**0.626****
NaP	–0.121	–0.039	0.342	–0.024
AcPy	–0.218	–0.200	0.086	0.182
Acp	–0.048	–0.065	0.282	–0.270
FL	0.138	0.040	0.100	–0.112
PA	–0.179	–0.233	–0.083	0.246
Ant	–0.053	–0.196	0.033	0.226
Flu	**–0.410***	**–0.377***	0.280	0.074
Pyr	**–0.368***	–0.333	0.328	0.016
BaA	**–0.403****	–0.269	0.335	–0.054
CHR	**–0.397****	–0.244	0.351	–0.042
BbF	–0.250	–0.116	**0.425***	–0.232
BkF	**–0.430****	–0.235	**0.383***	–0.108
BaP	–0.215	–0.080	**0.465***	–0.314
DBA	–0.236	–0.044	**0.449****	–0.280
BghiP	–0.206	–0.088	**0.442***	–0.335
IND	–0.278	–0.121	**0.447***	–0.272
$\sum_{\text{PAHs}}$	–0.342	–0.215	**0.428***	–0.151

** Significant at $p<0.01$; * Significant at $0.01<p<0.05$

PCA identified four factors which explained 85.1% of the variance of the $PM_{2.5}$-bound chemical concentrations included in the analysis. The first factor was characterised by positive loadings of 12 PAHs (NaP, AcPy, Flu, Pyr, BaA, CHR, BbF, BkF, BaP, DBA, IND and BghiP). Factor 2 included 3 PAHs (FL, PA and Ant with positive loading) and Ca (negative loading). Factor 3 was characterised by positive loadings of Mn, Al and Cu. The last factor included K and Na with positive loadings (Table A4.4).

Multiple regression analysis showed that these four factors explained 45.0% (F=5.12; p=0.004) and 50.1% (F=6.28, p=0.001) of the variations in viable cell numbers at the low and high exposure doses, respectively. For IL-8 production, these factors accounted for 47.5% (F=5.65; p=0.002) and 64.8% (F=11.5, p<0.001) of the variations at the low and high exposure doses, respectively. Factor 2 had no significant impact on the toxicity of $PM_{2.5}$, whilst factor 3 only influenced IL-8 production. The cell viability and IL-8 production of exposed cells were mainly influenced by factors 1 and 4 (Table 6.2).

Table 6-2. Coefficients and their relative p-values of regressions between factors and cellular responses.

Cellular response		**Factor 1**	**Factor 2**	**Factor 3**	**Factor 4**
Number of viable cells – 50 µg.mL^{-1}	Coefficient	**–0.446**	–0.048	0.098	**–0.489**
	p-value	*0.006*	*0.747*	*0.514*	*0.003*
Number of viable cells – 500 µg.mL^{-1}	Coefficient	–0.192	–0.091	0.172	**–0.653**
	p-value	*0.187*	*0.524*	*0.235*	*<0.001*
IL-8 (pg.mL^{-1}) – 50 µg.mL^{-1}	Coefficient	**0.347**	0.076	**0.502**	**–0.310**
	p-value	*0.024*	*0.604*	*0.002*	*<0.042*
IL-8 (pg.mL^{-1}) – 500 µg.mL^{-1}	Coefficient	**–0.256**	0.169	**–0.289**	**0.686**
	p-value	*0.041*	*0.168*	*0.022*	*<0.001*

Significant coefficients (at p<0.05) are presented in bold

6.4. Discussion

6.4.1. Toxicity of $PM_{2.5}$ at different exposure doses

The dose-response relationship between cell viability and PM dose observed in this study was similar to that reported in other studies examining the *in vitro* toxicity of particulate matter from biomass burning (Franzi et al., 2011; Jalava et al., 2010). Jalava et al. (2010) reported a decrease of cell viability from about 97% to 48% when exposing mouse macrophages RAW264.7 to extracts of PM_1 from wood burning at doses of 50 µg.mL^{-1} and 300 µg.mL^{-1}, respectively. When treating the RAW264.7 cells with coarse PM collected in a rural area affected by wildfire smoke at different PM amounts of 0, 2.5 and 25 µg (equivalent to doses of 0, 25 and 250 µg.mL^{-1} in the current study), Franzi et al. (2011) observed a decline of live cells from 65% to 50% and then 25%, respectively.

Theoretically, cells experience greater levels of inflammation when exposed to higher doses of PM and induce more inflammatory proteins (Akhtar et al., 2014; Jalava et al., 2006). Mouse macrophages were reported to induce 2 to 3.5-fold and 2 to 3-fold higher amounts of TNF-α and IL-6, respectively, when exposed to wood burning $PM_{2.5-1}$ (particulate with diameter of between 1

and 2.5 µm) at a dose of 300 µg.mL^{-1} than those exposed to a dose of 50 µg.mL^{-1} (Jalava et al., 2006). The cytokine production of cells when exposed to $PM_{2.5}$ from Veldt grass and Banksia burns in this study was similar to the result of Jalava et al. (2006) with increased IL-8 production observed in the higher exposure dose. However, markedly lower production of IL-8 was induced at the higher dose compared with the lower dose for both Jarrah and Spinifex burning derived $PM_{2.5}$. Cell viability was also decreased in response to high concentrations of Jarrah derived $PM_{2.5}$ and it is plausible that occurrence of rapid cell death prevented the induction of cytokine production. Similar results were reported in other *in vitro* toxicological studies which also observed lower cytokine production of cells exposed to extremely high doses of particulates (Akhtar et al., 2010; Michael et al., 2013). The production of IL8 in cells exposed to the higher dose of Spinifex derived PM was lower than that of controls. Given the enhancing impact of the grassland $PM_{2.5}$ extracts on cell number growth, it follows that a component of the Spinifex $PM_{2.5}$ could act to reduce the production of inflammatory cytokines. For example, zinc (Zn) is known to have antioxidant and anti-inflammatory abilities and has been found to reduce cytokine production in cell culture models (Prasad, 2008; Varin et al., 2008), which makes Zn a possible candidate for mediating this effect (see 6.4.3), although in this study Zn concentrations were correlated with cell viability but not IL-8 production. Further, the potential adsorption of IL-8 on PM might also be another reason for the low IL-8 concentrations at the higher exposure dose, particularly in the case of Spinifex $PM_{2.5}$. Akhtar et al. (2010) reported that particulates had the capacity to adsorb IL-8 and this capacity varied depending on PM characteristics such as carbon content, surface area and solubility. This leads to the possibility that the differing composition of PM samples arising from different vegetation types in this study were able to adsorb IL8 to varying degrees, thus further confounding results.

6.4.2. Toxicity of $PM_{2.5}$ from combustion of different vegetation types

The number of *in vitro* toxicological studies on PM from biomass burning and toxicity of PM derived from combustion of different biomass types is limited (Dong et al., 2017). Only one study by Karlsson et al. (2006) examined the toxicity of PM (size was not mentioned) from burning wood and wood pellets and found that combustion of these fuel types generated PM having similar genotoxicity for both sources. This finding was likely due to the same wood nature of these fuel types. However, this study showed the difference in the toxicity of $PM_{2.5}$ derived from burning grassland (Spinifex) compared with forest and woodland (Banksia and Jarrah). Grassland generated $PM_{2.5}$ with no remarkable cytotoxicity (higher cell viability) and less inflammatory effect (lower IL-8 production) on the A549 cells. Between forest and woodland, Jarrah seemed to

be more toxic (lower cell viability and higher IL-8 production) than the Banksia at a dose of 50 $\mu g.mL^{-1}$ but the toxicological difference became less noticeable at the higher exposure dose. Since PM constituents have effects on the biological responses of exposed cells (Jalava et al., 2012; Pavagadhi et al., 2013; Verma et al., 2009), the differences in toxicity between vegetation types found in this study might be explained by the differences in $PM_{2.5}$-bound water-soluble metals and PAH concentrations (Chapter 5). It is reasonable that $PM_{2.5}$ from Jarrah was the most toxic due to its highest concentrations of PAHs and water-soluble metals compared with those derived from combustion of other vegetation types. This finding was supported by a recently published result from an *in-vivo* study by Kim et al. (2018) who tested the toxicity of PM (size was not mentioned) from wildland fuel (including red oak, peat, pine needles, pine and eucalyptus) burns in mice lungs and also found that particulates from eucalyptus had higher lung toxicity (assessed by neutrophil counts) due to their highest concentrations of some PAHs such as PA, Ant and Flu.

6.4.3. Association between chemical composition and toxicity of $PM_{2.5}$

The stronger Spearman's correlation coefficients between total water-soluble metals concentration and number of viable cells compared with those between total PAH concentrations and cell viability (Table 6.1) suggests that cell viability was more likely associated with the water-soluble metals content of the PM. This was confirmed by the results of the PCA and multiple regression analysis which showed that factor 4 (characterised by the positive loadings of K and Na) had significant negative correlation with number of viable cells at both doses (Table 6.2). Similar results were obtained by Bølling et al. (2012) who examined the toxicity of $PM_{0.1\text{-}2.5}$ in woodsmoke on a co-culture of A549 pneumocytes and THP-1 monocytes and observed that the number of viable cells had a stronger correlation with total concentration of elements (R^2=0.47) than with total concentration of PAHs (R^2<0.1). Potassium (K), the abundant element, was found to have the strongest correlation with cell viability (R^2 = 0.64) (Bølling et al., 2012), which is similar to the findings of this current study. Kasurinen et al. (2018) also observed positive correlations between the concentrations of K and Na in PM_1 in woodsmoke and cytotoxicity of PM_1 on A549 cells (assessed by the cellular metabolic activity), however the correlations were not strong. K is a marker of bushfire emissions and the relatively strong negative correlation between K concentration and cell viability observed in this current study emphasises the potential health effects of $PM_{2.5}$ emitted from bushfires.

PAHs were also observed to have a negative impact on cell viability, especially at the low exposure dose, with a significant negative correlation between factor 1 and the cellular response. At the high exposure dose, the correlation was also negative but weaker since the extremely low cell viability

in some samples might have obfuscated the strength of PAH effects. Negative correlations between PAHs and cell viability have also been reported in several studies on *in vitro* toxicity of PM from biomass burning (Jalava et al., 2012; Kasurinen et al., 2018).

Cellular production of IL-8 was associated with both $PM_{2.5}$-bound metal and PAH concentrations. The influence of PM-bound metal contents on IL-8 production of cells was pronounced at the higher exposure dose with a strong and significant positive correlation between IL-8 and total concentrations of metals. In contrast, PAHs showed a clearer influence on IL-8 concentrations at the lower exposure dose with positive correlations obtained between amounts of IL-8 released and concentrations of total PAHs as well as most individual PAHs. Our results were similar to the findings of Jalava et al. (2012) who tested the toxicity of woodsmoke PM on mouse macrophages RAW264.7 and observed significantly positive correlation coefficients between concentrations of genotoxic PAHs including BaA, BbF, BkF, BaP and TNF-α release (ρ=0.56–0.76). Kasurinen et al. (2018) observed strong correlations (ρ>0.78) between IL-8 concentrations produced by human monocytes cells (THP-1) and concentrations of PAH in PM generated from wood burning.

Al, Mn and Cu in $PM_{2.5}$ (associated with factor 3) also affected the IL-8 production of cells. Cells exposed to $PM_{2.5}$ with higher concentrations of these metals induced more IL-8 (Table 6.2). This finding was supported by results of other studies which also found higher inflammatory cytokines induced from cells exposed to biomass burning related PM with higher metal concentrations (Marchetti et al., 2019; Pavagadhi et al., 2013).

Meanwhile, IL-8 production at the higher exposure dose of 500 $\mu g.mL^{-1}$ might be affected by IL-8 adsorption potential of PM and/or extensive cell death for Spinifex and Jarrah PM as previously discussed. The results for these vegetation types distorted the strength and direction of correlations between $PM_{2.5}$ chemical composition and IL-8 induced by cells exposure at the high concentration of PM extracts.

It is noteworthy that there might be some component in $PM_{2.5}$ from Spinifex and Veldt grass which promoted cellular proliferation, resulting in the increase in cell number growth compared with the control at both exposure doses. Grassland-PM samples contained relatively high concentrations of zinc (Zn), which was positively correlated with cell viability (Table 6.1). As Zn is an essential element with many biological roles including in the processes of DNA synthesis and cell proliferation, as well as anti-apoptotic functions, it is a strong potential candidate for mediating the beneficial effects of grassland $PM_{2.5}$ on cell viability (MacDonald, 2000; Truong-Tran et al., 2001).

Limitations

This study has a number of limitations. Firstly, different matrices were used to extract the PM-bound chemical components (Milli-Q water for water-soluble metals and dichloromethane for PAHs) and $PM_{2.5}$ exposed to cells (methanol). The amounts of chemicals that cells were exposed to, therefore, might be different to what was found in the chemical analysis which might result in uncertainty in the correlations between chemical composition of $PM_{2.5}$ and biological responses of the cells. Secondly, endotoxin is often analysed in toxicological studies of ambient aerosols to identify the potential contribution to the inflammatory effects of particulates (Cavanagh et al., 2009). Due to the limited amount of $PM_{2.5}$ collected, endotoxin analysis could not be performed in this study. However, PM derived from biomass burning has been found to generate negligible levels of endotoxin (Franzi et al., 2011). Therefore, endotoxin has not been investigated in many studies on toxicity of biomass burning PM (Danielsen et al., 2011; Jalava et al., 2012, 2010; Karlsson et al., 2006; Kasurinen et al., 2018; Kubátová et al., 2006). Thirdly, the extraction, concentration and resuspension of the PM preparation process might lead to differences in the natural characteristics of the particulates, e.g. size or morphology might change which in turn might also influence their cellular toxicity. Furthermore, the method of exposing cells to PM extracts *in vitro* does not replicate the physiological conditions of PM exposure in individuals, therefore the biological responses of cells *in vitro* may differ to those in the airways of human exposed to bushfire smoke. Finally, as an experimental study with the limitations outlined above care must be taken when interpreting the results of this study. While relative toxicity can be implied, the results cannot be generalised to population or human exposure to bushfire smoke.

6.5. Conclusions

This study examined the biological response of human epithelial cells to $PM_{2.5}$ from burning different vegetation types in experimental conditions in order to investigate the effects of fuel types and $PM_{2.5}$ chemical composition on the toxicity of PM. This study demonstrated that the vegetation fire derived $PM_{2.5}$ provoked adverse impacts on human epithelial cells. The toxicity of $PM_{2.5}$ also varied for different fuel types. The toxicity of different vegetation types was associated with their relative PM-bound chemical compositions. $PM_{2.5}$ from Jarrah burns was the most hazardous, followed by those from Banksia and then Spinifex. Between the two types of grassland, Veldt grass $PM_{2.5}$ was more toxic than Spinifex $PM_{2.5}$. These findings emphasise the need to consider the public health impacts of exposure to bushfire smoke in Australia, since Jarrah, Banksia and Veldt grass are vegetation types growing in close proximity to dense communities. There are several other endpoints for assessing the *in vitro* toxicity such as the generation of oxidative stress

or genotoxicity, which should also be considered as part of a suite of endpoints to better understand the health impacts of bushfire derived PM. Finally this study only measured *in vitro* toxicity of particulate matter, whereas smoke is a complex mixture and studies of whole bushfire smoke would be more informative.

Chapter 7. SYNTHESIS

This study investigated the chemical composition of emissions from combustion of common vegetation types in Western Australia to determine the effect of fuel types and/or combustion conditions on the chemical composition and *in vitro* toxicity of the smoke. To do this pollutants emitted from burning different vegetation types and in different combustion conditions were measured. It also examined the biological effects of $PM_{2.5}$ in vegetation fire smoke using an *in vitro* approach to investigate the role of fuel type on toxicity of bushfire-derived particulates. The study found that emissions profiles of pollutants from burning different vegetation types did vary, as did the *in vitro* toxicity of the $PM_{2.5}$ derived. The effects of combustion conditions on emissions also varied, depending on the pollutant of interest. This section summarises the overall findings of this study and addresses some limitations and implications of this work for future research and considerations for practitioners in their planning and responses to managing smoke impacts on human health.

Chemical composition of smoke produced from the combustion of different vegetation types

Emissions from the combustion of different vegetation types including grassland, woodland and forest varied. Vegetation type did not have a strong influence on the emissions of CO_2, NO_x and most carbonyls (formaldehyde, acetaldehyde, acetone and propionaldehyde). Emissions of other gaseous pollutants such as CO, SO_2, butyraldehyde and benzaldehyde were strongly dependent on the type of vegetation burned, with higher emissions generated from combustion of Jarrah (forest) and Banksia (woodland) than from burns of Spinifex (grassland) (Chapter 3 and 4). Similarly the emission of $PM_{2.5}$ and its components were highly affected by vegetation type with greater $EF_{PM2.5}$, elemental carbon (EC) (referred from mass absorption coefficient), concentrations of $PM_{2.5}$-bound water-soluble metals and $PM_{2.5}$-bound PAHs generated from burns of Banksia and Jarrah compared with those from Spinifex (Chapter 5). Overall among the three types of vegetation, woodland and forest burns yielded similar emissions of pollutants to each other, but emitted significantly higher amounts of air pollutants than the grassland burns.

When the three different types of grassland were considered, the EF_{CO} of the Veldt grass (temperate grass) was 2-fold higher than those of Spinifex and Kimberley grasses (tropical grasses). Consequently, the EF_{CO2} of the Veldt grass was significantly lower than those from the two remaining grasses since a high proportion of Veldt grass burned carbon emitted in the form of CO. These big differences are possibly a consequence of Veldt grass burns occurring mainly in the smouldering phase, whilst the combustion of the two tropical grasslands was largely in the flaming

phase (Chapter 3). The two tropical grasses generated similar emissions for most of the other pollutants, except for SO_2, NO_x and butyraldehyde. The temperate grass emitted much higher amounts of pollutants including CO, formaldehyde, acetaldehyde, VOCs, PM and EC (Chapter 3, 4 and 5). This difference was especially notable for the $EF_{PM2.5}$ of Veldt grass which was 5-fold higher than those from the combustion of Spinifex and Kimberley grass (Chapter 5).

This study has demonstrated that vegetation type plays an important role in influencing the amount of air pollutants emitted from bushfires. The big differences observed in this study were mainly between vegetation types, which is not surprising since each have distinct differences in plant structures (e.g. grassland vs. woodland/forest), they occur in different geographical regions (e.g. tropical vs. temperate) and each also has a distinctive and characteristic chemical composition. These differences should be considered when predicting emissions of bushfires since predictions made from inappropriate reference vegetation types are unlikely to be accurate.

Air pollutant profiles produced from burning Western Australian vegetation types compared with those in other Australian and international studies

Compared with the available data on field studies of bushfire emissions in Australia, the emission factors for CO_2 and CO in this laboratory-based study were similar to those reported for similar vegetation types such as tropical savanna and temperate forest (Chapter 3). The EFs for formaldehyde from this study were lower than those reported in field-based studies (Paton-Walsh et al., 2014, 2005), which might be due to the secondary formation of formaldehyde and the bias toward the smouldering phase of measurements in field studies (Chapter 4). When compared with international data (Hosseini et al., 2013; Liu et al., 2017; McMeeking et al., 2009; Sinha et al., 2003), emission factors for pollutants that were rarely reported in Australian studies such as NO_x, SO_2 and $PM_{2.5}$ were different with those reported for other vegetation types in the world, which most likely reflects the distinct differences in fuel composition (Chapters 3 and 5). These differences also support the need to use EFs obtained from the combustion of local vegetation types to better predict the bushfire emissions of the unique vegetation types of Australia and more particularly Western Australia. The need is more pronounced for vegetation types growing in close proximity to communities, in order to better estimate pollutant concentrations and evaluate the potential for adverse health effects.

Combustion conditions (fuel moisture and air flow rate) and the chemical composition of smoke

Moisture content (in the range <10%–25%), in this experimental study, was not a significant predictor of emissions of gaseous pollutants (Chapters 3 and 4), but it did significantly influence the emissions of $PM_{2.5}$, with greater $EF_{PM2.5}$ obtained from burns of moister fuel (Chapter 5). Although the concentrations of $PM_{2.5}$-bound chemical species (water-soluble metals) were not affected by the fuel moisture content (Chapter 5), the emission factors for these $PM_{2.5}$-bound species were increased with the increase of vegetation moisture content since they were calculated from the $EF_{PM2.5}$. These results suggest that the prescribed burns may have higher emission factors for particulates compared with wildfires since the fuel moisture content in prescribed burns is typically higher than that experienced in wildfires. However, it should be noted that in addition to the fuel moisture content, there are many other factors that differ between prescribed burns and wildfires (e.g. weather conditions, woody and foliage proportions of fuel burned, and fire intensity) which strongly impact the emissions of particulates (Burling et al., 2011; Wardoyo et al., 2006).

Air flow rate was found to have a strong effect on emissions from vegetation fires, with much higher EFs for most of the measured pollutants observed from burns in higher flow rates. It was initially expected that with higher flow rates (meaning higher oxygen supply) the combustion would be more effective and less pollutants would be emitted. However, given the relatively small amount of vegetation sample in each burn (25 or 50g) and the air spread mode (causing heading fires), the high flow rate applied was possibly too intense and strongly reduced the burning temperature, resulting in a reduction in flaming and thus leading to higher emissions of pollutants associated with the smouldering phase, such as CO and carbonyls (Chapters 3 and 4). Furthermore, high air flow rates might have disturbed the ash remaining after the burn, leading to the mobilisation and subsequent extraction of more pollutants (Chapters 3 and 5). With the known significant influence of wind on air pollutant emissions, it is suggested that care should be taken when undertaking prescribed burns to avoid combustion conditions where the flame ceases rapidly (i.e. heading fires) since burning in these conditions is not only less efficient in respect to the fuel load reduction but also may generate more pollutants to the atmosphere.

Toxicity of $PM_{2.5}$ and vegetation type

The *in vitro* toxicity of $PM_{2.5}$ was found to be significantly affected by vegetation type being burned, with particulates from forest and woodland more toxic to A549 cells than those from grasslands. Cells exposed to $PM_{2.5}$ from Banksia and Jarrah burning had lower cell viability and produced more inflammatory cytokine IL-8 than those exposed to Spinifex derived $PM_{2.5}$.

Between forest and woodland, Jarrah seemed to be more toxic than Banksia at the lower exposure dose of 50 $\mu g.mL^{-1}$ but the toxicological difference became less pronounced at the higher exposure dose (Chapter 6).

The influence of vegetation type on relative *in vitro* toxicity of PM from bushfires has not been previously investigated and reported (Dong et al., 2017). The findings of this study have provided preliminary evidence showing the important role of fuel type on the toxicity of $PM_{2.5}$ emitted from vegetation fires. These results were supported by a recently published *in vivo* study which also found that particulate matter derived from burning eucalyptus was more toxic to mice lung cells than those from other fuel types including red oak, peat, pine needles and pine (Kim et al., 2018). However, our knowledge and understanding of the toxicity of emissions from the combustion of different vegetation types is still very limited and requires further investigation.

Higher toxicity of $PM_{2.5}$ was observed at higher exposure doses. Cytotoxicity of $PM_{2.5}$ from burning all types of vegetation followed a dose-response trend with lower cell viability observed when cells were exposed to extracts at higher concentrations of $PM_{2.5}$. Cells exposed to Banksia and Veldt grass burning derived $PM_{2.5}$ also showed cytokine production increasing with increased doses of $PM_{2.5}$ (Chapter 6). This finding confirms the higher risk to human lung health arising from exposure to more concentrated smoke.

Chemical components of $PM_{2.5}$ associated with toxicity

The higher toxicity of $PM_{2.5}$ derived from Jarrah burns compared with $PM_{2.5}$ from other vegetation types appeared to be well explained by the fact that the highest total concentrations of 13 water-soluble metals and 16 PAHs were found in Jarrah $PM_{2.5.}$ This confirms the association between $PM_{2.5}$ chemical composition and its toxicity. The concentrations of 16 PAHs and 6 metals (K, Na, Al, Mn, Cu and Ca) explained 45 to 50% of the variations of cellular responses (except for IL-8 at the high exposure dose which was potentially affected by the adsorption capacity of PM). The cytotoxicity of vegetation fire derived $PM_{2.5}$ was associated with $PM_{2.5}$-bound K and Na concentrations, while the inflammatory impact (IL-8 production) was more strongly associated with the content of genotoxic PAHs (BbF, BkF, BaP, DBA, BghiP and IND), Al, Cu and Mn in the $PM_{2.5}$ (Chapter 6). These results provide useful insights into issues associated with the toxicological evaluation and risk assessment of bushfire-derived $PM_{2.5}$. The potential toxicity of $PM_{2.5}$ can be initially assessed based on concentrations of adsorbed chemical compounds. However, it is noteworthy that the assessed toxicity is only related to the responses investigated in this study (i.e. cellular cytotoxicity and inflammatory cytokine IL-8 production) since different chemicals may have distinct toxicological pathways.

Most of the literature on *in vitro* toxicity of bushfire and open biomass burning smoke has focused on the PM sourced from bushfires after ageing of the particulates during long-distance transport. To date no studies on the *in vitro* biological effects of PM generated at, or near, bushfires in the field have been found, except for a study byLeonard et al. (2007) who examined the PM collected in the field during prescribed burns. The toxicity of PM after long-distance transport might change due to the chemical transformation of unstable components, e.g. organic compounds (Jalava et al., 2006). Therefore, the toxicity of bushfire/open biomass burning PM reported in the majority of studies may not reflect the potential effects on people who are in close proximity to the source of the smoke. The results of my study have provided more information on the toxicity of newly generated particulates, a topic which, together with consideration of ageing during long-range transport, needs attention in future studies both in the field and laboratory.

Study limitations

When compared with field-based measurements, laboratory-based experiments have the advantage that they can capture emissions for the complete fire process (including both flaming and smouldering phases). Field-based measurements, on the other hand, may be biased towards the flaming phase (for measurements using aircraft) or the smouldering phase (for ground-based measurements) (Paton-Walsh et al., 2014). However, the limited amount of fuel burned under experimental conditions in this study may not be fully representative of emissions in practice. In addition, other practical factors such as surface soil conditions, higher variability of fuel components and higher proportions of woody fuel may affect the emissions of bushfires in the field (Possell et al., 2015). This study was also limited by the instrumentation used to measure and sample the smoke. For example the potential cross inference of SO_2 and NO_2 sensors and the inability to quantify VOCs emitted are recognised problems. This study was also limited in its ability to measure PM emissions under isokinetic conditions, which might also have affected the results (Misra et al., 2003).

The study was also limited in its ability to compare with field results due to time, budget and logistics constraints. Even though the MCE and EFs for CO_2 and CO from this study were comparable with other field-based studies of Australia, further studies on smoke emissions in the field should be conducted to validate this laboratory-based approach, especially for pollutants ($PM_{2.5}$, SO_2, NO_x) or fuel types (Banksia woodland and temperate grass) which have not been previously sufficiently studied. It should also be noted that the ageing of pollutants in the field situation may change their emission profiles and impacts compared with laboratory-based data.

Implications and recommendations

This study has shown that the fuel type significantly influenced the emission factors for pollutants from vegetation fires and it provides a set of data on emission factors for the main pollutants from fires of typical vegetation types of Western Australia. With the limited amount of bushfire emission data available from Australian systems, this study provides a significant resource for calculating the emissions inventories of bushfires in Australia. This is especially important for pollutants such as NO_x, SO_2, carbonyls and $PM_{2.5}$. The functions to extrapolate EFs for acetaldehyde, acetone and propionaldehyde from the EF for formaldehyde obtained in this study (Chapter 4) may also help in estimations of the emissions of these non-commonly measured carbonyls from bushfires.

Woodland and forest fires generated the highest emissions of pollutants with a higher toxicity $PM_{2.5}$ than tropical grassland. Among the grassland types, the temperate grass burns generated significantly higher emissions and slightly more toxic $PM_{2.5}$ than the tropical grass. These results suggest greater potential health effects from bushfires in woodland, forest and temperate grassland compared with tropical grassland fires, noting that the area, combustion conditions and proximity of populations will be important factors that affect subsequent human exposure and the potential for impact. Therefore, suitable and effective protection equipment and methods for firefighters who fight bushfires in these ecosystems (woodland, forest and temperate grassland) should be considered. Given the close proximity to communities, the potential for enhanced toxicity with these types of fires also needs further consideration to protect community health.

The biological effects of particulates from vegetation fires on human lung cells was investigated in this study, however, bushfire emissions consist of gaseous pollutants such as CO, SO_2, NO_x, aldehydes and PAHs which also have potential human health impacts. Hence, future research assessing the toxicity of whole bushfire smoke is recommended to enhance our understanding of the potential health impacts of bushfire smoke exposure.

Even though the results of *in vitro* studies may not be able to replicate the physiological responses of the human body to pollutants exactly, this study showed that *in vitro* toxicological testing methods are adequate to provide a valuable preliminary indication and comparison of the toxicity of pollutants from different sources. Different toxicological effects of pollutants can be *in vitro* tested by varying the types of cells used and the biological endpoints measured. Furthermore, *in vitro* toxicity testing also enhances our in-depth understanding of the mechanisms through which a pollutant can affect human health (U.S. National Research Council, 2007). Further studies using *in vitro* approaches are therefore recommended to investigate the toxicity of emissions, not only

from bushfires but also from other sources, to enable better understanding of the health risks that humans face.

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APPENDICES

APPENDIX 1. Supplementary material for Chapter 3

Table A1.1. Agreement in PM mass collected at different positions on a cross-section of the duct at sampling point confirming the well-mixing of the smoke

Flow condition	Position	PM mass collected (µg)	Mean PM mass between positions (µg)	SD of PM mass between positions (µg)	CoV =SD*100/Mean
No - 1	1	1444	1,387	53	3.8%
	2	1381			
	3	1338			
No - 2	1	420	428	31	7.3%
	2	463			
	3	402			
No - 3	1	606	635	27	4.3%
	2	661			
	3	637			
Low - 1	1	288	281	11	4.1%
	2	286			
	3	268			
Low - 2	1	273	268	8	3.0%
	2	273			
	3	259			
Low - 3	1	364	369	14	3.8%
	2	385			
	3	359			
High - 1	1	95	103	9	8.7%
	2	100			
	3	113			
High - 2	1	319	314	6	2.1%
	2	315			
	3	307			
High - 3	1	166	171	8	4.9%
	2	180			
	3	166			

SD: Standard deviation

CoV: Coefficient of Variation

Position 1, 2: close to the edge of the duct; position 3: in the middle of the duct.

Table A1.2. Examples of change in moisture content of vegetation samples after storing at 4°C (before burning)

Weight (g) before storing at 4ºC	Moisture content (%) before storing at 4ºC	Weight (g) before burning	Moisture content (%) before burning	Change in moisture content (%)
W_B	MC_B	W_A	MC_A	$W_A - W_B$
25.3	9.0	25.3	9.0	0
25.4	8.0	25.3	7.6	- 0.4
25.0	6.9	25.0	6.9	0
25.4	8.9	25.5	9.3	+ 0.4
51.0	22.3	51.2	22.6	+ 0.3
50.4	22.3	50.4	22.3	0
50.8	7.7	51.0	8.1	+ 0.4

Moisture content of vegetation sample before burning (after storing at 4ºC) was calculated by using following formula:

$$MC_A = \frac{W_A - W_B \times (1 - MC_B)}{W_A}$$

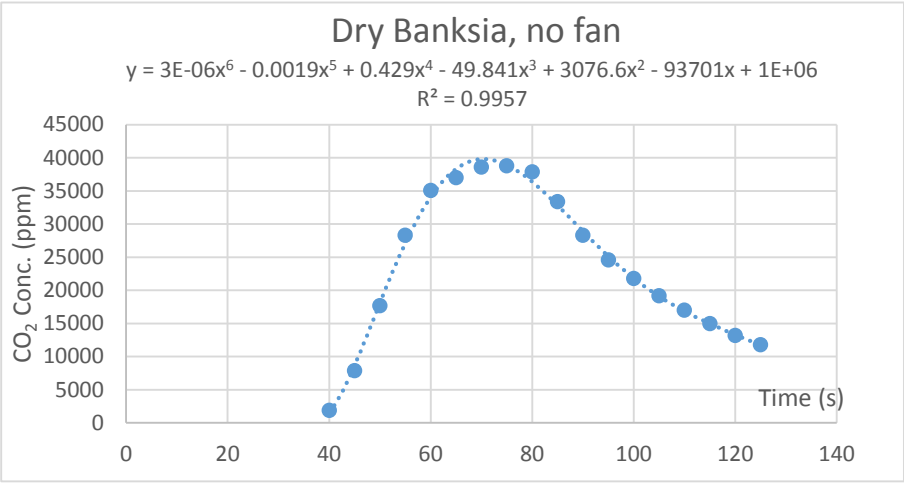
Table A1.3. The comparison of MCE and EFs (g.kg^{-1} dry fuel) of inorganic gases observed in this study with literature

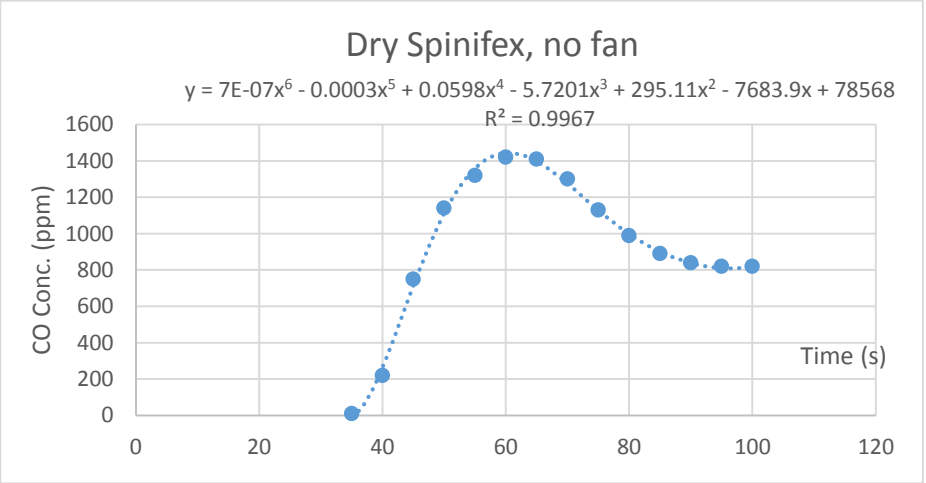
	MCE	EF_{CO2}	EF_{CO}	EF_{SO2}	EF_{NO}	EF_{NO2}	EF_{NOx}[b]	Author	Study type
Grassland									
Spinifex (n=27)	0.948 ± 0.012	1564 ± 20	55 ± 13	0.23 ± 0.16	2.8 ± 0.8	0.59 ± 0.19	3.1 ± 0.8	This study[a]	
Kimberley grass (n=9)	0.942 ± 0.004	1555 ± 6	60 ± 4	0.01 ± 0.01	1.1 ± 0.02	0.96 ± 0.14	1.7 ± 0.1	This study[a]	
Veldt grass (n=9)	0.868 ± 0.040	1432 ± 65	139 ± 42	0.08 ± 0.1	1.8 ± 1.02	0.93 ± 0.31	1.8 ± 0.3	This study[a]	
Dambo grass	0.98 ± 0.00	1607 ± 10	20.1 ± 4.0		1.7 ± 0.1	0.8 ± 0.1		(Chen et al., 2007)	Lab
Australian savanna		1674 ± 56	87 ± 33					(Smith et al., 2014)	Field
		1613 ± 86	88 ± 7					(Shirai et al., 2003)	Field
	0.86 - 0.99	1466 - 1698	15 - 147					(Desservettaz et al., 2016)	Field
African savanna	0.94	1700 ± 60	65 ± 20	0.43 ± 0.30			3.3 ± 0.6	(Sinha et al., 2003)	Field
Savanna		1686 ± 38	63 ± 17	0.48 ± 0.27			3.9 ± 0.8	(Akagi et al., 2011)[b]	Compilation
Woodland and forest									
Banksia woodland (n=27)	0.904 ± 0.011	1592 ± 20	107 ± 13	1.1 ± 1.0	3.2 ± 1.0	0.4 ± 0.3	3.4 ± 1.0	This study[a]	
Coastal plain fuel	0.930 ± 0.029	1632 ± 150	78 ± 28	0.9 ± 1.4	4.5 ± 2.4	0.7 ± 0.4		(McMeeking et al., 2009)	Lab
Chaparral	0.909 ± 0.029	1538 ± 125	93 ± 24	0.4 ± 0.2	1.7 ± 2.2	0.5 ± 0.2		(McMeeking et al., 2009)	Lab
Chaparral	0.945 ± 0.005	1772 ± 41	66 ± 6	0.68 ± 0.13	2.3 ± 0.2	0.6 ± 0.2	2.3 ± 0.3	(Burling et al., 2010)	Lab
Jarrah forest (n=27)	0.896 ± 0.015	1577 ± 27	117 ± 17	1.3 ± 0.8	2.8 ± 0.3	0.30 ± 0.12	2.9 ± 0.3	This study[a]	
Amazon forest species	n/a	1565 ± 128	50 ± 17				2.8 ± 0.8	(Soares Neto et al., 2011)	Lab
Boreal forest	0.917 ± 0.068	1311 ± 325	71 ± 40	0.1 ± 0.1	3.3 ± 1.8	1.6 ± 1.1		(McMeeking et al., 2009)	Lab
Australian temperate forest	0.88 - 0.91	1620 ± 160	120 ± 20					(Paton-Walsh et al., 2014)	Field
	0.92 ± 0.01	1660 ± 170	93 ± 15					(Guérette et al., 2018)	Field
US temperate forest	0.912	1454 ± 78	89.3 ± 28.5	0.32 ± 0.37	0.11 ± 0.11	0.58 ± 0.50	0.5 ± 0.4	(Liu et al., 2017)	Field
Temperate forest		1637 ± 71	89 ± 32				2.5 ± 1.0	(Akagi et al., 2011)	Compilation

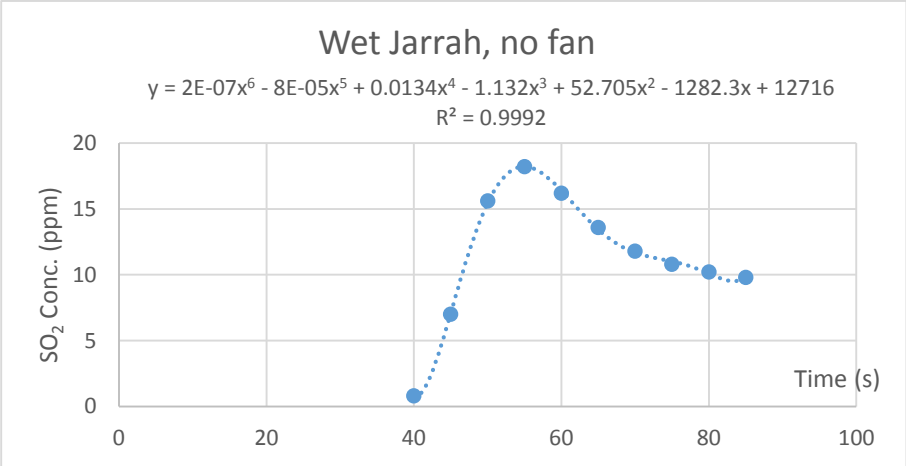
Note: [a]The EF values (of this study) obtained under no fan conditions were used for comparison in order to have combustion (natural ventilation) and sampling conditions (without the effect of exhaust fan) most similar with other studies. [b]NOx calculated as NO.

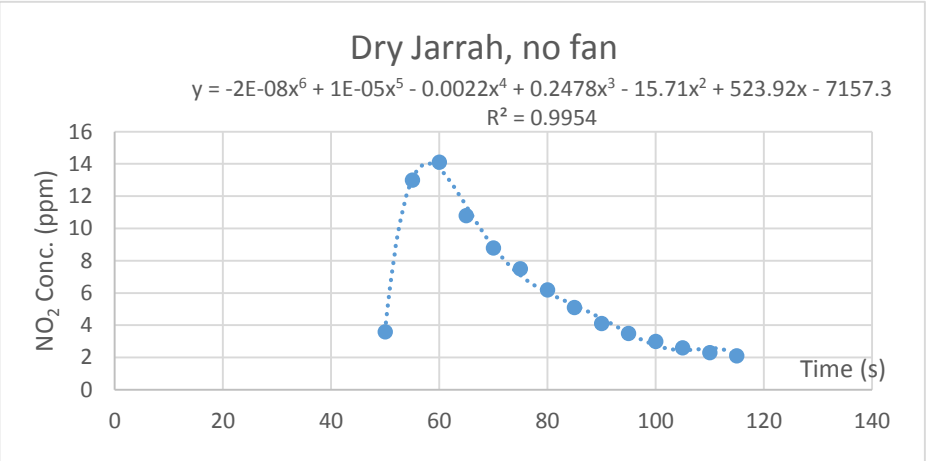
Figure A1.1. Examples of extrapolation of inorganic gases concentration peaks

a) Distribution of concentration peaks with sampling time of samples not over the working range of instrument followed the **6th degree polynomial function**

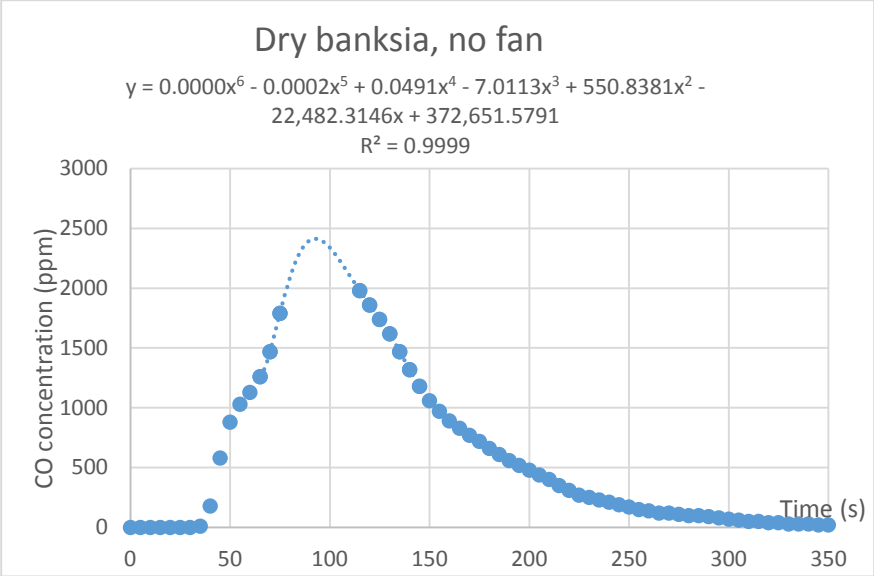








b) Examples of extrapolated concentration peaks



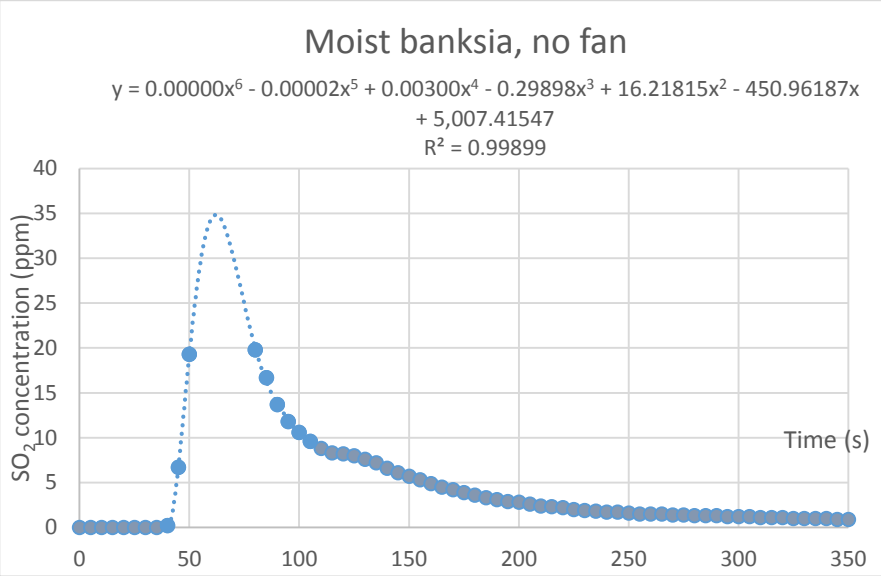
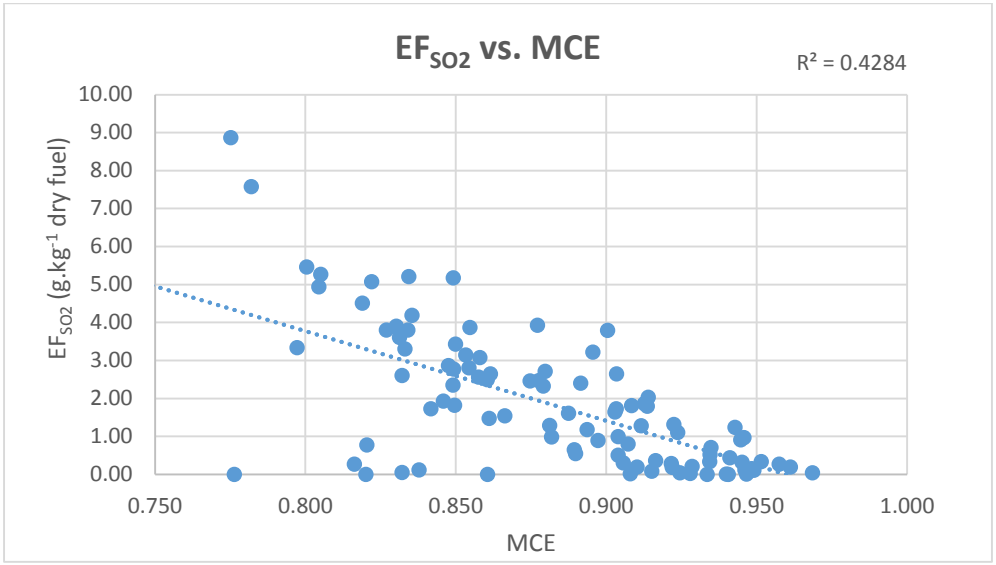
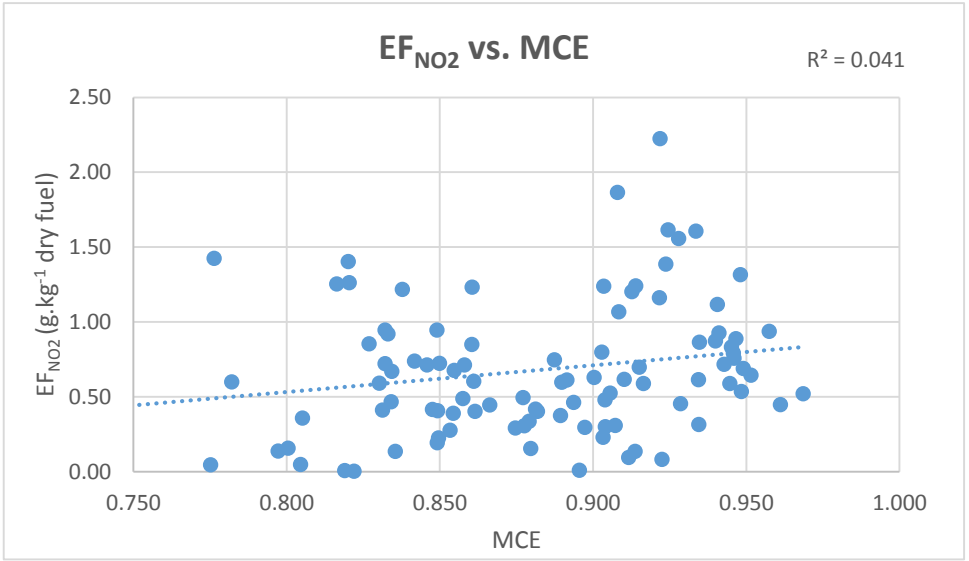
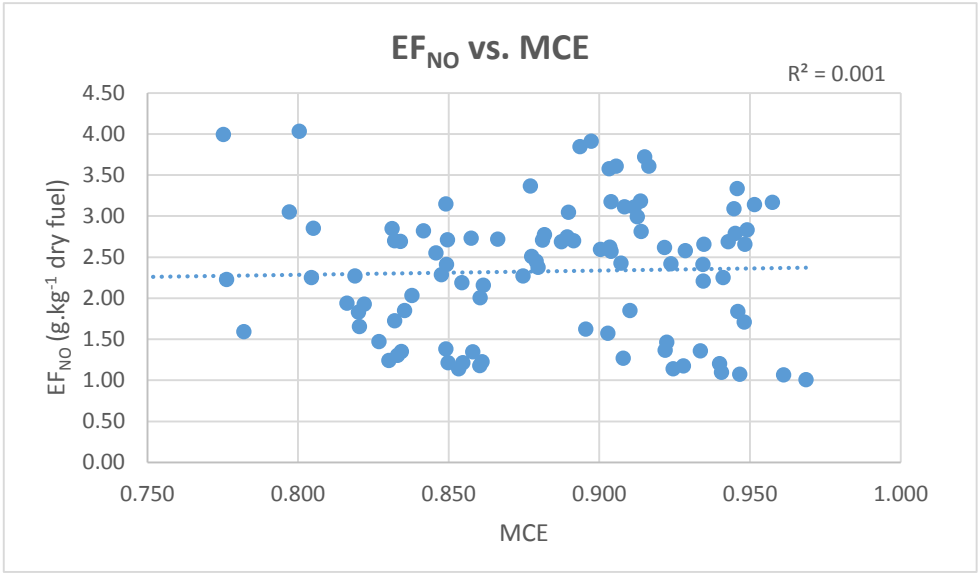


Figure A1.2. Relationship between EFs for SO_2, NO, NO_2 and NO_x (as NO) and MCE of vegetation burns (n=99, for all combustion conditions)







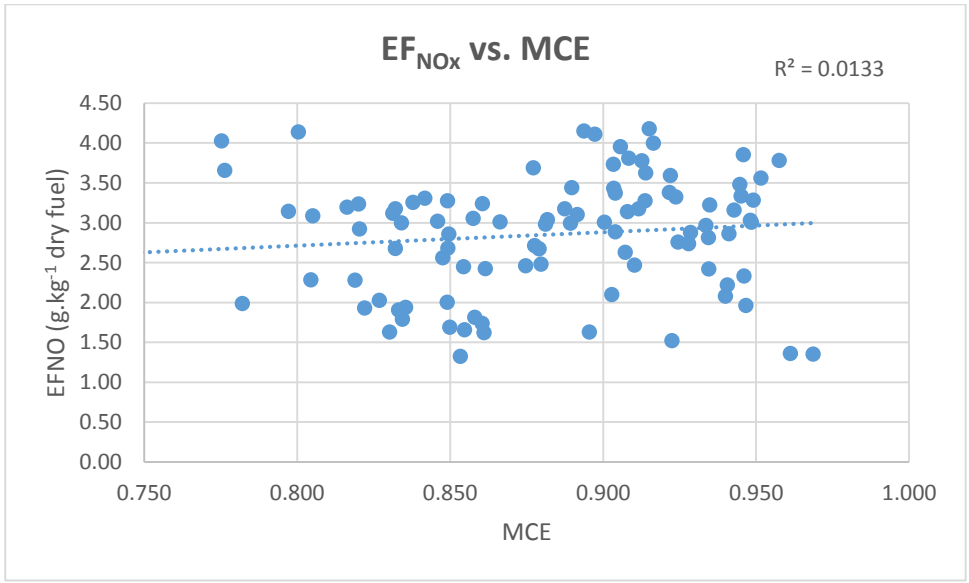
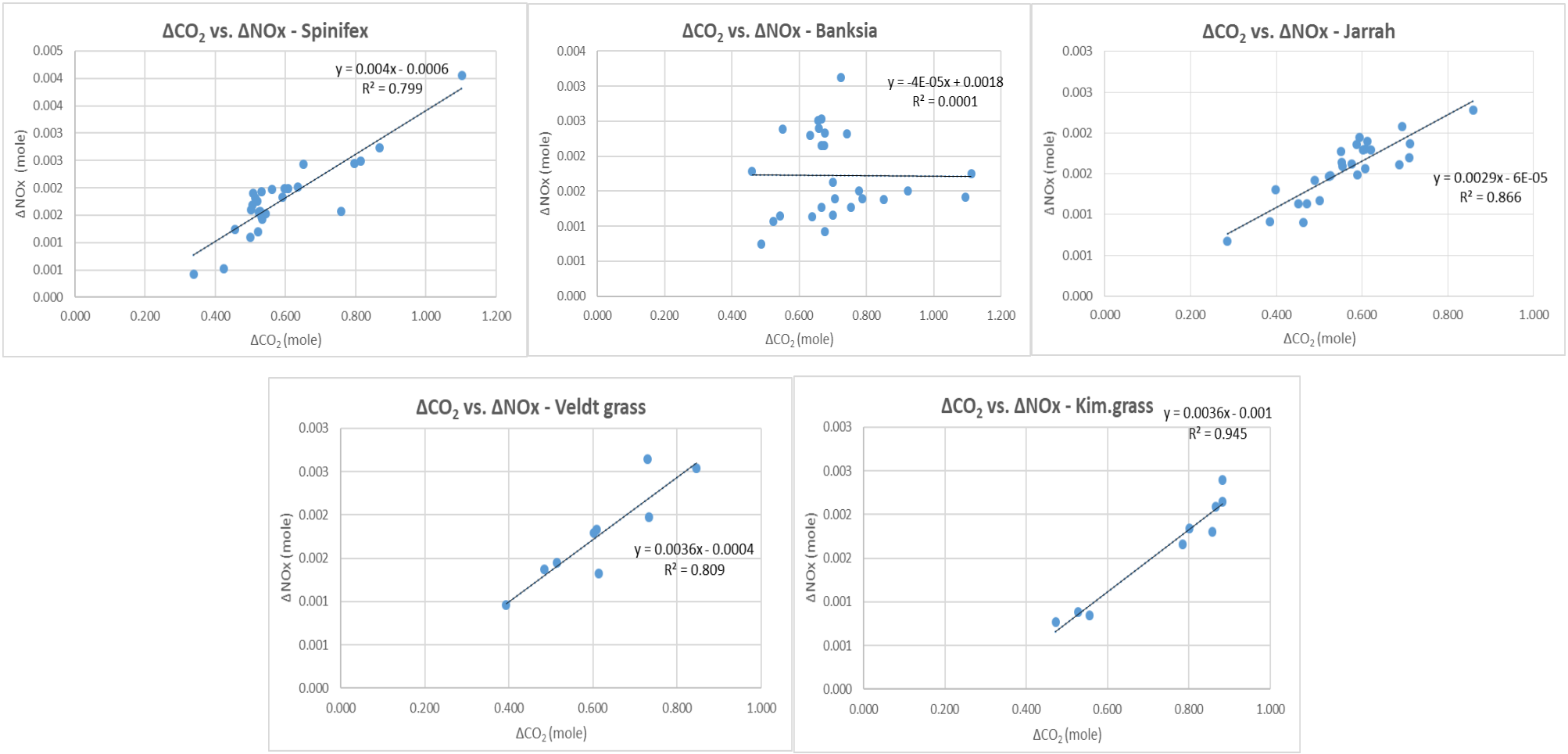


Figure A1.3. Relationship between number of moles of CO_2 and NO_x (as NO) emitted from burns of individual vegetation types



APPENDIX 2. Supplementary material for Chapter 4

Table A2.1. Concentrations of carbonyls in blank burns ($mg.m^{-3}$) and ratio of blank burns to the vegetation fire samples

	n	CH_2O	CH_3CHO	$CH_2=CHCHO$	CH_3COCH_3	CH_3CH_2CHO	$CH_3CH_2CH_2CHO$	C_6H_5CHO
Blank burns	12	0.071	0.005	<LoD	0.0003	0.001	0.006	0.006
No fan	4	0.145	0.010	<LoD	0.0004	0.002	0.007	0.0004
Low fan	4	0.046	0.003	<LoD	0.0002	0.0003	0.005	0.002
High fan	4	0.021	0.002	<LoD	0.0002	0.001	0.005	0.0004
Samples (average)	90	4.942	2.813	0.865	0.588	0.394	0.197	0.470
No fan	36	7.598	4.375	1.883	0.633	0.548	0.233	0.932
Low fan	27	4.116	2.290	0.420	0.645	0.350	0.203	0.307
High fan	27	3.113	1.775	0.293	0.486	0.283	0.154	0.171
Blank burns - samples ratio		1.4%	0.2%	NA	0.1%	0.3%	3.0%	1.3%
No fan		1.9%	0.2%	NA	0.1%	0.4%	3.0%	0.0%
Low fan		1.1%	0.1%	NA	0.0%	0.1%	2.5%	0.7%
High fan		0.7%	0.1%	NA	0.0%	0.4%	3.2%	0.2%

Formaldehyde - CH_2O; Acetaldehyde - CH_3CHO; Acrolein - $CH_2=CHCHO$; Acetone - CH_3COCH_3; Propionaldehyde - CH_3CH_2CHO; Butyraldehyde - $CH_3CH_2CH_2CHO$; Benzaldehyde - C_6H_5CHO

Table A2.2. Result on testing breakthrough (pilot test). Numbers in table are the ratios of carbonyl amounts found in the 2nd section to those in the 1st section of the DNPH tubes

Amount of vegetation	Time of sampling	CH_2O	CH_3CHO	$CH_2=CHCHO$	CH_3COCH_3	CH_3CH_2CHO	$CH_3CH_2CH_2CHO$	C_6H_5CHO
250 g	15 min	61%	22%	>100%	33%	0%	35%	22%
250 g	15 min	25%	82%	>100%	8%	0%	17%	18%
50 g	6 min	1%	23%	26%	0%	8%	0%	0%
50 g	6 min	31%	25%	>100%	5%	0%	12%	0%
50 g	6 min	10%	18%	0%	0%	32%	24%	13%

The 2,4-DNPH tube consists of two sections of sorbent. The 1st section includes 300 mg sorbent, the 2nd one contains 150 mg sorbent. In order to determine whether there was breakthrough, two sections were extracted and analysed for carbonyls separately. Since the sorbent amount of 2nd section was half of that of the 1st section, there was potential breakthrough if the amounts of carbonyl found in 2nd section was >50% of those in the 1st section.

Table A2.3. QA/QC parameters for carbonyl analysis

	Chemical formula	R^2 of the standard curve	Intra-day coefficient of variation (%)	Accuracy of standard (%)	LoD ($mg.L^{-1}$)	Conc. in reagent[#] ($ng.L^{-1}$)
Formaldehyde	CH_2O	0.9994-0.9999	0.4 - 2.5	97-99	0.007	1.47
Acetaldehyde	CH_3CHO	0.9982-0.9998	0.4 - 2.9	95-99	0.006	ND
Acrolein	CH_2=CHCHO	0.9986-0.9995	0.2 - 5.7	94-100	0.001*	ND
Acetone	CH_3COCH_3	0.9984-0.9995	0.0 - 8.5	98-100	0.08	ND
Propionaldehyde	CH_3CH_2CHO	0.9984-0.9995	0.3 - 5.8	96-98	0.001*	ND
Butyraldehyde	$CH_3CH_2CH_2CHO$	0.9984-0.9994	0.4 - 9.6	97-99	0.001*	ND
Benzaldehyde	C_6H_5CHO	0.9985-0.9994	3.3 - 8.9	96-101	0.001*	ND

Limit of detection (LoD) of a compound was calculated as 3 times of the standard deviation of this compound in blank tubes. In cases that the standard deviations could not be identified (e.g. concentration was lower than the method detection limit), method detection limits were used (marked as asterisk). [#] The purity of reagent (acetonitrile) was accessed as instructed by TO-11A (USEPA, 1999b). Acetonitrile reagent was mixed in a ratio 4:1 with desorbing solution of a blank tube. Then both the blank tube desorbing solution and the mixed solution were analysed for carbonyl contents. The carbonyl concentrations in the acetonitrile reagent were then calculated using mass balance method (USEPA, 1999b). ND – not detected (n=3).

Table A2.4. Mean concentrations of carbonyls from burning vegetation (Unit: mg.m^{-3}) and distributions of emission (concentration/EF) of individual carbonyls (presented as % of emission of total carbonyls)

Type of vegetation	n		CH_2O	CH_3CHO	CH_3COCH_3	CH_3CH_2CHO	$CH_3CH_2CH_2CHO$	C_6H_5CHO	Total carbonyls
Spinifex	27	Conc.	3.46	2.09	0.38	0.29	0.11	0.25	6.50
		% of total	52%	32%	6%	5%	2%	3%	100%
Kimberley grass	9	Conc.	4.51	2.27	0.25	0.19	1.38	0.31	12.6
		% of total	51%	25%	2%	2%	16%	3%	100%
Veldt	9	Conc.	11.0	5.65	0.66	0.79	0.25	0.37	21.3
		% of total	57%	31%	4%	4%	1%	2%	100%
Banksia	27	Conc.	4.35	2.99	0.68	0.41	0.23	0.48	10.1
		% of total	48%	32%	8%	4%	3%	5%	100%
Jarrah	27	Conc.	5.17	2.52	0.69	0.36	0.23	0.71	10.6
		% of total	53%	26%	8%	4%	3%	6%	100%

Note: The mean values for Spinifex, Banksia and Jarrah were the average values of nine combustion conditions (3 moisture contents × 3 flow rates); whilst the mean value for Kimberley grass and Veldt grass were the average values of three combustion conditions (1 moisture content × 3 flow rates).

Table A2.5. R^2 coefficient of correlations between EFs for carbonyls (n=99)

	EF_{CH2O}	EF_{CH3CHO}	$EF_{CH3COCH3}$	$EF_{CH3CH2CHO}$	$EF_{CH3CH2CH2CHO}$	$EF_{C6H5CHO}$
EF_{CH2O}	1.000	0.930**	0.720**	0.894**	0.153**	0.447**
EF_{CH3CHO}		1.000	0.736**	0.957**	0.111**	0.365**
$EF_{CH3COCH3}$			1.000	0.806**	0.045*	0.476**
$EF_{CH3CH2CHO}$				1.000	0.044*	0.366**
$EF_{CH3CH2CH2CHO}$					1.000	0.113*
$EF_{C6H5CHO}$						1.000

**Correlation was significant with p<0.01; * Correlation was significant with 0.01<p<0.05.

Table A2.6. Coefficient R^2 and p-value of regression between MCE and EFs for carbonyls

Correlation	All veg. types (n=33)		Spinifex (n=9)		Kim. grass (n=3)		Veldt (n=3)		Banksia (n=9)		Jarrah (n=9)	
	R^2	**p-value**	**R^2**	**p-value**	**R^2**	**p-value**	**R^2**	**p-value**	**R^2**	**p-value**	**R^2**	**p-value**
MCE - EF_{CH2O}	**0.362**	**<0.001**	0.236	0.185	0.826	0.274	0.851	0.253	**0.888**	**<0.001**	0.381	0.077
MCE - EF_{CH3CHO}	**0.340**	**<0.001**	0.194	0.236	0.755	0.314	0.800	0.295	**0.836**	**0.001**	0.353	0.091
MCE - $EF_{CH3COCH3}$	**0.513**	**<0.001**	0.260	0.160	0.734	0.345	0.884	0.222	**0.897**	**<0.001**	0.386	0.074
MCE - $EF_{CH3CH2CHO}$	**0.383**	**<0.001**	0.241	0.180	0.808	0.289	0.808	0.289	**0.868**	**<0.001**	0.435	0.053
MCE - $EF_{CH3CH2CH2CHO}$	0.005	0.692	0.108	0.389	0.826	0.274	0.770	0.319	**0.809**	**0.001**	0.346	0.096
MCE - $EF_{C6H5CHO}$	**0.383**	**<0.001**	0.001	0.934	0.697	0.371	0.747	0.336	**0.768**	**0.002**	0.005	0.854

Significant R^2 and p-value are presented in bold.

Table A2.7. Detected percentage of VOCs in smoke from vegetation fires

VOCs	All veg.	Spinifex				Veldt		Banksia			Jarrah			
	type	Dry	Moist	Wet	Total	Dry	Dry	Moist	Wet	Total	Dry	Moist	Wet	Total
Benzene	**88%**	56%	78%	100%	**78%**	**100%**	89%	89%	89%	**89%**	100%	100%	78%	**93%**
Toluene	**81%**	33%	56%	100%	**63%**	**100%**	89%	89%	67%	**81%**	89%	100%	89%	**93%**
Furfural	**49%**	78%	89%	67%	**78%**	**67%**	11%	-	-	**4%**	67%	44%	67%	**59%**
Ethylbenzene	**40%**	-	44%	22%	**22%**	**22%**	44%	56%	11%	**37%**	67%	78%	56%	**67%**
Xylene	**53%**	11%	56%	33%	**33%**	**33%**	78%	78%	0%	**52%**	100%	100%	44%	**81%**
Styrene	**71%**	78%	78%	56%	**70%**	**67%**	78%	78%	22%	**59%**	89%	89%	78%	**85%**
Benzaldehyde	**53%**	89%	100%	56%	**81%**	**56%**	-	-	-	**-**	44%	89%	100%	**78%**
Benzonitrile	**17%**	44%	22%	22%	**30%**	**22%**	-	-	11%	**4%**	22%	-	22%	**15%**
Isopropylbenzene	**10%**	-	-	-	**-**	**-**	11%	-	-	**4%**	33%	56%	-	**30%**
Phenol	**21%**	78%	11%	44%	**44%**	**11%**	11%	-	-	**4%**	11%	11%	33%	**19%**
Benzofuran	**42%**	78%	22%	56%	**52%**	**33%**	11%	33%	-	**15%**	78%	56%	56%	**63%**
m-Cymene	**23%**	-	-	-	**-**	**-**	-	-	-	**-**	78%	89%	67%	**78%**
Indene	**69%**	11%	56%	89%	**52%**	**100%**	78%	67%	56%	**67%**	100%	89%	44%	**78%**
p-Cymenene	**4%**	-	-	-	**-**	**-**	-	-	-	**-**	-	-	44%	**15%**
Naphthalene	**40%**	56%	56%	56%	**56%**	**33%**	11%	-	-	**4%**	100%	78%	11%	**63%**

Percentage was calculated by dividing the number of burns in which VOCs were detected to the total number of burns conducted. 9 burns were conducted for each type of vegetation in each level of moisture content, so there were 27 burns for Spinifex, Banksia and Jarrah; 9 burns for Veldt grass; and total of 90 burns for all vegetation types. "-" means 0%.

Table A2.8. Comparison of EFs for carbonyls from vegetation fires obtained in this study and from literature

	EF_{CH2O}	EF_{CH3CHO}	$EF_{CH3COCH3}$	$EF_{CH3CH2CHO}$	Author	Study type
Grassland						
Spinifex	1.01 ± 0.66	0.57 ± 0.35	0.11 ± 0.07	0.09 ± 0.06	This study	Lab
Kimberley grass	0.86 ± 0.41	0.42 ± 0.18	0.02 ± 0.02	0.03 ± 0.03	This study	Lab
Veldt grass	2.25 ± 1.28	1.34 ± 0.97	0.26 ± 0.28	0.21 ± 0.17	This study	Lab
Dambo grass	0.60	2.13	1.20	0.19	(Yokelson et al., 2008)	Lab
African savanna	1.12	0.76	0.19		(Christian et al., 2003)	Lab & Field
Australian savanna	1.5 ± 0.5				(Paton-Walsh et al., 2010)	Field
	1.6 ± 0.4				(Smith et al., 2014)	Field
Savanna	1.73 ± 1.22	1.55 ± 0.75	0.63 ± 0.17		(Akagi et al., 2011)	Compilation
Woodland						
Banksia woodland	0.94 ± 0.66	0.57 ± 0.35	0.17 ± 0.13	0.08 ± 0.05	This study	Lab
Chaparral	0.35 ± 0.14				(Burling et al., 2010)	Lab
Temperate forest						
Jarrah forest (n=27)	1.18 ± 0.74	0.60 ± 0.42	0.20 ± 0.16	0.10 ± 0.07	This study	Lab
Eucalyptus	0.27	0.92	0.61	0.10	(Yokelson et al., 2008)	Lab
Australian temperate	1.7 ± 0.4				(Guérette et al., 2018)	Field
forest	2.6 ± 1.2				(Paton-Walsh et al., 2005)	Field
US Temperate forest	2.29 ± 0.27	1.64 ± 0.52			(Liu et al., 2017)	Field
Forest (Portugal)	0.09 - 0.96	1.03 - 1.87		0.03 - 0.17	(Vicente et al., 2012)	Field, Tedlar bags
Temperate forest	2.27 ± 1.13				(Akagi et al., 2011)	Compilation

There is no comparison for butyraldehyde and benzaldehyde due to the lack of data on these compounds from other studies.

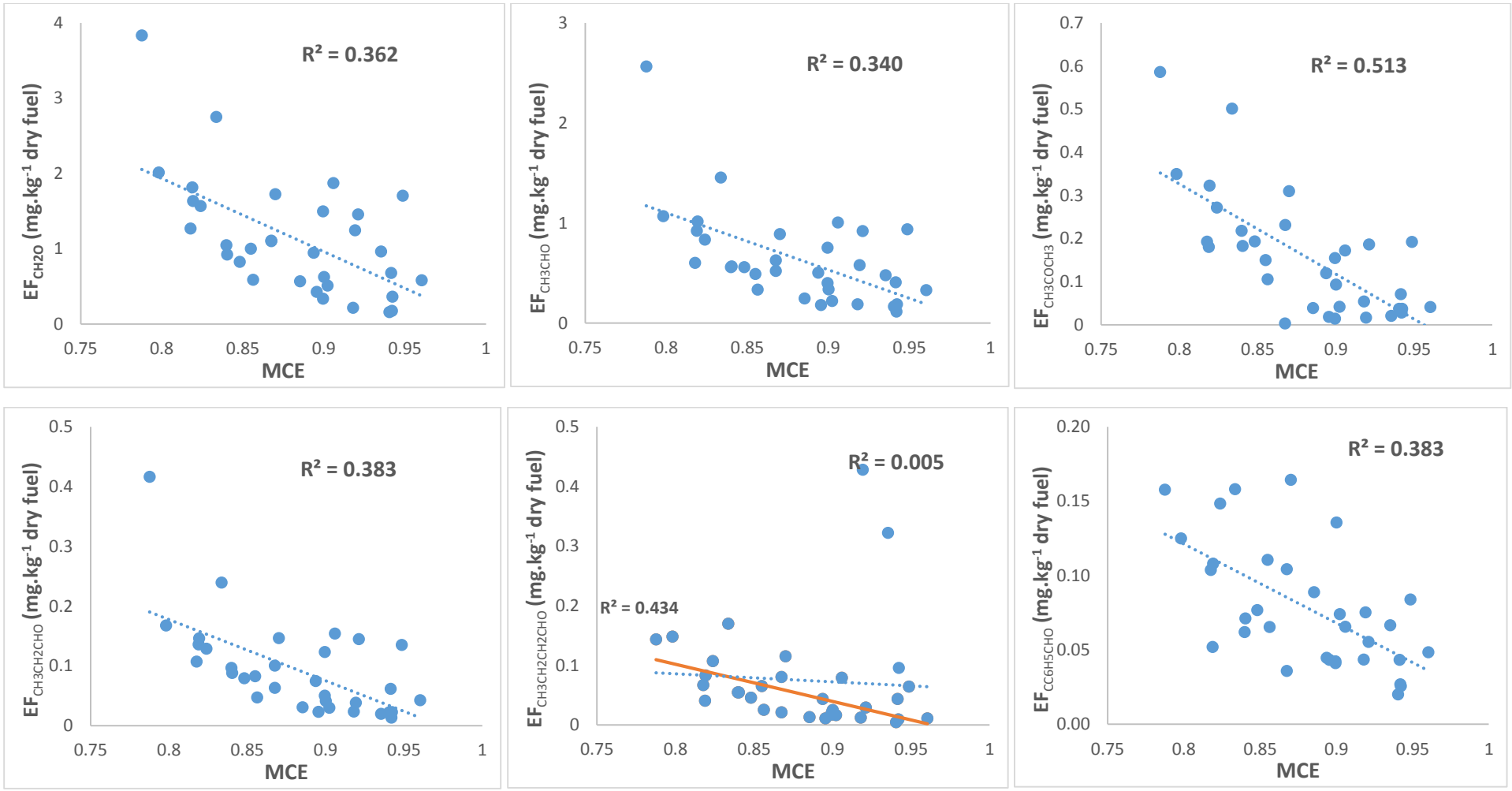
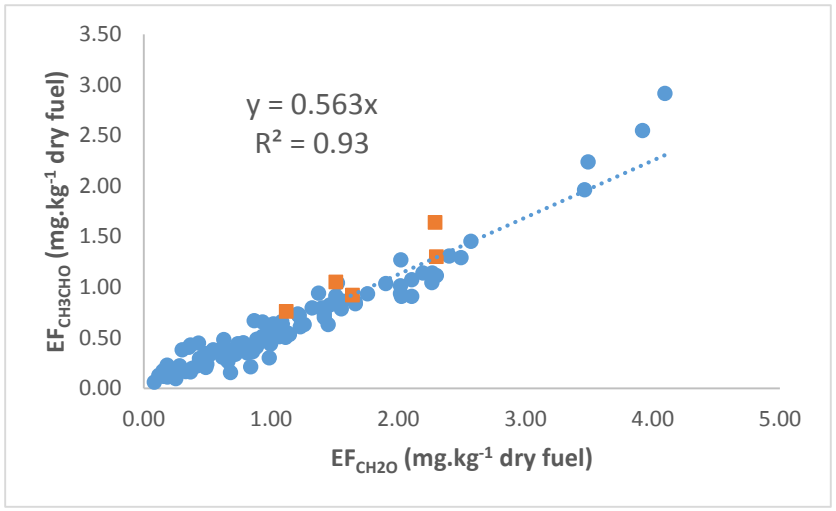
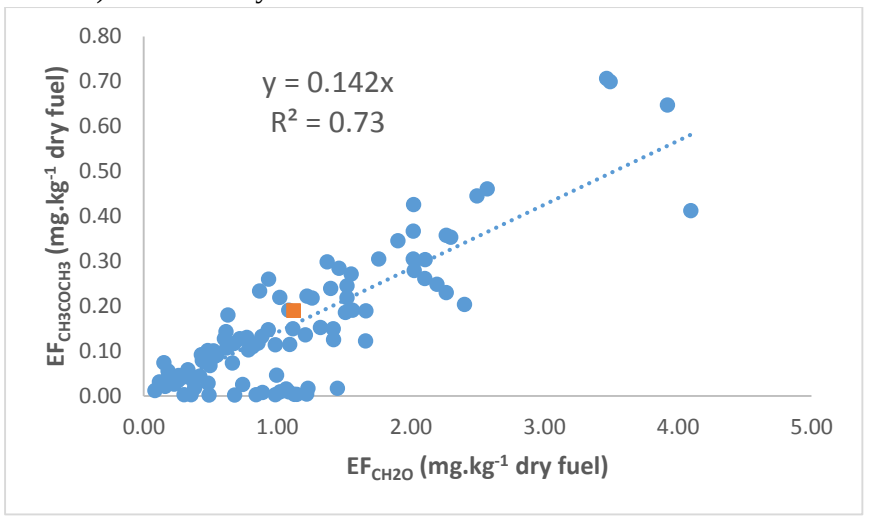


Figure A2.1. Regression relationships between MCE and EFs for carbonyl. The solid orange line in the graph of MCE-EF $_{CH3CH2CH2CHO}$ shows the regression between MCE and EF for butyraldehyde when excluding data from burns of Kimberley grass

a) Formaldehyde - Acetaldehyde



b) Formaldehyde - Acetone



c) Formaldehyde – Propionaldehyde

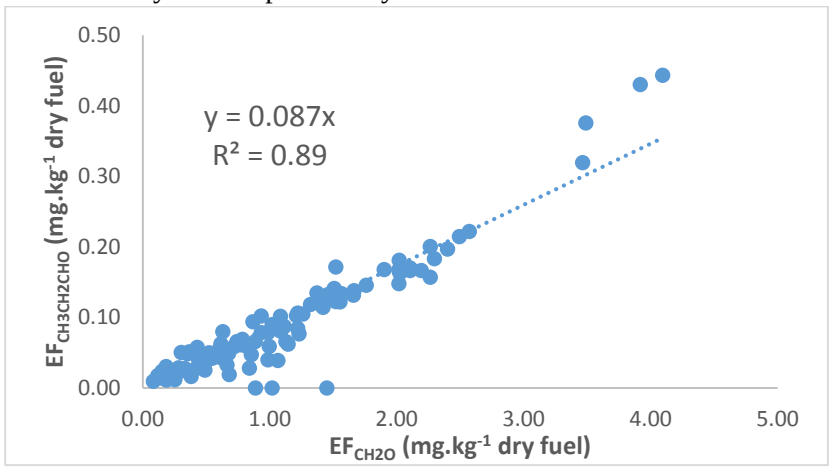


Figure A2.2. Linear relationships between EFs for formaldehyde and acetaldehyde (a), acetone (b) and propionaldehyde (c) (n=99). Round blue dots showing the data obtained in this study; square orange dots showing the values reported in other studies (Christian et al. (2003) - African savanna; Burling et al. (2011) – US conifer forest; Lawson et al. (2015) – Australian coastal heathland; Liu et al., (2017) – US temperate forest; and Guérette et al. (2018) – Australian temperate forest)

APPENDIX 3. Supplementary material for Chapter 5

Table A3.1. QA/QC parameters for PM water-soluble metal analysis

	R^2 of the standard curve	Recovery rate (%)	Intra-day coefficient of variation (%)	Accuracy of standard (%)	Limit of detection ($\mu g.mg^{-1}$)
Na	0.9997	106	1.0 - 2.3	102 - 107	<0.004
K	0.9999	106	NA	101 - 109	<0.0004
Ca	0.9996	112	1.8 - 3.6	101	<0.002
Ni	0.9999	112	1.7 - 12.3	105 - 107	<0.0001
Fe	0.9999	109	2.4 - 13.8	108 - 109	<0.003
Cu	0.9998	110	1.7 - 12.8	107 - 108	<0.003
Cd	1.0000	105	0.2 - 12.9	102 - 107	<0.0001
Cr	1.0000	111	2.4 - 9.3	103 - 104	<0.0001
Pb	1.0000	86	NA	104 - 107	<0.0002
Zn	1.0000	111	0.8 - 6.3	98 - 99	<0.0001
Mg	0.9997	102	0.5 - 11.3	97 - 102	<0.004
Mn	0.9999	113	3.3 - 5.6	102 - 103	<0.0002
Al	1.0000	98	1.6 - 18.3	96 - 114	<0.0005

Limit of detection (LoD) in $\mu g.mg^{-1}$ of PM was calculated by dividing the LoD (in µg of metals/sample) to the mean $PM_{2.5}$ mass (in mg/sample)

Table A3.2. QA/QC parameters for 16 USEPA polycyclic aromatic hydrocarbon (PAHs) in $PM_{2.5}$

PAHs	Abbreviation	Number of rings	LoD ($ng.mg^{-1}$)	% of measured and certified values of SRM
Naphthalene	NaP	2	0.21	90%
Acenaphthylene	AcPy	3	0.001	NA
Acenaphthene	Acp	3	0.013	115%
Fluorene	FL	3	0.12	NA
Phenanthrene	PA	3	0.17	NA
Anthracene	Ant	3	0.011	NA
Fluoranthene	Flu	4	0.098	103%
Pyrene	Pyr	4	0.003	91%
Benzo(a)anthracene	BaA	4	0.003	120%
Chrysene	CHR	4	0.005	112%
Benzo(b)fluoranthene	BbF	5	0.064	85%
Benzo(k)fluoranthene	BkF	5	0.029	101%
Benzo(a)pyrene	BaP	5	0.006	99%
Dibenzo(a,h)anthracene	DBA	5	0.006	108%
Benzo(g,h,i)perylene	BghiP	6	0.012	88%
Indeno(1,2,3-cd)pyrene	IND	6	0.008	108%

Limit of detection (LoD) in $ng.mg^{-1}$ of PM was calculated by dividing the LoD (in ng of PAHs/sample) to the mean PM mass (in mg/sample)

Table A3.3. Mean concentration of PM_{10}, $PM_{2.5}$ and ratio of $PM_{2.5}$ and PM_{10} from vegetation burning

Vegetation type	n	PM_{10} concentration ($mg.m^{-3}$)	$PM_{2.5}$ concentration ($mg.m^{-3}$)	$PM_{2.5}/PM_{10}$
Spinifex	27	29.7	28.5	94.7%
Kimberley grass	9	23.3	22.8	97.5%
Veldt grass	9	158	155	98.4%
Banksia	27	103	101	97.9%
Jarrah	27	67.2	66.3	98.3%

Table A3.4. Kruskal-Wallis test results for effect of vegetation type on concentrations of $PM_{2.5}$-bound water-soluble metals

Metal	Different vegetation type			Different types of grasses		
	$\chi^2(2)$	p	Pairwise comparison	$\chi^2(2)$	p	Pairwise comparison
K	48.1	**<0.001**	Spinifex vs. Banksia/Jarrah	21.8	**<0.001**	Kimberley vs. Spinifex/Veldt
Na	65.2	**<0.001**	Spinifex vs. Banksia vs. Jarrah	21.8	**<0.001**	Kimberley vs. Spinifex/Veldt
Ca	6.78	**0.034**	Spinifex - Jarrah	12.1	**0.002**	Veldt vs. Spinifex
Zn	7.69	**0.021**	Banksia - Jarrah	8.80	**0.012**	Veldt vs. Spinifex
Mg	2.63	0.268	NA	2.00	0.368	41.6
Al	18.8	**<0.001**	Banksia vs. Jarrah/Spinifex	0.268	0.875	53.7
Cr	29.6	**<0.001**	Banksia vs. Jarrah/Spinifex	2.23	0.329	48.8
Mn	33.2	**<0.001**	Banksia vs. Jarrah/Spinifex	3.39	0.184	44.5
Fe	35.8	**<0.001**	Banksia vs. Jarrah/Spinifex	4.12	0.127	55.7
Ni	30.1	**<0.001**	Banksia vs. Jarrah/Spinifex	4.24	0.120	56.4
Cu	19.4	**<0.001**	Banksia vs. Jarrah/Spinifex	13.0	**0.002**	Veldt vs. Spinifex/Kimberley
Cd	44.2	**<0.001**	Spinifex vs. Banksia vs. Jarrah	2.11	0.349	63.1
Pb	45.1	**<0.001**	Spinifex vs. Banksia vs. Jarrah	4.42	0.110	63.4
∑metals	50.1	**<0.001**	**Spinifex vs. Banksia/Jarrah**	22.1	**<0.001**	**Kimberley vs. Spinifex/Veldt**

Significant values of p are in bold

Table A3.5. Emission factors for $PM_{2.5}$ (Mean ± SD), dominant water-soluble metals and PAHs in $PM_{2.5}$ generated from vegetation burning

	Different types of grassland (dry) (n=9)			Different vegetation types (n=27)		
	Spinifex	**Kimberley grass**	**Veldt grass**	**Spinifex (grassland)**	**Banksia (woodland)**	**Jarrah (forest)**
$EF_{PM2.5}$	4.8 ± 3.1	3.6 ± 1.9	31.8 ± 17.3	7.4 ± 4.0	17.2 ± 8.4	18.0 ± 11.2
Water-soluble metals						
EF_K	0.2 ± 0.02	0.02 ± 0.01	2.6 ± 1.1	0.4 ± 0.3	2.3 ± 0.9	2.7 ± 1.5
EF_{Na}	0.032 ± 0.019	0.001 ± 0.002	0.420 ± 0.188	0.055 ± 0.042	0.679 ± 0.380	0.432 ± 0.292
EF_{Ca}	0.015 ± 0.017	0.001 ± 0.002	0.002 ± 0.002	0.010 ± 0.013	0.018 ± 0.028	0.011 ± 0.026
EF_{Mg}	0.001 ± 0.001	0.0002 ± 0.0003	0.001 ± 0.002	0.001 ± 0.001	0.006 ± 0.009	0.009 ± 0.019
EF_{Zn}	0.001 ± 0.001	0.001 ± 0.001	0.020 ± 0.013	0.001 ± 0.001	0.002 ± 0.002	0.001 ± 0.001
$EF_{16\ PAHs}$ *	5.6 ± 4.3	6.3 ± 1.4	29.7 ± 19.2	14.1 ± 10.6	25.8 ± 15.1	44.8 ± 24.0

EFs for $PM_{2.5}$ and water-soluble metals are presented in $g.kg^{-1}$ dry fuel; EFs for PAHs are presented in $mg.kg^{-1}$ dry fuel.

*n=3 for dry grasses, n=9 for vegetation types.

Table A3.6. The comparison of MCE and EFs for $PM_{2.5}$ (g.kg-1 dry fuel) obtained in this study with literature

	MCE	$EF_{PM2.5}$	Author	Type of study
Grassland				
Spinifex (n=9)	0.948 ± 0.012	3.1 ± 1.8	This study	Lab-based
Kimberley grass (n=3)	0.942 ± 0.004	2.5 ± 1.2	This study	Lab-based
Dambo grass	0.98 ± 0.00	2.2 ± 1.1	(Chen et al., 2007)	Lab-based
Australian savanna[1]	0.86 - 0.99	12 ± 3	(Desservettaz et al., 2017)	Field-based
African savanna[2]	0.94	10.0 ± 7.5	(Sinha et al., 2003)	Field-based
Savanna		7.2 ± 3.4	(Akagi et al., 2011)	Compilation
Veldt grass (n=3)	0.868 ± 0.040	15.3 ± 4.3	This study	Lab-based
Montana grass	0.98-0.97	10.3 ± 0.9	(Chen et al., 2007)	Lab-based
Rangeland	0.905 ± 0.043	18.9 ± 13.9	(McMeeking et al., 2009)	Lab-based
Woodland and forest				
Banksia woodland (n=9)	0.904 ± 0.011	10.3 ± 5.3	This study	Lab-based
Jarrah forest (n=9)	0.896 ± 0.015	6.0 ± 1.6	This study	Lab-based
Chaparral	0.909 ± 0.029	11.6 ± 15.1	(McMeeking et al., 2009)	Lab-based
Chaparral	0.945 ± 0.005	7.1 ± 1.5	(Burling et al., 2011)	Field-based
Chaparral	0.946 ± 0.006	5.5 ± 1.3	(Hosseini et al., 2013)	Lab-based
Temperate forest		12.7 ± 7.5	(Akagi et al., 2011)	Compilation

The EF values (of this study) obtained under no flow conditions were used for comparison in order to have the most similar combustion (natural ventilation) and sampling conditions (without the effect of exhaust fan) with other laboratory-based studies.

[1]Aitken mode PM; [2] PM_4.

Table A3.7. Diagnostic ratios of PAHs obtained in this study and other studies and proposed diagnostic ratios for different emission sources

Diagnostic ratio				Fuel	Author
PA/PA+Ant	**Flu/Flu+Pyr**	**BghiP/BaP**	**IND/IND+BghiP**		
Vegetation fires					
0.85 ± 0.03	0.44 ± 0.02	0.69 ± 0.05	0.52 ± 0.02	All veg. types[1]	This study
0.83 ± 0.03	0.43 ± 0.02	0.71 ± 0.07	0.52 ± 0.01	Spinifex[2]	This study
0.85 ± 0.02	0.42 ± 0.03	0.69 ± 0.03	0.49 ± 0.01	Kimberley grass[3]	This study
0.86 ± 0.02	0.45 ± 0.01	0.69 ± 0.05	0.52 ± 0.01	Veldt grass[3]	This study
0.85 ± 0.01	0.45 ± 0.02	0.67 ± 0.06	0.53 ± 0.02	Banksia[2]	This study
0.88 ± 0.02	0.45 ± 0.02	0.70 ± 0.02	0.53 ± 0.01	Jarrah[2]	This study
0.72 ± 0.17	0.40 ± 0.04		0.40 ± 0.04	Chaparral	Hosseini et al., 2013
0.75	0.60	0.86	0.75	Shrubland	Alves et al., 2011
0.98	0.61	0.70	0.66	Shrubland	Garcia-Hurtado et al., 2014
0.83 - 1.0	0.25 - 0.70	0.33 - 0.58	0.15 - 0.49	Forest	Vicente et al., 2012
Other sources					
		>1.7		Traffic sources	Tobiszewskind & Namieśnik, 2012
0.77	0.61	3.34	0.04	Non-catalyst vehicle	Rogge et al., 1993
0.89	0.44	2.27	0.09	Catalyst vehicle	Rogge et al., 1993

[1] n=33 (average of all vegetation types in all combustion conditions); [2] n=9 (average of all combustion conditions); [3] n=3 (average of dry vegetation in 3 air flow rates).

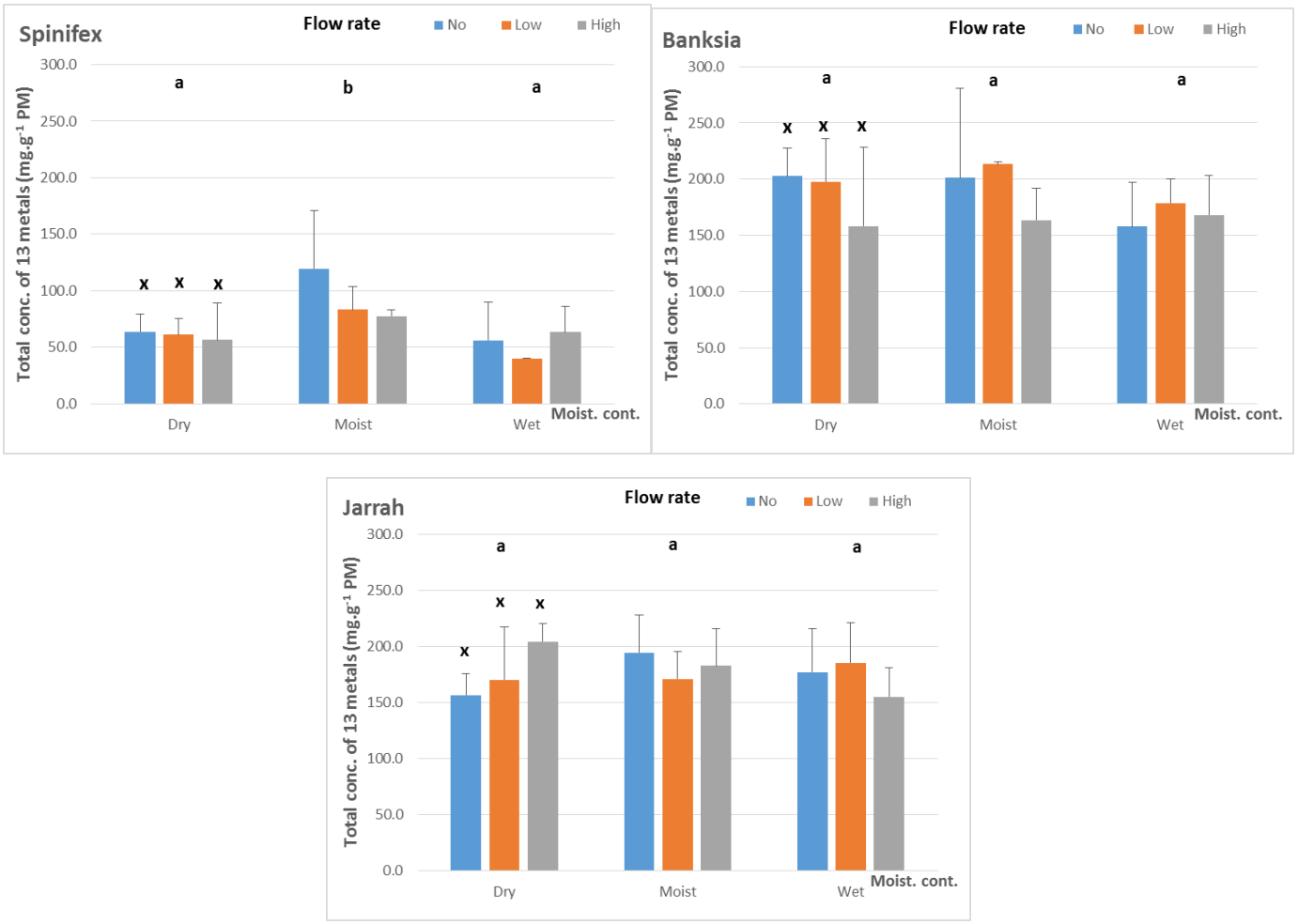
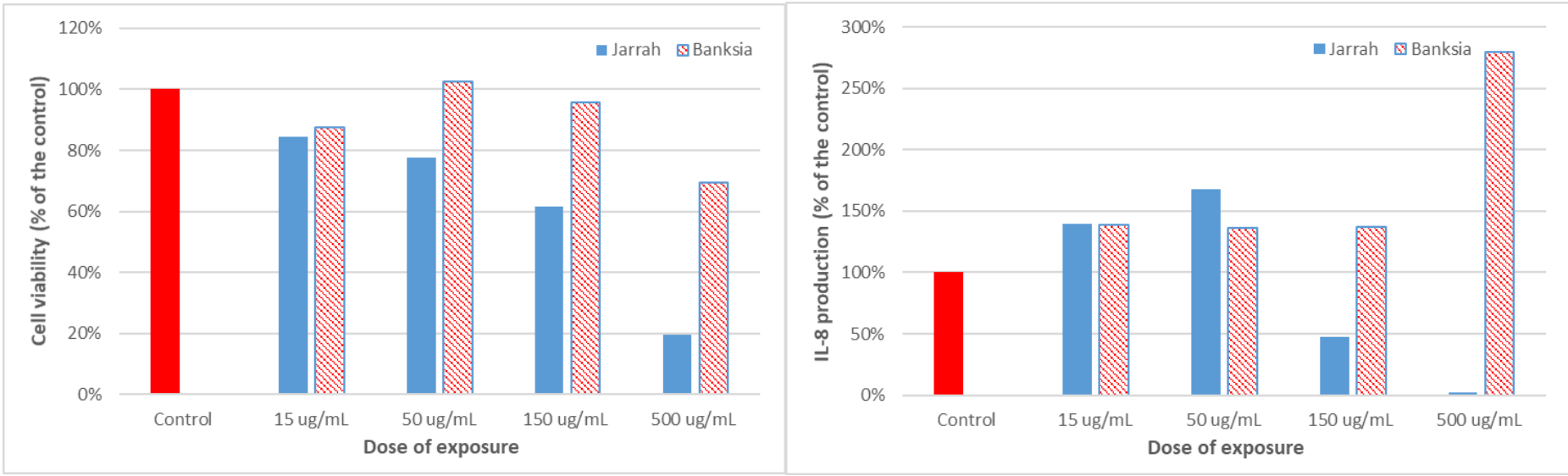


Figure A3.1. Influence of combustion conditions on the total concentrations of water-soluble metals in $PM_{2.5}$ from burning given vegetation types (n=27). Letters a, b, c at the top of each bar group represent the significant difference between different fuel moisture contents. Letters x, y, z at the top of each bar represent the significant difference between different flow rates. Influences of moisture content and flow rate were assessed using PERMANOVA with $p<0.05$

APPENDIX 4. Supplementary material for Chapter 6

Results of the pilot study to determine the doses of exposure

A pilot study was conducted with several exposure doses in range of 12.5 to 500 µg.mL^{-1} and found large differences in the dose response of cells exposed to PM depended on toxicity endpoint and type of vegetation.



$PM_{2.5}$ from Jarrah could negatively influence the cell viability at low doses of exposure (<150 µg.mL^{-1}) but Banksia $PM_{2.5}$ showed strong effects only at a dose of 500 µg.mL^{-1} (above figure, left). The effects of $PM_{2.5}$ on cellular cytokine production were also more pronounced at the 500 µg.mL^{-1}dose (above figure, right).

Based on the above results and due to the limited amount of collected $PM_{2.5}$, two doses of PM suspension, 50 and 500 µg.mL^{-1}, were chosen for the main experiments to ensure the cellular responses to exposure to $PM_{2.5}$ from all vegetation types measurable.

Table A4.1. Mean toxicological responses of A549 cells exposed to $PM_{2.5}$ from vegetation fires at two doses of exposure

Vegetation type	n	Cell viability (% of the control)		IL-8 concentration ($pg.mL^{-1}$)		Adjusted IL-8 concentration ($pg.mL^{-1}.10^{-5}$ cells)
		50 $\mu g.mL^{-1}$	500 $\mu g.mL^{-1}$	50 $\mu g.mL^{-1}$	500 $\mu g.mL^{-1}$	50 $\mu g.mL^{-1}$
Different vegetation types						
Spinifex	9	116	114	201	48.4	116
Banksia	9	84	34	171	274	138
Jarrah	9	50	34	197	128	370
Different dry grasses						
Spinifex	3	110	126	225	51.7	135
Veldt grass	3	129	103	172	224	88.4
Control		100	100	167	145	111
Blank filters		118	105	174	150	97.6

Table A4.2. Mean adjusted concentrations of water-soluble metals in $PM_{2.5}$ from vegetation combustion exposing to cells. Bracketed values represent standard deviations. Unit: $\mu g.mg^{-1}$ PM

	Different vegetation types			**Different kinds of dry grass**	
	Spinifex	**Banksia**	**Jarrah**	**Spinifex**	**Veldt grass**
K	60 (24)	140 (32)	154 (15)	49 (7)	87 (30)
Na	7.3 (2.3)	38 (13)	23.2 (4.7)	6.9 (1.2)	14 (2)
Ca	1.3 (1.3)	0.84 (0.88)	0.43 (0.59)	2.4 (1.7)	0.048 (0.020)
Mg	0.12 (0.14)	0.25 (0.34)	0.30 (0.49)	0.18 (0.19)	0.028 (0.030)
Zn	0.17 (0.16)	0.10 (0.03)	0.051 (0.051)	0.24 (0.23)	0.49 (0.11)
Al	0.036 (0.037)	0.021 (0.041)	0.020 (0.025)	0.050 (0.043)	0.0032 (0.0010)
Cr	0.010 (0.018)	<0.0001	0.0025 (0.0037)	0.027 (0.026)	0.0012 (0.0005)
Mn	0.012 (0.020)	0.0010 (0.0013)	0.010 (0.014)	0.031 (0.027)	0.0010 (0.0010)
Fe	0.036 (0.040)	<0.003	0.015 (0.013)	0.054 (0.043)	0.0035 (0.0026)
Ni	0.012 (0.023)	<0.0001	0.0019 (0.0028)	0.035 (0.032)	0.0022 (0.0003)
Cu	0.021 (0.032)	0.0019 (0.0019)	0.0093 (0.012)	0.053 (0.043)	0.0010 (0.0004)
Cd	0.009 (0.018)	<0.0001	<0.0001	0.027 (0.025)	0.0069 (0.0017)
Pb	0.007 (0.012)	<0.0002	<0.0002	0.018 (0.016)	0.0012 (0.0021)
$\sum_{\textbf{13 metals}}$	**69 (25)**	**180 (40)**	**180 (17)**	**59 (6)**	**100 (30)**

Table A4.3. Mean concentrations of 16 PAHs in $PM_{2.5}$ from vegetation combustion which cells were exposed to. Bracketed values represent standard deviations. Unit $ng.mg^{-1}$ PM

		Different vegetation types (n=27)			Different kinds of grass (n=9)	
		Spinifex	**Banksia**	**Jarrah**	**Spinifex**	**Veldt grass**
Naphthalene	**NaP**	0.57 (0.42)	0.49 (0.34)	0.84 (0.52)	0.45 (0.39)	0.39 (0.30)
Acenaphthylene	**AcPy**	0.37 (0.36)	0.56 (0.35)	1.3 (0.60)	0.24 (0.04)	0.83 (0.58)
Acenaphthene	**Acp**	0.30 (0.12)	0.047 (0.019)	0.78 (0.37)	0.093 (0.055)	0.55 (0.21)
Fluorene	**FL**	0.51 (0.37)	0.37 (0.17)	0.61 (0.19)	0.17 (0.13)	0.86 (0.17)
Phenanthrene	**PA**	39 (35)	48 (23)	75 (37)	8.6 (1.5)	88 (22)
Anthracene	**Ant**	7.9 (6.7)	8.6 (4.2)	11 (6)	1.9 (0.4)	15 (4)
Fluoranthene	**Flu**	260 (160)	270 (98)	480 (180)	110 (55)	190 (30)
Pyrene	**Pyr**	330 (200)	330 (110)	590 (190)	150 (77)	230 (42)
Benzo(a)anthracene	**BaA**	120 (50)	120 (52)	240 (94)	73 (15)	59 (7)
Chrysene	**CHR**	140 (75)	140 (63)	320 (130)	77 (11)	67 (13)
Benzo(b)fluoranthene	**BbF**	170 (76)	130 (55)	240 (100)	110 (37)	57 (13)
Benzo(k)fluoranthene	**BkF**	110 (35)	96 (43)	210 (93)	100 (32)	49 (9)
Benzo(a)pyrene	**BaP**	220 (91)	150 (67)	290 (130)	160 (59)	68 (8)
Dibenzo(a,h)anthracene	**DBA**	33 (15)	20 (10)	60 (42)	33 (11)	13 (2)
Benzo(g,h,i)perylene	**BghiP**	160 (66)	100 (48)	230 (100)	120 (60)	45 (4)
Indeno(1,2,3-cd)pyrene	**IND**	160 (60)	110 (53)	200 (91)	130 (61)	50 (6)
$\sum_{16\ PAHs}$		**1700 (750)**	**1500 (570)**	**2900 (1100)**	**1100 (210)**	**930 (140)**

Table A4.4. Factor loadings of the chemical compounds obtained from PCA. Only loadings >|0.30| are presented

Chemical species	Factor 1	Factor 2	Factor 3	Factor 4
K				**0.843**
Na				**0.928**
Ca		**-0.736**		
Al			**0.811**	
Mn			**0.853**	
Cu			**0.841**	
NaP	**0.805**			
AcPy	**0.680**	**0.555**		
FL		**0.796**		
PA		**0.926**		
Ant		**0.910**		
Flu	**0.736**	**0.545**		
Pyr	**0.773**	0.495		
BaA	**0.933**			
CHR	**0.907**			
BbF	**0.970**			
BkF	**0.949**			
BaP	**0.971**			
DBA	**0.866**			
IND	**0.988**			
BghiP	**0.969**			

Loadings >|0.50| are presented in bold