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ENSP

Karen dos Santos Gonçalves

Cardiovascular diseases and the exposure to particulate air pollutants derived from forest fires in Porto Velho municipality, Rondônia state, Brazilian amazon rain forest region

Rio de Janeiro

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Orientador(a): Sandra de Souza Hacon

Coorientador(a): Paulo Roberto Benchimol-Barbosa

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Banca Examinadora

Dr, Nino Künzli
Swiss TPH, Universidade da Basiléia, Suíça

Dr., Christian Schindler
Swiss TPH, Universidade da Basiléia, Suíça

Dr., Mirko Winkler
Swiss TPH, Universidade da Basiléia, Suíça

Dr, Luiz Antonio Camacho
ENSP/FIOCRUZ

Dr, Cleber Nascimento do Carmo
ENSP/FIOCRUZ

Drª, Sandra de Souza Hacon (Orientador)
ENSP/FIOCRUZ

Dr, Paulo Roberto Benchimol-Barbosa (Co-orientador)
HUPE/UERJ

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Dedicada à minha filha Cecilia, que com um simples sorriso me dá a força necessária para nunca desistir...

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"What you write in ink in small black letters may be completely lost, through the action of a simple drop of water. But what is written in your mind stays there for eternity."

(Tsangyang Gyatso)

"Once we accept our limits, we go beyond them."

(Albert Einstein)

RESUMO

Este estudo pretende contribuir para o conhecimento sobre a poluição atmosférica proveniente da queima de biomassa florestal e os efeitos sobre a mortalidade e morbidade das doenças cardiovasculares na cidade de Porto Velho, região amazônica brasileira. A tese foi dividida em dois manuscritos. O primeiro manuscrito objetivou prever as concentrações diárias de $PM_{2,5}$ (Partículas com diâmetro menor que 2,5 µm) utilizando uma técnica de calibração de profundidade óptica do aerossol a partir de dados de satélite com resolução de 3km no município de Porto Velho. O segundo manuscrito analisou se os métodos de série temporal e caso cruzado produzem estimativas de risco equivalentes na avaliação dos efeitos da saúde relacionados à poluição do ar em Porto Velho e examinou o efeito de interação entre $PM_{2,5}$ e temperatura sobre o risco de mortalidade e morbidade por doenças cardiovasculares. O método proposto de validação utilizando os dados de satélite demonstrou ser um método importante e alternativo para descrever os impactos dos incêndios florestais na qualidade do ar e para avaliar os efeitos relacionados com a saúde. A comparação entre os desenhos epidemiológicos demonstra que tanto as séries temporais como as análises de caso cruzado estratificado por tempo produziram resultados comparáveis da relação entre a poluição do ar originada pela queima de biomassa florestal e os resultados de saúde. Os resultados sugerem um efeito de defasagem. O efeito da interação mostrou resultados complexos, mas indicou um forte e significativo efeito sinérgico entre poluentes e temperatura na região amazônica brasileira, que deve ser mais investigado. Os resultados descritos podem apoiar as políticas de saúde pública e a vigilância da saúde ambiental para minimizar os impactos sobre a queima de biomassa florestal.

Palavras-chave: Doenças cardiovasculares, incêndios florestais, poluição do ar, dados de satélites, efeito de interação, região amazônica brasileira.

ABSTRACT

This study intended to contribute with the knowledge on biomass burning air pollution and the effects on mortality and morbidity of cardiovascular diseases in the city of Porto Velho, Brazilian Amazon region. The Thesis was divided in two manuscripts. The first manuscript aimed to predict the daily $PM_{2.5}$ concentrations (Particulate matter with diameter less than 2.5μm) using an aerosol optical depth calibration approach from satellite data with a 3 km resolution in the municipality of Porto Velho, Brazil. The second manuscript analysed whether the time series and case crossover methods produce equivalent risk estimates in the assessment of the air pollution-related health effects in Porto Velho and examined the interaction effect between $PM_{2.5}$ and temperature on risk of mortality and morbidity for cardiovascular diseases. The validation approach using the satellite data showed an important and alternative method to describe the impacts of forest fires on air quality and to assess the related health effects. The comparison between the epidemiological designs demonstrate that both time series and time-stratified case-crossover analyses produced comparable results of the relationship between biomass burning air pollution and health outcomes. The results suggest a delayed and cumulative effect. The interaction effect showed complex results but indicated a strong and significant synergistic effect between pollutant and temperature in Brazilian Amazon region that must be more investigated. The results described can support the public health policies and environmental health surveillance to minimize the impacts regarding the forest biomass burning.

Key words: Cardiovascular diseases, forest fires, air pollution, satellite data, interaction effect, Brazilian Amazon region.

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ADOPTED STANDARDS

This thesis is in accordance with the following standards:

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1 INTRODUCTION

1 INTRODUCTION

Since mid-20th century, the direct effects of the respiratory system of children and elderly were measured and exhaustively investigated through emergency, hospital admissions and mortality, particularly in urban centres.[1-3] Currently, the impact of air pollutants on cardiovascular diseases has been draws attention internationally with the investigation and demonstration of evidences on its important magnitude and more acute effect on the exposed population.[1-3]

In Brazil, few studies have evaluated the association of air pollutants and the increase of cardiovascular disease[4], especially the studies that have considered as the main source of pollutants the emissions from biomass burning during the forest fires in Amazon region.[5]

The intensity and the indiscriminate use of fires have become a serious environmental problem in the country, affecting ecosystems' balance and human health with consequences on local, regional, and global levels.[5,6] In the case of the Amazon region, which has geographic and environmental circumstances distinct from the other regions of the country, including the historic process of territory occupation, the use of fire exposes every year increasingly larger portions of the population who becomes vulnerable to its effects.[7]

In spite of the existing scientific literature[8,9,10,11,12] and the progress made to demonstrate and to reverse the detrimental impact of atmospheric pollutants[13] on the environment, the potential health effects on populations exposed to biomass burning air pollution in the Amazon region still lack local information that may help the understanding of the dynamics on the mortality and morbidity of diseases, particularly cardiovascular diseases.[5]

It is extremely important to carry out a detailed study on the association between particulate matter and cardiovascular events, identifying vulnerable population groups, specific pathologies, and defining the environmental levels that correspond to the process of exposure, falling ill and/or deceased, in order to subsidise planning and evaluation of health programmes aimed at these issues.[15]

Considering this scenario, this thesis aimed to answer the following research question:

Is biomass burning air pollution related to the risk on mortality and morbidity of cardiovascular diseases during the seasons with increased occurrence of forest fires in the Porto Velho municipality, Brazilian Amazon region?

This study was developed in partnership among experts from National School of Public Health Sergio Arouca, Oswaldo Cruz Foundation, Brazil, from Pedro Ernesto Hospital, University of Rio de Janeiro State and from Swiss Tropical and Public Health Institute, University of Basel, Switzerland. The results were showed in two manuscripts formatted according to Journal rules. The first manuscript was on "*Validation of aerosol optical depth to predict fine particle concentrations in a municipality of Brazilian Amazon Region*" and it is in submission to Environmental Science and Technology Journal. The second was on "*Short-term effects of biomass burning on cardiovascular disease in a municipality of Brazilian Amazon Region: Comparison of time series and case crossover analyses*" and will be submitted to Epidemiology Journal.

The next chapters show the background concerning the environmental scenario of forest fires in Brazil. Emphasis was made on previous studies devoted to approach specific air pollution-related health effects, particularly cardiovascular diseases, and the conceptual framework showing the two epidemiological designs applied in this thesis: a traditional time-series and a case crossover analyses.

2 BACKGROUND

2 BACKGROUND

2.1 AIR POLLUTION AND HEALTH EFFECTS

The association among high levels of air pollution and its effects on human health has been constantly investigated for more than 50 years.[14-19] The first epidemiological studies began after some classic episodes of increase in mortality rates during extreme pollution concentration, such as the Meuse Valley in Belgium on December, 1930[20] and the London heat event in 1952.[21] However, the knowledge achieved and the progress made for improving air quality over the recent decades have not been enough to reduce the high morbidity and mortality rates of cardiorespiratory diseases due air pollutant exposure.[10-12]

Although many air pollutants may cause health effects, either individually or in combination, particulate matter (PM) became the main centre of several epidemiological studies.[13,22,23] The PM is composed of a heterogeneous mix of gas, solid and liquid particles suspended in the air varying in size, chemical composition and depend on the emission source.[24] The main emission sources are fossil fuel and forest biomass burning.[25,26] In Brazil, the PM emission sources varies according to the country regions.[27] In the South and Southeast Brazilian region, come from moved and fixed sources, such as cars and industries, while in the Northern region (Amazon), the expansion of agribusiness and forest fires are the main sources.[7,28]

Considering the forest fires in the Amazon region, the primary atmospheric particles are formed with smaller size than 30nm.[29,30] After the formation of primary particles, the increase of particles size reach diameters as 100-1000nm.[31] In atmosphere several interactions and physicochemical transformations react with other products of combustion, such as carbon monoxide (CO), nitrous oxides (NO_3), hydrocarbons and aerosol particles. These particles are incorporated in the atmosphere, transported and mixed[32]. Then, with new photochemical reactions started the formation of secondary pollutants such as ozone (O3), aldehydes and nitrates.[33]

Several environmental factors, especially the weather conditions can influence the forest biomass burning.[27] The conditions of flammability are controlled by the characteristics of biomass, relative humidity, temperature, precipitation and wind speed.[34]

The aerodynamic pattern of the particle controls the entrance in the respiratory system. Particles with diameters smaller than 10μm are inhaled and deposited in the upper and lower airways.[13,35] Particles with diameters less than 2.5µm, reach the lungs and the alveolar space. For this reason, the particles smaller than 10µm (PM_{10}) or 2.5µm ($PM_{2.5}$) are extensive assessed in air quality monitoring and the concentrations are calculated in µg/m³.[35]

The particle size determines the process of inhalation and deposition in the respiratory system.[13] The particles deposited in bronchi are removed by the mucociliary clearance. In the alveolar space, interact locally with the surfactant liquid and epithelial cells. Some solid particles are recognized by the immune system and are engulfed by macrophages. Ultrafine particles, with diameter less than 200nm, they are not recognized by the body's defence system and suffer diffusion mechanisms. A small fraction can reach the bloodstream and other organs such as the heart, liver and brain.[13,35,36]

Several recent experiments have shown that the biological effects of air pollution are very fast and efficients.[36] Considering the molecular level, evidence points to the key role of free radicals and dependent pathways multistage as on pulmonary oxidative stress, pro systemic responses-inflammatory, vascular dysfunction and atherosclerosis.[37,38,39] Three hypothetical paths of these effects have been raised. First, the proinflammatory mediators (cytokines, activation of immune cells or platelets) and vasoactive molecules (possibly histamine or micro particles) of lung cells. Second, the disturbing in balance of the autonomic nervous system or heart rate variability by interactions of particles with lung receptors or nerves. The third is the distribution of ultrafine particles or constituents (organic compounds, metals) for systemic circulation.[24,36]

In this context, the scientific community has been discussing about the current predictive models of risk assessment which still remain imperfect, with many gaps and uncertainties.[36] Cardiovascular events may be caused by additional factors, as yet unknown and, therefore, trigger a stochastic process within a population.[41] This is one of several reasons that include air pollution as an important public health issue among the list of new risk factors.[42]

The inhalation of the PM is a trigger for the development of cardiovascular events within hours or days of exposure.[24,36] Because of the nature and unintended exposure to air pollution, the risk of continued development of cardiovascular events among millions of susceptible individuals may occur slightly.[36] Furthermore, PM triggers several adverse biological responses, such as systemic inflammation, that increase the cardiovascular risk from months to years of exposure.[43]

2.2 BIOMASS BURNING IN THE BRAZILIAN AMAZON REGION

Since 60's, the Brazilian Amazon region experienced an accelerated process of urbanization, resulting from a national development policy associated with the natural resources exploitation.[44] The urban area has emerged and grown due to government pressures, producing significant changes in the land use, spatial organization, inequality and environmental impacts that have affected the region development.[45]

According to the Demographic Census developed by Brazilian Statistics and Geographic Institute (Instituto Brasileiro de Geografia e Estatística – IBGE) in 2010, it is possible to observe the Amazon urbanization process in the last years.[46] In 1960, the population that was around three million inhabitants, reached 16 million in 2010. Seventy six percent of the population is located in urban areas, representing growth rates higher than the average of the others parts of the country.[47]

The biomass burning and deforestation in the Brazilian Amazon region began in the 70's, with agricultural expansion and the opening of the *Transamazônica* highway.[7] Since then, every year, the use of fire increases in the region.[5,7] Among the known events, it is possible to highlight the forest fires in 1997 and 1998 in the state of Roraima due to the *El Niño* phenomenon and the severe drought in 2005 and 2010. These events affected 30% (1.7 million m^2) in the Amazon basin, with higher losses and effects on health.[5] Study conducted by Saatchi et al. (2012)[47], the authors showed that the impacts produced by the severe drought of 2005, may have contributed to the second major drought, five years later, in 2010, which affected almost half of the entire length of the Amazon.

Considering these important events and its effects on human health[5.7] policies to combat the forest fires have been implemented, in order to minimize the impact.[48] However, despite monitoring satellite reported a slightly decrease in the occurrence of forest fires in the last year (2015), the practice of biomass burning in the Brazilian Amazon is still intense.[49]

Considering the case of Porto Velho municipality, state of Rondônia, the human occupation intensified from the 70's and 80's as a result from the implementation of a government policy and the National Institute of Agrarian Reform (Instituto Nacional de Reforma Agrária - INCRA).[50] In the region, the land use is focused on agricultural practices and public policies of incipient housing infrastructure. This situation provided significant environmental liabilities due to deforestation unplanned and precarious living conditions for certain vulnerable population.[5,7,51]

3 CONCEPTUAL MODEL

3 CONCEPTUAL MODEL

3.1 EPIDEMIOLOGICAL DESIGNS ON AIR POLLUTION-RELATED CARDIOVASCULAR DISEASES

Over the past 20 years, the number of studies on air pollution-related with cardiovascular disease increased substantially.[13.22] Different methods were applied to evaluate the short-term and long-term effect of exposure.[36]

There are several studies assessing the short-term effects. The traditional time series method has the advantage to examine high concentrations of air pollutants and the outcomes in the population (symptoms, hospital admissions, emergency visits and mortality).[1,52-54] It is possible to combine aggregated data from datasets and assume temporal variations to count data of health outcomes and environmental factors, removing effects as trend, seasonality, weather conditions and autocorrelation.[55-57] The most common methods are generalized linear models (GLM) and generalized additive models (GAM).[56-58]

In the GLM models, the relationship between the health outcome and the exposure can be assume as linear. However, the model can be more complex to interpretate.[55,56] The GAM model allow non-linear relationships, with the use of smoothing functions (*splines*), facilitating the interpretation of the relationship between the pollutant, health outcomes and potential confounding factors.[57,58]

The time series studies also assume the independence of the varibles.[56-58] However, weather conditions and air pollutant are not truly independent and have temporal variability, producing possible interactions or variability that lead to error. For this reason, the time-stratified case-crossover design has emerged as an alternative to the traditional time series analysis.[58-60]

Introduced as an epidemiological design in 1991, the case-crossover evaluate the transient effects of exposure in the short term on the acute events.[60] It is similar to a traditional case-control design and produce statistically significant analysis. The main assumption used in this design is that the exposure should be higher and more frequent in the immediately time before the occurrence of the outcome.[58,61] To analyse is necessary apply samples where each individual corresponds to a case and serves as its own control. The case is defined as the period of risk during or immediately before the exposure. The controls are periods of time, not coincident with the case, which provide an estimate of the expected baseline exposure frequency for each case. The case-controls are derived from the same individual at different times.[62-64] Thus, it is possible to control variables related to the individual characteristics such as diet, habits, body type and others.[58]

The method applied to estimate the association in the case-crossover design is the conditional logistic regression model (CLR) and can be used to compare cases and controls. The daily count of the outcome is weighted in the regression.[58] The time-stratified analysis has the possibility to control the seasonality and trends, comparing different exposure periods such as years, months and days of the week. The weather conditions and air pollutants can be considered as having straight or no association with the outcome.[62-64]

Considering the epidemiological designs described above, it was developed a conceptual model for this study using the traditional time series and case crossover analyses (Figure 1). The outcome was the cardiovascular diseases (CVD) and the exposure was the $PM_{2.5}$ as air pollutant. In this study, the cases were the individuals over 45 years with CVD, residents in the city of Porto Velho, Rondonia state during the period from 2009 to 2014. The controls strategies were the time-stratified exposure considering the year, month and weekdays during the dry season and whole study period. It was analysed the mortality using the daily CVD deaths and morbidity with the daily CVD emergency hospital admissions (EHAs). The models was fitted using the weather conditions such as average temperature, relative humidity and precipitation. Additionally, it was analysed the interaction effect between the average temperature and the $PM_{2.5}$ on risk estimates for CVD.

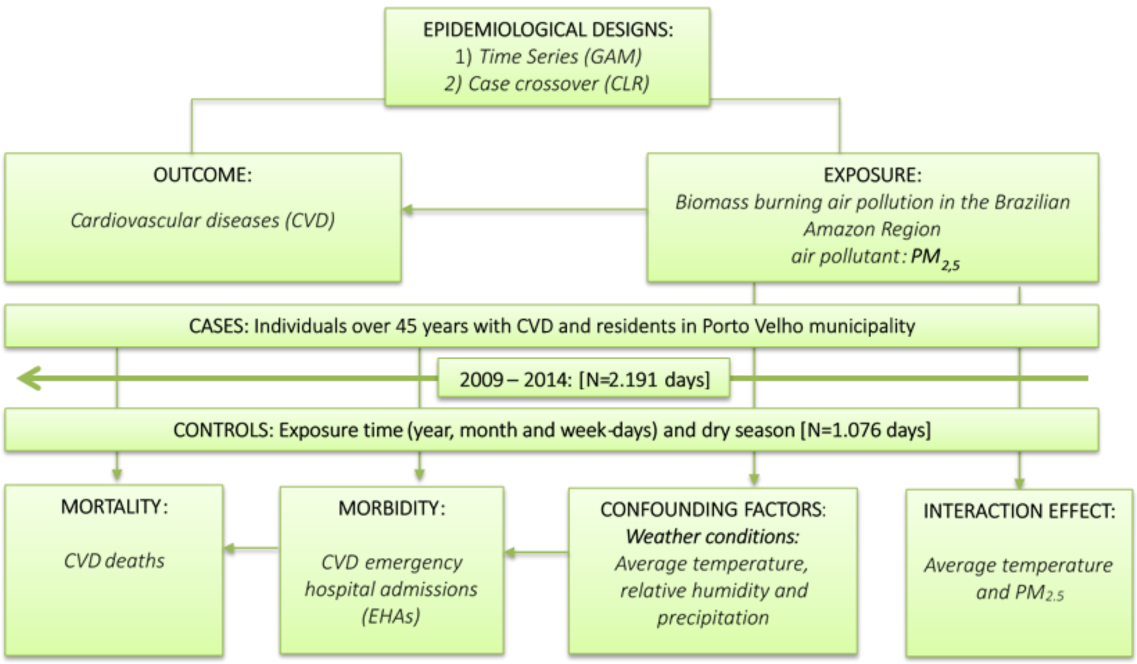

Figure 1: Framework of conceptual model applied

4 THESIS AIMS

4 THESIS AIMS

4.1 OVERALL

Examine the effect of biomass burning air pollution during and after the occurrence of forest fires on mortality and morbidity of cardiovascular diseases in Porto Velho municipality, Rondonia state, Brazilian Amazon region. Period from 2009 to 2014.

4.2 SPECIFICS

1. Predict the daily $PM_{2.5}$ concentrations using an aerosol optical depth calibration approach from satellite data with a 3 km resolution in the municipality of Porto Velho, Brazil;

2. Analyse whether the time series and case crossover methods produce equivalent risk estimates in the assessment of the air pollution-related health effects in Porto Velho;

3. Examine the interaction effect between $PM_{2.5}$ and temperature on risk of mortality and morbidity for cardiovascular diseases.

CHAPTER I – First manuscript

VALIDATION OF AEROSOL OPTICAL DEPTH TO PREDICT FINE PARTICLE CONCENTRATIONS IN A MUNICIPALITY OF BRAZILIAN AMAZON REGION

In submission to Environmental Science and Technology Journal

(Formatted according to Journal rules)

5 CHAPTER I - VALIDATION OF AEROSOL OPTICAL DEPTH TO PREDICT FINE PARTICLE CONCENTRATIONS IN A MUNICIPALITY OF BRAZILIAN AMAZON REGION

5.1 ABSTRACT

Epidemiological studies generally use particulate matter measurements with diameter less 2.5μm ($PM_{2.5}$) from monitoring networks. Satellite aerosol optical depth (AOD) data has considerable potential in predicting $PM_{2.5}$ concentrations, and thus provides an alternative method for producing knowledge regarding the level of pollution and its health impact in areas where no ground $PM_{2.5}$ measurements are available. This is the case in the Brazilian Amazon region, where forest fires are frequent sources of high pollution. In this study, we applied a non-linear model for predicting $PM_{2.5}$ concentration from AOD retrievals using interactions terms between average temperature, relative humidity, sine, cosine of date in a period of 365.25 days and the square of the lagged relative residual. Regression performance statistics were tested comparing the goodness of fit and R^2 based on results from linear regression and non-linear regression for six different models. The regression results for non-linear prediction showed the best performance, explaining on average 82% of the daily $PM_{2.5}$ concentrations when considering the whole period studied. In the context of Amazonia, it was the first study predicting $PM_{2.5}$ concentrations using the latest high-resolution AOD products also in combination with the testing of a non-linear model performance. Our results permitted a reliable prediction considering the AOD-$PM_{2.5}$ relationship and set the basis for further investigations on air pollution impacts in the complex context of Brazilian Amazon Region.

Key words: Aerosol Optical Depth, Particulate matter, Air pollution, Forest fire, Validation approach, Brazilian Amazon Region.

5.2 INTRODUCTION

In spite of the efforts to improve air quality during the past decades in light of the knowledge on the effects of atmospheric pollution on human health, levels of air pollution experienced by human populations continue to cause a large burden of disease.[1,2,3] Atmospheric aerosols and particulate matter that are breathable (< 2.5 µm diameter = $PM_{2.5}$) and inhalable (< 10 µm = PM_{10}), generated from natural and anthropogenic emission sources present known effects for a number of causes of death, particularly the increase on cardio-respiratory diseases especially in areas with high concentrations.[4,5]

Intensive and indiscriminate occurrence of forest fire has become a serious environmental problem in Brazil, affecting ecosystems' balance and human health with consequences at the local, regional and global level.[6,7] In the case of the Brazilian Amazon region, which has geographic and environmental circumstances that are distinct from other world regions, the occurrence of fire and emissions of $PM_{2.5}$ exposes every year increasingly large portions of vulnerable populations.[8,9]

To understand the association between $PM_{2.5}$ and effects on human health, epidemiological studies have employed $PM_{2.5}$ measurements from monitoring sites. However, due to cost and lack in appropriate infrastructure, especially in rural and remote areas, no fixed site $PM_{2.5}$ measurements are available in many regions of Brazil. This is a major limitation for estimating exposure to $PM_{2.5}$ and assessing health impacts associated with forest fires as one of its major source.[10,11,12,13,14,15]

An alternative approach to estimate the air quality in areas without direct $PM_{2.5}$ measurements is by means of satellite remote sensing using the aerosols products from the Moderate Resolution Imaging Spectroradiometer (MODIS). Aerosol optical depth (AOD) reflects the integrated amount of particles and is an important satellite-retrieved technique for predicting the $PM_{2.5}$ concentrations. The AOD has been successfully used in statistical models for estimating $PM_{2.5}$ levels. As shown by previous studies, parameters such as local meteorology and land use information influence the relationship between AOD and daily $PM_{2.5}$ concentrations, which need to be taken into account as additional predictors.[10,16,17,18,19,21,22,23]

The aim of this study was to predict the daily $PM_{2.5}$ concentrations using an AOD calibration approach with a 3 km resolution in the municipality of Porto Velho, Brazil. For Brazilian Amazon region, it is the first study to develop this approach considering a non-linear prediction model. The assessment is part of an investigation that aims at analysing the impact of $PM_{2.5}$ exposure on cardiovascular disease in Porto Velho.

5.3 MATERIAL AND METHODS

5.3.1 Ground-level $PM_{2.5}$ data

Over the study period, $PM_{2.5}$ concentrations were measured for 24h at one air quality monitoring station in Porto Velho municipality, which was implanted in partnership between Institute of Physics at University of São Paulo (USP), University of Rondônia (UNIR), Environmental Biogeochemistry Laboratory Wolfgang H. Pfeiffer and National School of Public Health-Oswaldo Cruz Foundation (Fundação Instituto Oswaldo Cruz - FIOCRUZ) in Brazil. The $PM_{2.5}$ monitor is located at 15 km to the centre of urban area (**Figure 2**). Porto Velho municipality is the third capital in the Brazilian Amazon region with 67 districts within the urban area. With an area of 34,096 km^2 Porto Velho has a population of 502,748 inhabitants (IBGE, 2015). $PM_{2.5}$ measurements were collected by means of a stacked filter unit (SFU) and were analysed gravimetrically according to Air quality guidelines for particulate matter, ozone, nitrogen dioxide and sulfur dioxide, World Health Organization (WHO, 2005)[24]. Daily averages were calculated during the period from 25 September 2009 to 21 October 2011 with a total of 757 days.

5.3.2 MODIS 3 km AOD retrieval

On board the National Aeronautics and Space Administration (NASA) satellites Terra and Aqua has been in operation since 1999 and 2002, respectively. These satellites were used to retrieve AOD aerosol products with a 3 km resolution (MOD04_3K and MYD04_3K) and operating at an altitude of approximately 700 km (http://modis-atmos.gsfc.nasa.gov/). In the Collection 6, Level 2 aerosol products, the most recent 3 km AOD dark target retrieval algorithm is similar to the 10 km standard product (Collection 5, Level 2) and has three different wavelength channels of 0.47, 0.66, and 2.12 µm employed for AOD retrieval over-land. The other channels are used for screening procedures (e.g., coverage of cloud, snow and ice).[25,26] Remer et al. (2013, 2005)[26,27] and Levy et al. (2007, 2010)[28,29] describe in more details the retrieval of MODIS satellite aerosol data. In this study, we used the algorithm retrieval in MATLAB (version 2015a, MathWorks) for AOD daily averages, the software ArcGIS (version 10, ESRI) to create 820 grid cells of 3 x 3-km^2 covering the study area for spatial analyses.

5.3.3 Statistical model and validation

In this study, we considered five different types of prediction models of $PM_{2.5}$ concentrations from AOD retrievals. They were all in the form $PM_{2.5}$ = exp (linear predictor), with the linear predictor involving terms composed of AOD and other influencing factors. The advantage of such a model over one for log- transformed outcome data is that it provides estimates of mean exposure levels while estimates of geometric mean levels are obtained otherwise. In Model 1, we took the linear predictor to be a cubic polynomial in AOD with time-independent coefficients:

(Model 1)

$$PM_{2.5} = \exp(\alpha + \beta_1(AOD) + \beta_2 (AOD)^2 + \beta_3 (AOD)^3)$$

In a next step, we considered the coefficients of Model 1 to be polynomials of second degree in average temperature (TEMP) and relative humidity (RH). Thus, each of the coefficients was assumed to be of the form $\beta_0 + \beta_1 \cdot TEMP + \beta_2 \cdot RH + \beta_3 \cdot TEMP^2 + \beta_4 \cdot RH^2 + \beta_5 \cdot TEMP \cdot RH$. Multiplying these polynomials with AOD, AOD^2 and AOD^3 and the intercept, respectively, provided:

(Model 2)

$$PM_{2.5} = \text{Model 1} \cdot \exp(\beta_4(AOD \cdot TEMP) + \beta_5(AOD \cdot TEMP^2) + \beta_6(AOD \cdot RH) + \beta_7(AOD \cdot (RH^2) + \beta_8(AOD \cdot TEMP \cdot RH) + \beta_9(AOD^2 \cdot TEMP) + \beta_{10}(AOD^2 \cdot TEMP^2) + \beta_{11}(AOD^2 \cdot RH) + \beta_{12}(AOD^2 \cdot RH^2) + \beta_{13}(AOD^2 \cdot TEMP * RH) + \beta_{14}(AOD^3 \cdot TEMP) + \beta_{15}(AOD^3 \cdot TEMP^2) + \beta_{16}(AOD^3 \cdot RH) + \beta_{17}(AOD^3 \cdot RH^2) + \beta_{18}(AOD^3 \cdot TEMP \cdot RH))$$

In an attempt to further improve the model, we added interaction terms between AOD, AOD^2 and AOD^3 with rainy season (Model 3) and tested interactions of the three AOD-terms with sine and cosine functions of date with a period of 365.25 days in the Model 4.

In the last step, we included the lagged relative residual and its square as additional predictor variables (Model 5). This was to reduce serial autocorrelation. The final model obtained after some backward elimination steps was of the form:

(Final model)

$$PM_{2.5} = \exp(\alpha + \beta_1 \cdot AOD + \beta_2 \cdot AOD^2) + \beta_3 \cdot AOD^3 + \beta_4 \cdot (AOD \cdot TEMP) + \beta_5(AOD \cdot RH) + \beta_6(AOD \cdot TEMP^2) + \beta_7(AOD \cdot RH^2) + \beta_8(AOD \cdot cos\_days) + \beta_9(AOD \cdot sin\_days) + \beta_{10}(AOD^2 \cdot cos\_days) + \beta_{11}(AOD^2 \cdot sin\_days) + \beta_{12}(AOD^3 \cdot cos\_days) + \beta_{13}(AOD^3 \cdot sin\_days) + \beta_{14}(residual) + \beta_{15}(residual)^2$$

In these equations, $PM_{2.5}$ denotes the predicted concentrations; *exp* is the exponential function; *cos_days* and *sin_days* denote the cosine and sine terms of date with a period of 365,25 days; *residual* denotes the lagged relative residual. Relative residuals were defined as ratio between residuals and predicted values. Model performance was evaluated by comparing the predictions with the ground measurements using the adjusted coefficient of determination (R^2 adj), residual standard deviation (RMSE), Akaike's information criterion (AIC), and partial autocorrelation of residuals by lags. High values of adjusted R squared suggest that MODIS AOD data can be used to estimate ambient concentrations. Furthermore, we calculated mean, standard deviation and maximum / minimum values to summarize the descriptive statistics of our sample for the whole period, the dry season (months June to November, when forest fires occur in Brazilian Amazon region) and the rainy season (months from December to May).

The spatial distribution of the 3x3km resolution MODIS AOD average during the study period was derived by spatial interpolation using the inverse distance weighting (IDW). We present the results for all states in Brazil and for our study area. It is important to highlight that all regression results were presented with the original AOD and $PM_{2.5}$ datasets. The software R (version 3.1.3) was used for statistical analyses.

5.3.4 Ethics

This manuscript is part of a larger project on *Cardiovascular diseases and the exposure to forest fires in Porto Velho municipality, Rondônia state, Brazil.* The project was submitted and accepted by the Research Ethics Committee of the Sergio Arouca National Public Health School (*Comitê de Ética em Pesquisa da Escola Nacional de Saúde Pública Sergio Arouca* – ENSP / FIOCRUZ) according to Resolution number 466/2012 from National Research Ethics Council (*Conselho Nacional de Pesquisa* – CONEP) under CAAE number 41732615.4.0000.5240.

5.4 RESULTS

5.4.1 Descriptive statistics

Table 1 shows the descriptive statistics of daily measured $PM_{2.5}$, values of AOD, relative humidity, average temperature and precipitation from 25 September 2009 to 21 October 2011, as well as for the dry and rainy seasons. Average daily level of $PM_{2.5}$ from the ground-level monitor was 11µg/m³±20µg/m³ over the three years studied. Of note, the analysis of the data for 2010, the year when one of the biggest dry seasons in Brazilian Amazon region occurred, revealed an annual-mean of 36µg/m³±46µg/m³.

Considering the differences between seasons, the maximum daily value was exceptionally high (164µg/m³) during the dry seasons of the period studied compared to 27µg/m³ in the rainy seasons.

Over the entire study period, the daily AOD values observed varied from 0.03 to 2.19. On average, 649 AOD values were retrieved per grid cell, which corresponds to 86% of the entire study period of 757 days.

The meteorological variables such as relative humidity, average temperature and precipitation were consistent with the climatic patterns expected for the Brazilian Amazon region and thus support the analysis in the regression models.

5.4.2 Non-linear prediction models

To test the performance of the five regressions models we use 649 valid days for the model fitting. The comparisons between the models analysed were shown in **Table 2**.

The Model 1 shows an R^2 adj of 0.54, RMSE of 13.59µg/m³ and AIC of 5234.3 for whole period. Model 2 including interactions between AOD, AOD^2 and AOD^3 and linear and quadratic terms in temperature and relative humidity provided a better fit with (R^2 = 0.67. After adding interactions between the three AOD-terms and rain the fit only slightly improved (R^2=0.70). In the Model 4 we excluded the rain term and added interactions of the three AOD-terms with sine and cosine of date with a period of 365.25 days. This model performed considerably better and explained 77% of the variance (RMSE=9.59µg/m³; AIC=4803.1).

After adding the lagged relative residual and its square as additional predictor variables (Model 5) the adjusted R^2 further increased to 0.82 (RMSE=8.60µg/m³;

AIC=4797.6). This means that this non-linear prediction model explain 82% of the variance of daily $PM_{2.5}$ concentrations. The introduction of these two terms also led to a drastic reduction in residual autocorrelation, in that lag1-autocorrelation of residuals was no longer significant (**Figure 3).** As visualized in **Figure 3**, the time series of predicted $PM_{2.5}$ concentrations follows a very similar pattern as the measured $PM_{2.5}$ confirming the high performance of the prediction models. The period from mid-July to end of Octobre 2010 – a dry period with plumes of biomass burning – is characterized by very high AOD values, reaching peaks 50-100 times above the typical values observed before and after this period. The comparisons between the measured and predicted $PM_{2.5}$ concentrations for Model 1 and Model 5 were illustrated in **Figure 4**.

The spatial distributions of the 3 km resolution MODIS AOD average during the study period for all Brazilian states and for our study area were shown in **Figure 5.** The highest satellite AOD values were observed in the Brazilian Amazon region. In our study areas, mean AOD values reached 0.21 in the urban area of Porto Velho, and 0.54 across the Rondônia state. Information about the distribution of $PM_{2.5}$ within the urban districts of Porto Velho and the relation with the health data will be presented in a separate manuscript about the impacts of $PM_{2.5}$ on human health in Brazilian Amazon region.

5.5 DISCUSSION

The results of our final non-linear prediction model for $PM_{2.5}$ showed a good performance, explaining on average 82% of the variance in $PM_{2.5}$ concentrations during the period studied. This result is similar and in accordance with the findings presented by Lee et al (2011)[10] and Xie et al (2015)[23], who showed prediction models that explained 92% and 82% of the variance in $PM_{2.5}$ concentrations in the North-eastern, US and in Beijing, China, respectively. Our model has the advantage that it does not produce negative predictions and fits the mean of the data as a function of the predictor variables. Moreover, by including the lagged relative residual and its square as additional predictor variables it was possible to remove the significant lag1-autocorrelation, and for further improve the model fit.

Observing the temporal distribution of predicted and measured $PM_{2.5}$ concentrations it is important to highlight the enormous peak of $PM_{2.5}$ observed between days 300 and 400 during the dry periods in our study area with the maximum daily value of 164$\mu g/m^3$. This value is 3.6 times higher than the guideline annual mean value proposed by WHO to protect public health (10$\mu g/m^3$), currently adopted by policy makers in only a few countries and is considered with major impacts on morbidity and mortality.[30] On the other hand, during the rainy seasons concentrations were low ($2\mu g/m^3$; $\pm 3\mu g/m^3$). This confirms the dominant role of fires as source of ambient air pollution in the Amazon region. This result highlights the importance to set limits for $PM_{2.5}$ in the Brazil Air quality Standards defined by the National Environmental Agency (CONAMA) that currently set limits only for PM_{10}.[30]

The non-linear prediction model demonstrated a high performance in predicting the daily $PM_{2.5}$ concentrations. However, some limitations, such as cloud properties and uncertainties need to be mentioned. The use of only one air monitoring station for the development and evaluation of the model is a major limitation of this study. However, although this limits our ability to draw any conclusions about the applicability of the model all across Brazil, it may provide a valid approach to predict $PM_{2.5}$ across our main study area. $PM_{2.5}$ is spatially rather homogenously distributed, thus extrapolation of the model from the measurement site to the urban area of Porto Velho is expected to be reliable. The model gives also good indications of possible hot spots of pollution where it may be worth installing additional monitors operating continuously or at least during dry seasons. With the use of mobile stations, one could characterize the spatial pattern of air pollution across a larger area with only one or a few monitors while using the current central monitoring station as a reference point to understand the temporal variation. The installation of air quality monitoring networks is an important step for the future evaluation of progress in clean air management and the assessment of its health impact.

The predictions were based on different spatial scales, which may be a source of uncertainties. The AOD satellite data was based on a grid cell with 3km resolution while $PM_{2.5}$ ground level is measured at a fixed point. It is also important to highlight the complex relationship between AOD and $PM_{2.5}$ due the nature of the forest aerosol in Brazilian Amazon. Other important predicting factors such as wind speed, atmosphere physical-chemical components were not analysed in this study.

Despite the uncertainties, this study is the first to predict $PM_{2.5}$ concentrations using a non-linear prediction model and the most recent higher-resolution MODIS AOD products in Porto Velho. By modelling the AOD-$PM_{2.5}$ relationship in a time-dependent manner reflecting seasonal fluctuations and influences of temperature and relative humidity, we were able to develop a prediction model for $PM_{2.5}$ with good fitting properties.

5.6 CONCLUSIONS

Satellite data has an important potential for the spatio-temporal prediction of $PM_{2.5}$ concentrations. It offers an alternative method to describe the impacts of forest fires on air quality with a high potential to assess the related health effects in the Brazilian Amazon region. Our method can help to strengthen the $PM_{2.5}$ monitoring networks with well-placed complementary measurement sites to understand the impact of air pollution in Brazil and to demonstrate the improvements of air quality and the related health benefits due to the adoption of clean air policies.

5.7 ACKNOWLEDGEMENT

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TABLES AND FIGURES

Table 1: Descriptive statistics of the parameters analysed during the study period (September, 25th 2009 to October, 21th 2011).

	Entire period (25 September 2009 to 21 October 2011)				
Variable	**N**[a]	**Mean**	**SD**[b]	**Min**	**Max**
MODIS AOD 3km (unitless)	649	0,29	0,36	0,03	2,19
$PM_{2.5}$ (µg/m³)	649	11,36	20,06	1.68	164,41
Average temperature (ºC)	649	26,82	1,41	16,24	31,26
Relative humidity (%)	649	84,93	5,80	61,50	98,75
Preciptation (mm)	649	5,06	11,37	0	71,40
	Dry season (June to November)				
Variable	**N**	**Mean**	**SD**	**Min**	**Max**
MODIS AOD 3km (unitless)	323	0,44	0,46	0,03	2,19
$PM_{2.5}$ (µg/m³)	323	20,51	25,19	1.68	164,41
Average temperature (ºC)	323	27,00	1,68	16,24	31,26
Relative humidity (%)	323	82,18	5,90	61,50	98,75
Preciptation (mm)	323	0	0	0	0
	Rainy season (December to May)				
Variable	**N**	**Mean**	**SD**	**Min**	**Max**
MODIS AOD 3km (unitless)	326	0,14	0,07	0.03	0,76
$PM_{2.5}$ (µg/m³)	326	2,28	2,87	1,68	26,62
Average temperature (ºC)	326	26,66	1,07	22,07	29,05
Relative humidity (%)	326	87,65	4,21	73,80	98,00
Preciptation (mm)	326	7,76	14,09	0,10	71,40
	Pearson correlation				
Variable	**AOD**	**PM2.5**	**TEMP**	**RH**	**PRECIP**
MODIS AOD 3km (unitless)	1				
$PM_{2.5}$ (µg/m³)	0.5811	1			
Average temperature (ºC)	0.245	0.1007	1		
Relative humidity (%)	-0.3957	-0.4455	-0.4411	1	
Preciptation (mm)	-0.101	-0.1454	-0.1638	0.2413	1

Note: a= Number of values observed in days; b=Standard deviation (SD)

Table 2: Comparison between prediction models.

Prediction models	N	df[a]	R^2	R^2 adj[b]	RMSE[c]
Model 1	649	3	0.544	0.541	13.59
Model 2	649	18	0.683	0.674	11.47
Model 3	649	21	0.707	0.697	11.04
Model 4	649	27	0.782	0.772	9.58
Model 5	649	15	0.823	0.816	8.60

Note: a=degrees of freedom; b= R-squared adjusted; c= Residual standard deviation (RMSE).
Model 1 = simple model with linear, quadratic and cubic term of AOD;
Model 2 = Model 1 + interactions of AOD, AOD^2 and AOD^3 with linear and quadratic terms in temperature and relative humidity;
Model 3 = Model 2 + interactions between AOD, AOD^2 and AOD^3 and rain;
Model 4 = Model 3 + interactions of AOD, AOD^2 and AOD^3 with sine and cosines of date with a period of 365.25 days (without the term for rainy season);
Model 5 = Model 4 + lagged relative residual and its square as additional predictor variables;

Figure 2: Study area with the locations of $PM_{2.5}$ and weather monitors. Municipality of Porto Velho, Rondônia state, Brazilian Amazon region.

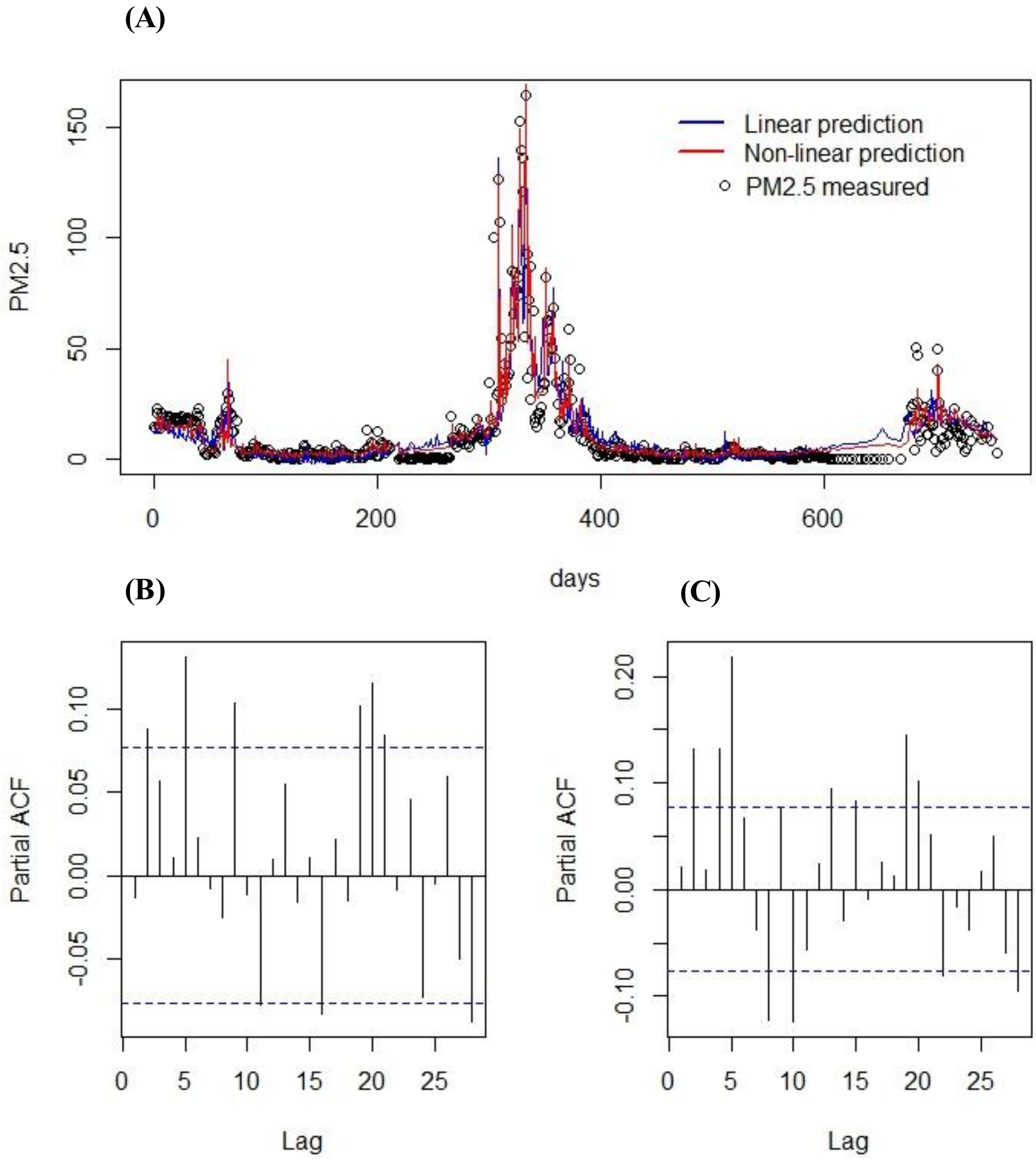
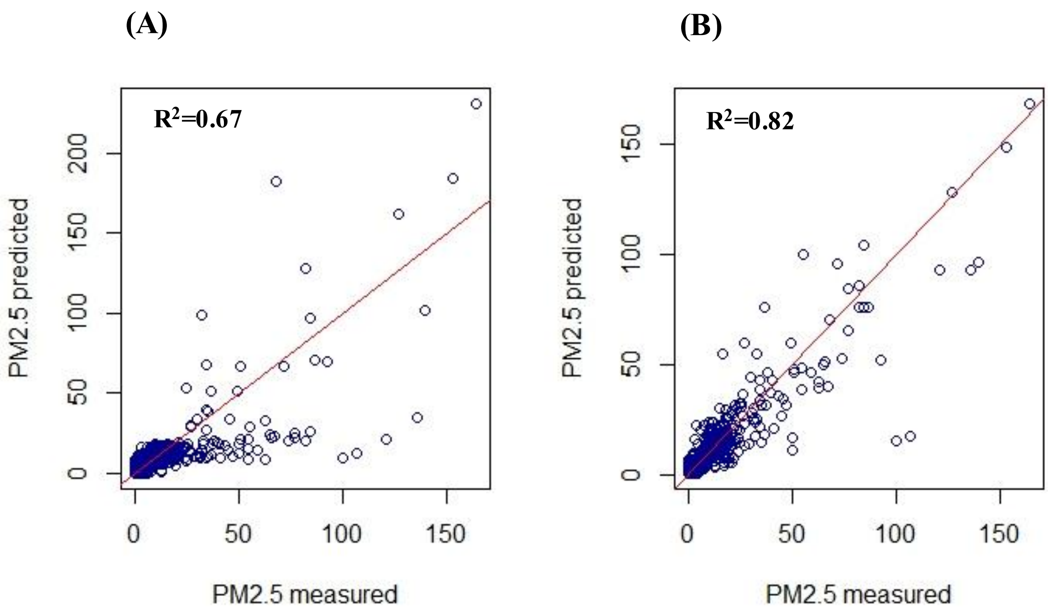


Figure 3: (A) Comparisons between $PM_{2.5}$ measured and $PM_{2.5}$ predictions ($\mu g/m^3$) across time. (B) Partial autocorrelation plot of residuals before introducing the lagged relative residual and its square as additional predictor variables into the model (C) Partial residual autocorrelation plot after after adding the the two variables to the model.



del 2

Figure 4: Comparisons between the measured and predicted $PM_{2.5}$($\mu g/m^3$) for (A) Model 2 and (B) non-linear prediction (Final model). The red line represents the regression line.

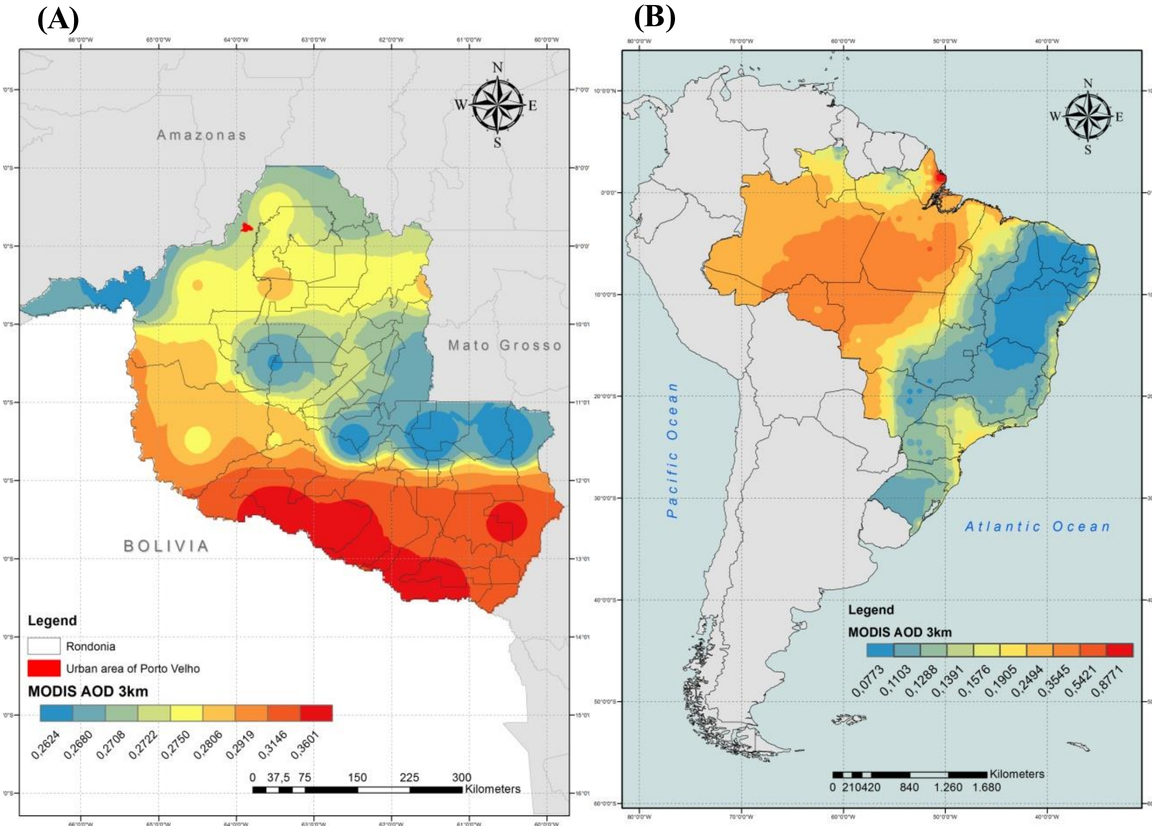


Figure 5: Spatial distribution for MODIS AOD 3 km values from IDW interpolation, September 25th 2009 to October 21th 2011. (A): Satellite AOD average after the interpolation in municipalities of Rondônia State including the study area of Porto Velho; (B): Spatial distribution of 3x3km Satellite AOD averages interpolated to all Brazilian states.

CHAPTER II – Second manuscript

SHORT-TERM OF BIOMASS BURNING ON CARDIOVASCULAR DISEASE IN A MUNICIPALITY OF BRAZILIAN AMAZON REGION: COMPARISON OF TIME SERIES AND CASE CROSSOVER ANALYSES

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6 CHAPTER II - SHORT-TERM OF BIOMASS BURNING ON CARDIOVASCULAR DISEASE IN A MUNICIPALITY OF BRAZILIAN AMAZON REGION: COMPARISON OF TIME SERIES AND CASE CROSSOVER ANALYSES

6.1 ABSTRACT

BACKGROUND: In the Amazon region, the haze events produced by forest fires during the dry season exposes more than 17 millions of people every year in Brazil and other South American countries. We aimed to analyse whether the time series and case crossover methods produce equivalent risk estimates in the assessment of the air pollution-related health effects in a municipality of Brazilian Amazon region. We also examined the interaction effect between air pollution and temperature on the risk of mortality and morbidity from cardiovascular diseases (CVD).

METHODS: We applied a quasi poisson additive model (time series analysis) and conditional logistic regression (case crossover analysis) to estimate the associations between the daily counts of cardiovascular deaths and emergency hospital admissions and daily levels of $PM_{2.5}$ adjusting for weather conditions, holidays, weekdays and interaction terms during dry season from 2009 to 2014 in Porto Velho municipality, Brazilian Amazon region.

RESULTS: During the occurrence of biomass burning), the RR and OR of CVD deaths associated with a 10 µg/m3 increment in $PM_{2.5}$ on the day of the event was 1.16 (95% CI: 1.04; 1.29) and 1.09 (95% CI: 0.75; 1.60), respectively. For CVD EHAs the corresponding RR was 1.06 (95% CI: 0.91; 1.23) and the OR was 1.09 (95% CI: 0.98; 1.20). Concerning the whole period, the case crossover analysis for CVD EHAs showed a significant association with $PM_{2.5}$ at lag 6 (OR: 1.02; 95%CI: 1.00; 1.03). In the time series analysis, we also observed significance in the smooth interaction term ($PM_{2.5}$-temperature) for CVD deaths ($p<0.00571$) and EHAs ($p<0.000401$).

CONCLUSIONS: In our study, time-stratified case-crossover and time series analyses produced comparable results of the relationship between biomass burning air pollution and health outcomes. There was also a strong interaction effect between the particulate matter and temperature that must be investigated in more detail.

Key words: Air pollution, biomass burning, particulate matter, cardiovascular diseases, lagged effect and interaction effect.

6.2 BACKGROUND

Many epidemiological studies have used particulate matter as indicator of exposure to ambient air pollution especially particulates with an aerodynamic diameter of less than 2.5µm ($PM_{2.5}$).[1-7] In 2013 the Global Burden of Disease (GBD) estimated 2.9 million attributable deaths related to $PM_{2.5}$ as a leading risk factor.[1,2] In the Amazon region, the haze events produced by forest fires during the dry season exposes more than 17 millions of people every year in Brazil and other South American countries.[8-13] Fires are used as an historical and sociocultural practice in agriculture associated with the deforestation.[9,10] Moreover, the Amazon region is sensitive to concomitants of climate change such as the drought events experienced in 2005 and 2010 that exposed the population to high air pollutant concentrations. The characteristics of the weather conditions contribute to increasing the number of fires and consequently the impacts on human health.[8,12]

The local infrastructure of the Amazon region, the high demand and problems in the health services along with the difficulties for maintenance of air quality monitoring station are one of the reasons that can explain the few studies developed in the region.[8,12] It is extremely important to carry out a detailed study on the association between particulate matter and cardiovascular diseases, identifying vulnerable population groups and defining the environmental levels that correspond to the process of exposure in Brazil in order to inform planning and evaluation of health programs aimed at these issues.[14]

In this context, two common epidemiological designs have been used to examine the air pollution-related health effects. For many years, time series analysis has been used to investigate the health impact of time varying environmental exposures (eg, air pollution and temperature)[14-16]. In more recent years, the case-crossover design introduced by MaClure in 1991, has been increasingly used to assess associations between transient exposures (eg, temperature or air pollution) and acute health outcomes[16,17]. This design is a case-control study with days playing the role of cases and controls. Each event defines a case day and associated control days are selected in the same month and a multiple of 7 days apart, which automatically controls seasonal and day of the week effects. There are few studies on whether the case crossover method is applicable to the assessment of health effects by air pollution from biomass burning. Additionally, the $PM_{2.5}$ exposure and meteorological conditions occur simultaneously in the complex atmosphere of Brazilian Amazon region. The previous studies that investigated the interaction effect between PM2.5 and meteorological conditions have supported the hypothesis that these factors may act as effect modifiers.[18,19] The assessment of the interaction effects can produce more realistic risk estimates in Amazon region and support policy makers in Brazil guiding the development of public policies and health vigilance.

This study aimed to analyse whether the time series and case crossover methods produce equivalent risk estimates in the assessment of the health effects of biomass burning in a municipality of Brazilian Amazon region. We also examined the interaction effect between $PM_{2.5}$ concentrations during the biomass burning and temperature on the risk of mortality and emergency hospital admissions due to cardiovascular diseases.

6.3 METHODS

6.3.1 Study location

Located in the Brazilian Amazon region, Porto Velho is the capital of Rondônia state (08°45'43"S, 63°54'14"W) and the third largest municipality in Brazil's Northern region with a population density of 12.57 inhabitants/km^2. The urban area covers 41.7km^2 and the forest covers around 88% of the municipality. Porto Velho has a humid tropical climate with transitions between semi-humid and equatorial climate. The population increased from 385,434 inhabitants in 2009 to 511,219 in 2016. 21% of the residents in Porto Velho were aged 20-29 years, while 26% of them were aged 40 years and over. The city has a historic importance in the socio-environmental vulnerability scenario of the Brazilian Amazon region.[13]

6.3.2 Data collection

Daily data on mortality was obtained from 1st January 2009 to 31st December 2014 and provided by the Mortality Information System – DATASUS, MS (*Computer department of the Brazilian public health system, Ministry of Health*). We also analysed daily emergency hospital admissions (EHAs) from 1st January 2009 to 31st December 2014 provided by the

HOSPUB (*Integrated health system of emergency and hospital admissions*) – DATASUS, MS. Deaths and emergencies from cardiovascular diseases (CVD) in adults over 45 years were categorised according to the International Classification of Diseases (revision 10) (ICD 10, Chapter IX – Diseases of the circulatory system).

Daily meteorological data on average temperature and relative humidity were obtained for the period 1st January 2011 to 31st December 2014, from the Porto Velho monitoring station of the INMET (*National Meteorology Institute*). The information is public and available on the website (www.inmet.gov.br). Daily data on aerosol optical depth were retrieved from National Aeronautics and Space Administration (NASA) - Terra and Aqua satellite data. We predicted the $PM_{2.5}$ concentrations using values of Aerosol Optical Depth (AOD) from the Moderate Resolution Imaging Spectroradiometer (MODIS). We applied a non-linear prediction model also involving mean daily temperature and relative humidity to estimate $PM_{2.5}$ concentrations for the whole study period. This model was derived using $PM_{2.5}$ concentrations from one air quality monitoring station in Porto Velho municipality operating between 25 September 2009 and 21 October 2011. The Chapter I describes the prediction model of daily $PM_{2.5}$ concentrations in the Porto Velho municipality in detail.

6.3.3 Statistical analysis

According to Amazon climate conditions, Porto Velho has a strong climatic seasonality divided into dry season and rainy season. The biomass burning occurs during the dry season taking advantage of the favourable conditions of high temperature and low relative humidity. In this study, we analysed the short-term effects of biomass burning on CVD mortality and on CVD EHAs during the dry season (months from June to November) and the whole study period (2009-2014).

We used time-series and case-crossover analyses to estimate the risk resulting from exposure to $PM_{2.5}$ during the biomass burning and to compare the performance of each method. Quasi poisson generalized additive models (GAM) were used to perform a conventional time-series analysis of daily counts of deaths and EHA's.

The case-crossover design is similar to that of a matched case-control study, with the difference that characteristics of event and control days and not characteristics of cases and controls are compared. By selecting control days in the same month and on the same day of the week, effects of seasonality and weekly cycles are automatically controlled. Thus, each case of death and of EHA defined an event day and the other days of the same month which were 7, 14, 21 or 28 days apart were selected as its controls. To perform case-crossover analysis we applied conditional logistic regression (CLR)

In both time-series and case-crossover analyses, we adjusted for concentrations, average temperature, relative humidity and holidays. In each model, we analysed the performance results with or without interaction effect between $PM_{2.5}$ concentrations and temperature. A dummy variable was created to represent the dry season and consequently occurrence of biomass burning. In this study, we assume that the relationship between $PM_{2.5}$ and mortality or EHAs is linear. As temperature and humidity could be non-linearly-related to the risk of death or emergency visits natural cubic splines of temperature and relative humidity at the day of the event with 2-4 degrees of freedom were used. For interaction term between the $PM_{2.5}$ and temperature in the time-series analysis (GAM) was applied a tensor smooth at 2-4 degrees of freedom.

Additionally, lagged effects of exposure to $PM_{2.5}$ concentrations during the biomass burning with lags between 1 and seven days after the exposure were assessed using the same adjustment variables. Contour plots on the log-scale of relative risk represented estimated interaction effects between $PM_{2.5}$ concentration and temperature. These plots were generated from time-series models. Results are reported as relative risks (RRs) for time series models and as odds ratios (ORs) for time-stratified case–crossover models along with their 95% confidence intervals (CIs). Spearman's correlation coefficient was used to evaluate the relations between all the variables studied. R software (version 3.1.3), packages "mgcv" and "survival (COXPH)" were applied to fit the time-series (GAM) and case-crossover models (CLR), respectively.

6.3.4 Ethics

This analysis is part of a larger project on *Cardiovascular diseases and the exposure to forest fires in Porto Velho municipality, Rondônia state, Brazil.* This project was submitted and accepted by the Research Ethics Committee of the Sergio Arouca National Public Health School (*Comitê de Ética em Pesquisa da Escola Nacional de Saúde Pública Sergio Arouca* – ENSP / FIOCRUZ), according to Resolution number 466/2012 from National Research Ethics Council (*Conselho Nacional de Pesquisa* – CONEP) under CAAE number 41732615.4.0000.5240.

6.4 RESULTS

There were 10,454 CVD deaths and 52,843 CVD EHAs recorded in Porto Velho during the whole study period (2009-2014). Table 3 shows the descriptive statistics of daily $PM_{2.5}$ concentration, meteorological parameters, mortality and emergency hospital admissions for the whole study period and the two seasons in Porto Velho. There was some variation in the variables across the seasons. The mean values of predicted concentrations of $PM_{2.5}$ differed between dry season (23μg/m³) and rainy season (7μg/m³). The difference of e maximum daily values of $PM_{2.5}$ between seasons was higher with values of 168μg/m³ and 41μg/m³, respectively. The daily mean numbers of e CVD deaths and CVD EHAs were slightly higher during the dry season (4.9 deaths and 36.7 EHAs) than during the rainy season (3.8 deaths and 35.6 EHAs). The meteorological conditions were in accordance with Amazon climate. The dry season was characterized by higher temperature values (Mean=27°C and Max=31ºC) and lower values of relative humidity (Mean=84% and Min=61.5%). The daily numbers of CVD deaths, and EHAs and the daily mean values of temperature were significantly and positively correlated with the daily mean values of $PM_{2.5}$. A moderate correlation between temperature and PM2.5 (R=0.27) was observed. The relative humidity was inversely correlated with $PM_{2.5}$.

Figure 6 shows the temporal distribution of biomass burning (satellite hotspots) and $PM_{2.5}$ concentrations for the whole period analysed. The peaks of $PM_{2.5}$ concentrations occurred in the same months as the highest frequencies of satellite hotspots. The months with the highest observed values were during the dry season, especially in September 2009 and 2010 and in August from 2011 to 2014.

The Table 4 and 5 show the estimated effects of daily levels of PM2.5 on CVD deaths and EHAs according to time-series and case-crossover models with or without interaction effect between temperature and $PM_{2.5}$ for dry season and whole study period. These models were fitted for temperature, humidity, holidays, year, month and days of the week. For mortality, higher risk estimates were obtained from the time-series analyses than from the case crossover models for both the dry season and the whole period. In contrast, for morbidity, the case crossover analysis provided higher values. During the occurrence of biomass burning (lag 0), the RR estimated for CVD deaths was 1.16 (95% CI: 1.04; 1.29) and the OR was 1.09 (95% CI: 0.75; 1.60). For CVD EHAs the RR was 1.06 (95% CI: 0.91; 1.23) and the OR was 1.09 (95% CI: 0.98; 1.20).

The lag-specific effects are shown graphically in Figures 7 and 8. Highest effects were observed for lag 0 both for mortality and for morbidity, in the time series analyses while effects disappeared or even got negative for lags 1 to 4. However, they became positive again at lags 5 to 7. This pattern might suggest harvesting. Associations between daily numbers of CVD deaths and PM2.5 were statistically significant at lags 0 and 6 (RR: 1.06; 95% CI: 1.00; 1.14). In contrast, the case crossover analysis showed a significant association between CVD EHAs and PM2.5 at lag 6 (OR: 1.02; 95%CI: 1.00; 1.03). Comparing the interaction effect for both analysed methods, it is important to highlight the consistently increase in RRs and ORs for CVD deaths and EHAs after adding the interaction term between temperature and $PM_{2.5}$. In the time-series analysis (GAM) the smooth term applied as the interaction effect was statistically significant for CVD deaths ($p<0.00571$) and EHAs ($p<0.000401$).

The Figure 9 shows a contour plot that represents the significant and strong effect of the interaction term in the GAM model during dry season. The highest risk of CVD death was observed at high temperatures (above 27ºC) and high level of PM2.5 (above 150µg/m³), On the other hand, the risk of CVD EHAs was higher with temperatures below 24°C with the concentration range of $PM_{2.5}$ from 50 to 150µg/m³.

6.5 DISCUSSION

Among the world's ten most populous countries, Brazil has one of the largest proportional increases in annual mean concentrations of $PM_{2.5}$, together with countries such as India, China, Bangladesh and Pakistan.[2] The occurrence of biomass burning has a high contribution in this increase, and therefore, it is important to characterise the forest fires related health risks. [11,12,20,21] Previous studies conducted in the Brazilian Amazon region have reported an increase of mortality and morbidity from respiratory diseases after exposure to high levels of air pollutants.[11,12,20-23] However, few studies in Brazil assessed the effects of biomass burning on cardiovascular diseases. Our findings in the city of Porto Velho support and extend these previous studies, demonstrating positive associations between $PM_{2.5}$ concentrations and CVD deaths and EHAs.

In general, the effects estimates using both time series and case crossover analyses were quite comparable.[17] For time series method, we found the lag-specific effect of CVD deaths statistically significant during the biomass burning air pollution (lag 0) and after the lag of 6 days. Concerning the time-stratified case crossover method, the lagged effect of CVD EHAs was significant for the lag of 6 days for whole study period. These results suggest a delayed effect and are in line with findings from other studies. The study developed in Mato Grosso, Amazonian municipality with the highest occurrence of biomass burning in Brazil, showed a significant cumulative effect of exposure to $PM_{2.5}$ over 6 and 7 days during dry season on morbidity from respiratory diseases in children.[11]. Even though the results obtained fromthe case crossover analysis for the dry season were not significant in this study, estimates were comparable in magnitude to those reported in other studies.[17,24-26]

We introduced an interaction term between $PM_{2.5}$ concentration and average temperature in the Quasi poisson generalized additive model to identify the isolated effect of each factor and how the combination of effects occurs. The results performed was complex and do not provide easily interpretable estimates for the relative risk but indicated a strong and significant interactive effect between pollutant and temperature in Brazilian Amazon region. The mortality risk at high temperatures (above 27°C) was higher than that found at low temperatures in the concentration range up 150µg/m³. Concerning the morbidity risk at temperatures below 24°C was higher in concentration range from 50 to 150µg/m³. Our findings is similar to model developed by Pinheiro, et al., (2014)[18] in São Paulo, Brazil and Curriero et al.,(2002)[19] in 11 United States (US) cities. Both studies aimed to determine the synergistic effects of PM_{10} and temperature and identified decreases in some concentration ranges of pollutant.

The impact of high temperature on the patients with cardiovascular diseases have been reported in previous studies. They make our finding of a strong interaction between high temperatures and high levels of PM2.5 plausible. .[27] Concerning the heat events, the recent findings suggest that cardiovascular deaths tend to happen quickly during hot days due to several pathophysiological mechanisms. For example, the stress during heat events can reduce cerebral blood velocity and consequently orthostatic tolerance.[28,29] Furthermore, the reduced plasma volume and water loss may modify the production of platelets, red and white cells, blood viscosity and plasma cholesterol level. All these mechanisms may be related with the increase in the mortality risk for arterial thrombosis during hot days.[30]

In the context of Brazilian Amazon region, where the socio-economical conditions are worse than in others parts of Brazil, the scenario of the exposure to environmental risk factors is complex. Other effect modifiers such as socioeconomic status, demographic developments, use of utilities as air conditioners and the synergistic effects with climate may presuppose health effects in the susceptible individuals and needs to be more investigated.[17-19, 31]

This study has two main strengths: (1) it is the first study to compare the results of time series and case crossover analyses regarding the effects of biomass burning on mortality and EHAs; and (2) sophisticated statistical methods were applied to assess lagged effects of biomass burning on both mortality and EHAs after adjusting for confounding factors. Nevertheless, some limitations need to be mentioned. We only focused on one city and the results might not be generalizable to other areas. However, the approaches applied in this study can be used in further research and in different areas. Moreover, we did not consider cause-specific mortality and morbidity because event numbers would have been too small. . Despite the small risks estimates on mortality and morbidity of CVD, the $PM_{2.5}$ exposure produced a substantial health effect when we consider the population size. Additionally, there might be exposure misclassification, as we used exposure daily estimates derived from satellite (AOD/MODIS) and meteorological data rather than daily measurements from an air quality monitoring station.

6.6 CONCLUSION

In our study, time-stratified case-crossover and time series analyses produce comparable estimates of the short-term relationship between air pollution from biomass burning and daily cardiovascular deaths and emergency hospital admissions. There was also a strong interaction effect between particulate matter and temperature that must be investigated in more detail. This finding may have implications for future studies and it may guide public health authorities in Brazil in developing public policies and health vigilance in the Amazon region regarding the environmental problems associated with air pollution from biomass burning. In particular, public health campaigns to reduce a prospective advancement of cardiovascular diseases in the population would be needed. International cooperation for reducing air pollution from biomass burning could provide significant public health benefits to Brazil and other South America countries.

6.7 ACKNOWLEDGMENTS

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TABLES AND FIGURES

TABLE 3. Descriptive statistics of daily $PM_{2.5}$ concentrations, meteorological parameters, numbers of deaths and of emergency hospital admissions, for the whole period and different seasons. Porto Velho municipality, Brazilian Amazon region.

Whole period: 1st January 2009 to 31st December 2014 (N=2.191)						
Variables	**Min**	**1st Q**	**Median**	**Mean**	**3rd Q**	**Max**
Outcome - Cardiovascular disease						
Mortality (deaths/day)	1.0	3.0	5.0	4.9	6.0	14.0
Emergency (admissions/day)	2.0	27.0	35.0	36.7	44.0	94.0
Exposure - $PM_{2.5}$ (µg/m³)	1.0	3.2	6.9	15.0	16.1	167.7
Controls - Meteorology						
24hr mean of temperature (°C)	16.2	25.9	26.7	26.6	27.4	31.3
24hr-mean of relative humidity (%)	61.5	83.0	87.0	86.4	90.0	98.8
24hr Preciptation (mm)	0	0	0	5.5	4.2	124.2
Dry season: June to November (N=1.076)						
Variables	**Min**	**1st Q**	**Median**	**Mean**	**3rd Q**	**Max**
Outcome - Cardiovascular disease						
Mortality (deaths/day)	1.0	3.0	5.0	4.9	6.0	14.0
Emergency (admissions/day)	5.0	27	36	36.7	45.0	94.0
Exposure - $PM_{2.5}$ (µg/m³)	1.0	4.1	12.6	23.1	27.9	167.7
Controls - Meteorology						
24hr mean of temperature (°C)	16.2	26.1	26.9	26.7	27.6	31.3
24hr-mean of relative humidity (%)	61.5	80.6	84.5	84.0	88.1	98.8
24hr Preciptation (mm)	0	0	0	2.5	0.6	68.8
Rainy season: December to May (N=1.015)						
Variables	**Min**	**1st Q**	**Median**	**Mean**	**3rd Q**	**Max**
Outcome - Cardiovascular disease						
Mortality (deaths/day)	1.0	2.0	4.0	3.8	5.0	13.0
Emergency (admissions/day)	2.0	27	34	35.6	43.0	93.0
Exposure - $PM_{2.5}$ (µg/m³)	1.0	2.5	5.3	7.2	10.5	40.5
Controls - Meteorology						
24hr mean of temperature (°C)	22.1	25.8	26.5	26.5	27.2	30.4
24hr-mean of relative humidity (%)	66.8	86.0	88.8	88.6	91.5	98.8
24hr Preciptation (mm)	0	0	1.0	8.5	10.2	124.2
Spearman's rank correlation						
	EHA	**DEATH**	**$PM_{2.5}$**	**TEMP**	**RH**	**PRECIP**
EHA[a]	1					
DEATH[b]	-0.0237	1				
$PM_{2.5}$[c]	0.0304*	0.0457*	1			
TEMP[d]	-0.0263	0.0550*	0.2663*	1		
RH[e]	-0.0009	-0.0363	-0.1996*	-0.6251*	1	
PRECIP[f]	0.019	-0.0579*	-0.0338	-0.2447*	0.3090*	1

Note: [a]EHA= Emergency hospital admissions for cardiovascular disease; [b]DEATH= Mortality for cardiovascular disease; [c]$PM_{2.5}$=$PM_{2.5}$ concentrations; [d]TEMP= Average temperature; [e]RH= Relative humidity; [f]PRECIP= Precipitation.

TABLE 4. Relative risks (RR), odds ratio (OR) and 95% confidence interval (95% CI) for CVD deaths and EHAs for dry season. Porto Velho municipality, Brazilian Amazon region.

	Time series - CVD deaths (RR)	
	With interaction	Without interaction
lag0	1.16 (1.04; 1.29)	1.13 (0.94; 1.35)
lag1	0.98 (0.92; 1.05)	0.97 (0.90; 1.05)
lag2	0.93 (0.86; 0.99)	0.92 (0.85; 0.99)
lag3	0.92 (0.87; 0.98)	0.92 (0.87; 0.98)
lag4	0.96 (0.91; 1.02)	0.96 (0.91; 1.02)
lag5	1.02 (0.95; 1.09)	1.02 (0.95; 1.09)
lag6	1.06 (1.00; 1.14)	1.06 (1.00; 1.14)
lag7	1.06 (0.96; 1.18)	1.06 (0.96; 1.18)
	Case crossover - CVD deaths (OR)	
	With interaction	Without interaction
lag0	1.09 (0.75; 1.60)	1.07 (0.79; 1.45)
lag1	0.97 (0.78; 1.20)	0.97 (0.79; 1.18)
lag2	0.96 (0.76; 1.21)	0.96 (0.76; 1.20)
lag3	1.00 (0.82; 1.20)	1.00 (0.83; 1.20)
lag4	1.05 (0.86; 1.28)	1.05 (0.86; 1.28)
lag5	1.06 (0.84; 1.34)	1.06 (0.83; 1.34)
lag6	0.99 (0.79; 1.23)	0.98 (0.79; 1.23)
lag7	0.80 (0.54; 1.20)	0.80 (0.53; 1.20)
	Time series - CVD EHAs (RR)	
	With interaction	Without interaction
lag0	1.06 (0.91; 1.23)	0.99 (0.94; 1.04)
lag1	1.02 (0.97; 1.08)	1.00 (0.97; 1.03)
lag2	1.01 (0.97; 1.04)	1.01 (0.98; 1.04)
lag3	1.00 (0.96; 1.03)	1.00 (0.98; 1.03)
lag4	0.99 (0.96; 1.02)	1.00 (0.97; 1.02)
lag5	0.99 (0.96; 1.03)	0.99 (0.96; 1.02)
lag6	0.99 (0.95; 1.02)	0.99 (0.96; 1.02)
lag7	0.97 (0.92; 1.02)	1.00 (0.95; 1.04)
	Case crossover - CVD EHAs (OR)	
	With interaction	Without interaction
lag0	1.09 (0.98; 1.20)	1.04 (0.99; 1.10)
lag1	1.03 (0.97; 1.09)	1.01 (0.98; 1.04)
lag2	1.00 (0.96; 1.02)	0.98 (0.96; 1.01)
lag3	0.97 (0.94; 1.00)	0.97 (0.94; 1.00)
lag4	0.96 (0.93; 0.99)	0.97 (0.94; 1.00)
lag5	0.97 (0.94; 1.00)	0.98 (0.95; 1.00)
lag6	0.99 (0.96; 1.02)	1.00 (0.97; 1.02)
lag7	1.03 (0.97; 1.09)	1.03 (0.97; 1.09)

Note: Models adjusted for average temperature, relative humidity, holidays, year, month and days of the week. a=Interaction term ($PM_{2.5}$ and average temperature) using smooth (tensor); b= Interaction terms ($PM_{2.5}$ and average temperature) using smooth (tensor).

TABLE 5. Relative risks (RR), odds ratio (OR) and 95% confidence interval (95% CI) for CVD deaths and EHAs for whole period. Porto Velho municipality, Brazilian Amazon region.

	Time series - CVD deaths (RR)	
	With interaction[a]	Without interaction
lag0	1.11 (0.92; 1.35)	1.02 (0.87; 1.19)
lag1	1.08 (0.97; 1.19)	1.03 (0.94; 1.13)
lag2	1.05 (0.97; 1.13)	1.03 (0.96; 1.12)
lag3	1.02 (0.93; 1.12)	1.03 (0.94; 1.13)
lag4	1.00 (0.91; 1.09)	1.01 (0.93; 1.11)
lag5	0.98 (0.90; 1.06)	0.99 (0.91; 1.07)
lag6	0.96 (0.88; 1.06)	0.96 (0.87; 1.05)
lag7	0.95 (0.80; 1.12)	0.92 (0.78; 1.08)
	Case crossover - CVD deaths (OR)	
	With interaction	Without interaction
lag0	1.05 (0.92; 1.19)	1.01 (0.95; 1.07)
lag1	1.02 (0.97; 1.08)	1.01 (0.97; 1.05)
lag2	1.00 (0.97; 1.05)	1.01 (0.97; 1.05)
lag3	0.99 (0.96; 1.03)	1.00 (0.96; 1.03)
lag4	0.99 (0.95; 1.02)	0.99 (0.95; 1.02)
lag5	0.99 (0.95; 1.03)	0.98 (0.94; 1.02)
lag6	0.99 (0.95; 1.03)	0.99 (0.95; 1.03)
lag7	1.01 (0.95; 1.07)	1.01 (0.95; 1.07)
	Time series - CVD EHAs (RR)	
	With interaction[b]	Without interaction
lag0	1.02 (0.87; 1.18)	0.98 (0.91; 1.05)
lag1	1.02 (0.94; 1.11)	0.99 (0.95; 1.03)
lag2	1.02 (0.98; 1.07)	1.00 (0.97; 1.04)
lag3	1.02 (0.98; 1.06)	1.01 (0.97; 1.05)
lag4	1.02 (0.97; 1.07)	1.02 (0.98; 1.06)
lag5	1.02 (0.97; 1.06)	1.02 (0.99; 1.06)
lag6	1.02 (0.97; 1.06)	1.03 (0.98; 1.07)
lag7	1.01 (0.93; 1.09)	1.02 (0.95; 1.10)
	Case crossover - CVD EHAs (OR)	
	With interaction	Without interaction
lag0	1.01 (0.95; 1.07)	0.99 (0.96; 1.03)
lag1	1.00 (0.97; 1.04)	0.99 (0.98; 1.01)
lag2	1.00 (0.98; 1.02)	0.99 (0.98; 1.01)
lag3	1.00 (0.98; 1.02)	1.00 (0.98; 1.02)
lag4	1.00 (0.98; 1.02)	1.00 (0.98; 1.02)
lag5	1.01 (0.99; 1.03)	1.01 (0.99; 1.02)
lag6	1.02 (1.00; 1.03)	1.02 (1.00; 1.04)
lag7	1.03 (0.99; 1.06)	1.03 (0. 99; 1.06)

Note: Models adjusted for average temperature, relative humidity, holidays, year, month and days of the week. a=Interaction term ($PM_{2.5}$ and average temperature) using smooth (tensor); b= Interaction terms ($PM_{2.5}$ and average temperature) using smooth (tensor).

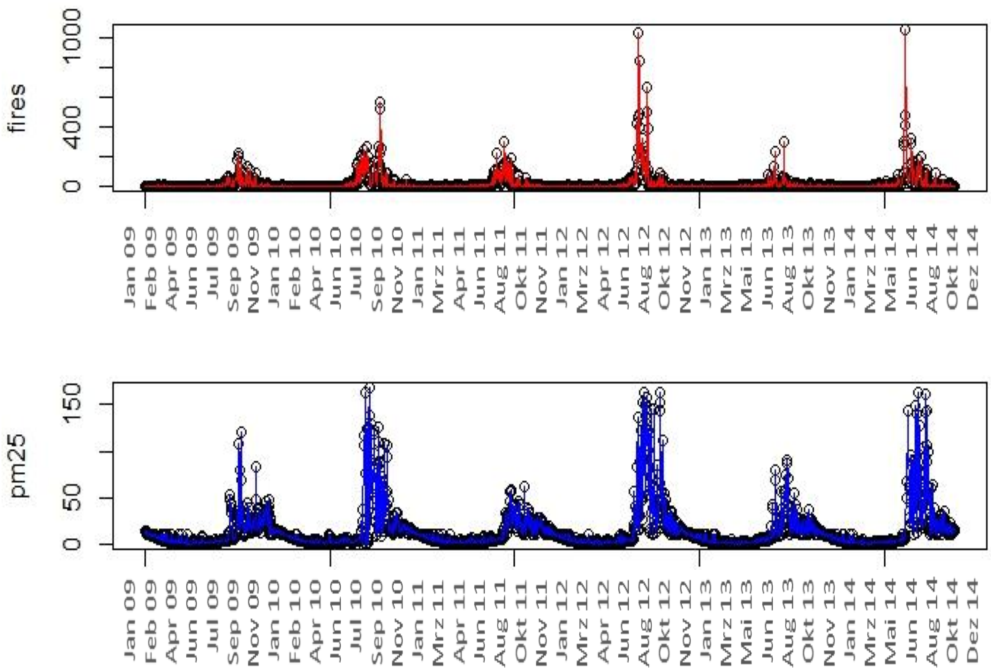

FIGURE 6. Temporal distribution of biomass burning (frequency of satellite hotspots) and $PM_{2.5}$ concentrations (μg/m³) for whole period analysed.

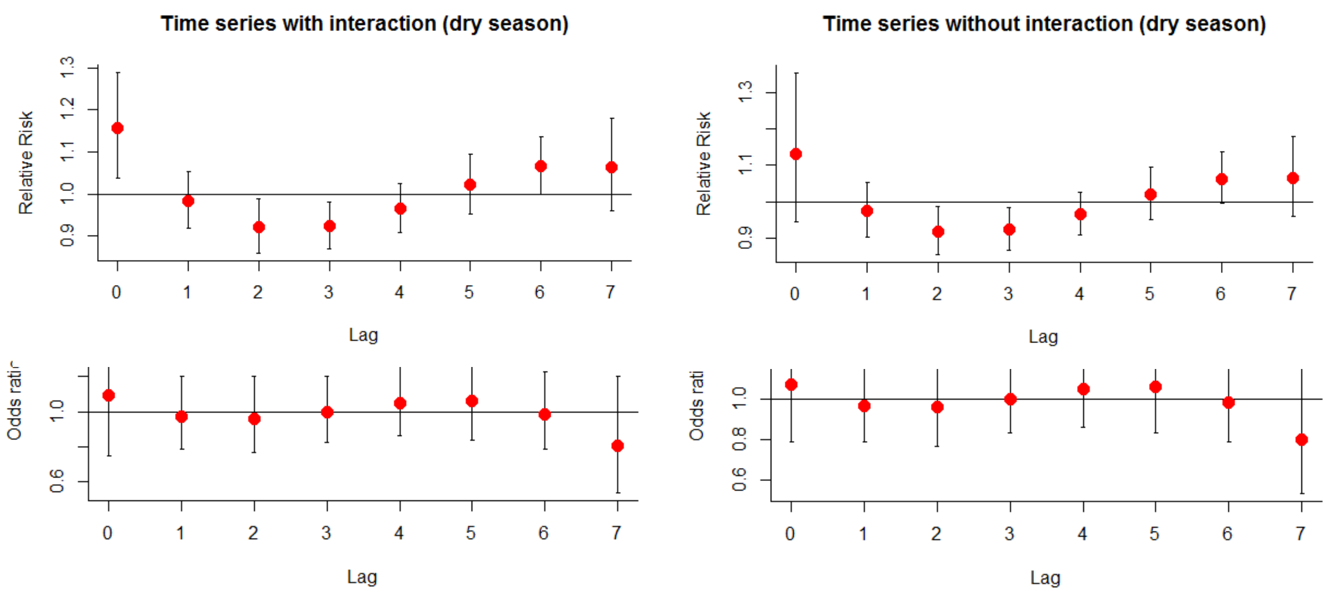

FIGURE 7. Lag-specific effects of $PM_{2.5}$ on the relative risk (RR) and odds ratio (OR) of CVD deaths for time series and case crossover models, respectively, considering the interaction term between $PM_{2.5}$ and average temperature for dry season.

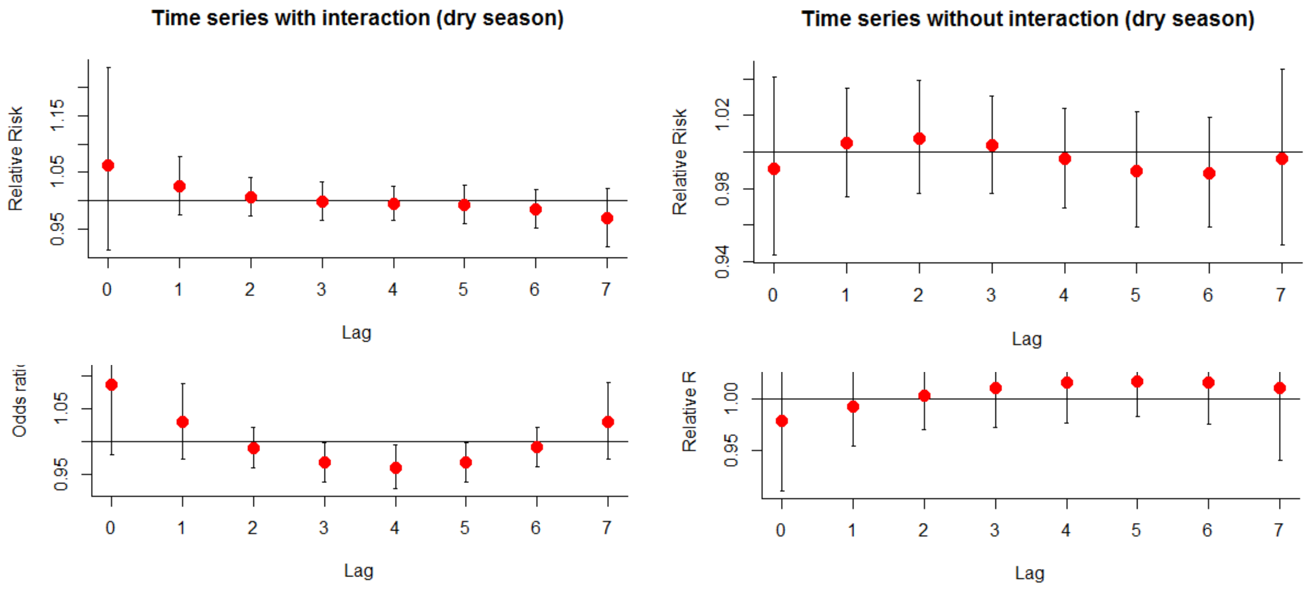

FIGURE 8. Lag-specific effects of $PM_{2.5}$ on relative risk (RR) and odds ratio (OR) of CVD EHAs for time series and case crossover models, respectively, considering the interaction terms ($PM_{2.5}$ and average temperature) for dry season.

[C]	Significance of smooth interaction term: te (Temperature, $PM_{2.5}$)				
Models	Deviance explained	Resid. Deviance	R^2 (adj)	df	p-value
GAM - CVD deaths	8.88%	2083.6	0.0174	2.993	0.00571
GAM - CVD EHAs	70.4%	1102.7	0.688	3.986	<0.000401

[A] **[B]**

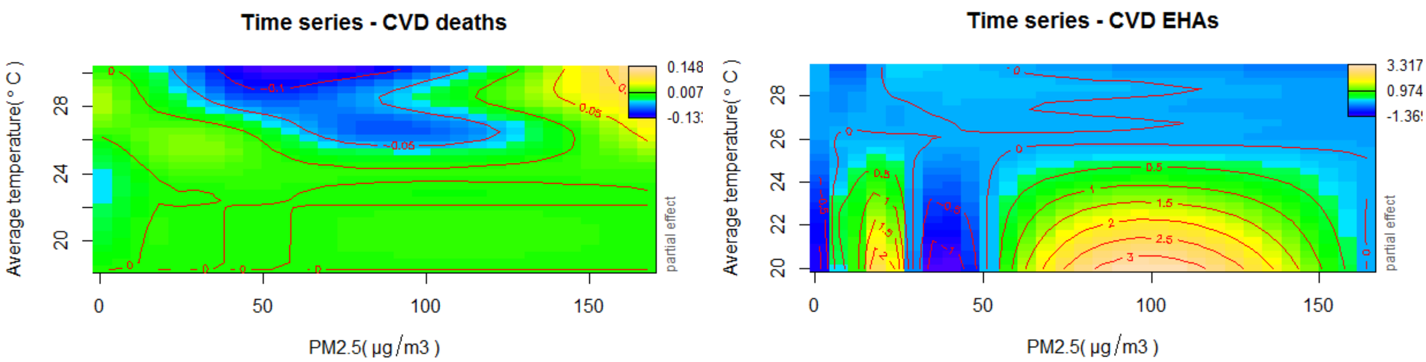

FIGURE 9. Interaction effect between $PM_{2.5}$ concentrations and average temperature on risk (log scale) of CVD deaths (A) and EHAs (B) during the dry season. (C) Partial effect from the time series model using the smooth interaction term for (GAM) statistically significant at 5%.

7 CONCLUSION

7 CONCLUSION

This study intended contribute with the knowledge on biomass burning air pollution and the effects on mortality and morbidity of cardiovascular diseases in the city of Porto Velho, Brazilian Amazon region. The results described in this Thesis can support the public health policies and environmental health surveillance to minimize the impacts regarding the forest biomass burning.

The findings presented may have implications for future studies and need to be highlighted. The validation approach to predict fine particle concentrations using the satellite data showed an important and alternative method to describe the impacts of forest fires on air quality and to assess the related health effects. The non-linear prediction model for $PM_{2.5}$ showed a good performance, explaining on average 82% of the variance in $PM_{2.5}$ concentrations during the study period. This model can be applied in other studies and in different municipalities, especially in areas with high cost for implementing air quality monitoring stations.

The comparison between the epidemiological designs demonstrate that both time series and time-stratified case-crossover analyses produced comparable results of the relationship between biomass burning air pollution and health outcomes. For time series method, the results showed a lag-specific effect of CVD deaths statistically significant during the biomass burning air pollution (lag 0) and after the lag of 6 days. Concerning the time-stratified case crossover method, the lagged effect of CVD EHAs was significant only for the lag of 6 days for whole study period. These results suggest a delayed and cumulative effect and support previous researches and discussed on the second manuscript. However, in general, the risk estimation using a case crossover analysis was lower and less accurate than the time series analysis.

The interaction effect showed complex results but indicated a strong and significant synergistic effect between pollutant and temperature in Brazilian Amazon region. The results suggested that the mortality risk at high temperatures (above 27°C) was higher than that found at low temperatures in the concentration range up 150μg/m³. Concerning the morbidity risk at temperatures below 24°C was higher in concentration range from 50 to 150μg/m³. This finding must be more investigated to increase the knowledge concerning the synergistic effect temperature-air pollutant and whether these terms can act as modifier effect in Amazon region.

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SUPPLEMENTARY 1

R CODES FOR CONDITIONAL POISSON ANALYSES

SUPPLEMENTARY 1 – R CODES FOR CONDITIONAL POISSON ANALYSES

The R codes used to perform the conditional logistic regression for case crossover analysis was based on the supplementary of the manuscript published by Armstrong, BG, Gasparrini, A and Tobias, A. Conditional Poisson models: a flexible alternative to conditional logistic case crossover analysis. BMC Medical Research Methodology 201414:122. DOI: 10.1186/1471-2288-14-122

###################

Variable name notation:

t:	time sequence
x:	exposure (eg pollution)
v1-v6:	covariates (eg temperature spline)
y:	outcome count
stratum:	stratum indicator (eg for yearXmonthXday-of-week)
dur	duration of time interval (if not equal)

Full worked example in R for CLR

```
################################################################################
# FIT CONDITIONAL LOGISTIC MODEL

# EXPAND THE DATA IN A CASE-CROSSOVER FORMAT (AND EXCLUDE STRATA WITH 0)
#  REQUIRED FUNCTION
funccmake <-  function(date,cases,vars=NULL,dow) {
#
  # DERIVE STRATUM VARIABLES
  if(missing(dow)) dow <- ifelse(class(date)=="Date",TRUE,FALSE)
  if(class(date)=="Date") {
    day <- if(dow) weekdays(date) else rep(1,length(date))
    month <- months(date)
    year <- format(date, format="%Y")
  } else {
    day <- rep(1,length(date))
    month <- date
    year <- rep(1,length(date))
    if(dow) stop("'dow' only available when 'date' is a date")
  }
#
  # DERIVE INDEXING VARIABLES
       gfactor <- factor(day):factor(month):factor(year)
       gnumber <- match(gfactor,unique(gfactor))
       gindex <- lapply(1:length(date),
               function(x) (1:length(date))[gnumber%in%gnumber[x]])
       gstatus <- lapply(1:length(date), function(x) gindex[[x]]==x)
#
  # EXPAND PREDICTORS
  if(!is.null(vars)) {
    varnames <- if(is.vector(vars)) deparse(substitute(vars)) else names(vars)
    vars <- as.matrix(vars)
    dimnames(vars) <- list(NULL,varnames)
  }
#
  # RESULTS
       res <- data.frame(
    index=unlist(gindex),
    status=unlist(gstatus)+0,
               stratum=rep(1:length(date),sapply(gindex,length)),
               weights=rep(cases,sapply(gindex,length))
       )
```

```
  if(!is.null(vars)) res <- cbind(res,vars[res$index,])
#
  return(res)
}

dataexp<- funccmake(data$stratum,data$numdeaths,vars=cbind(data$ozone10,data$temperature ))
dataexp <- dataexp[dataexp$weights>0,]
Xexp <- as.matrix(dataexp)[,-seq(4)]

# RUN CLR
library(survival)
timeout <- as.numeric(factor(dataexp$stratum))
timein <- timeout-0.1
model_clr <- coxph(Surv(timein,timeout,status) ~ Xexp, weights=weights, dataexp)
summary(model_clr)
```