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Genetic damage of organic matter in the Brazilian Amazon: A comparative study between intense and moderate biomass burning

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ABSTRACT

Background: The biomass burning that occurs in the Amazon region has an adverse effect on environmental and human health. However, in this region, there are limited studies linking atmospheric pollution and genetic damage.
Objective: We conducted a comparative study during intense and moderate biomass burning periods focusing on the genetic damage and physicochemical analyses of the particulate matter (PM).
Method: PM and black carbon (BC) were determined; organic compounds were identified and quantified using gas chromatography with flame ionization detection, the cyto-genotoxicity test was performed using two bioassays: cytokinesis-block micronucleus (CBMN) in A549 cells and *Tradescantia pallida* micronucleus (Trad-MCN) assay.
Results: The PM_{10} concentrations were lower than the World Health Organization air quality standard for 24 h. The *n*-alkanes analyses indicate anthropogenic and biogenic influences during intense and moderate biomass burning periods, respectively. Retene was identified as the most abundant polycyclic aromatic hydrocarbon during both sampling periods. Carcinogenic and mutagenic compounds were identified. The genotoxic analysis through CBMN and Trad-MCN tests showed that the frequency MCN from the intense burning period is significantly higher compared to moderate burning period.
Conclusions: This is the first study using human alveolar cells to show the genotoxic effects of organic PM from biomass burning samples collected in Amazon region. The genotoxicity of PM can be associated with the presence of several mutagenic and carcinogenic compounds, mainly benzo[a]pyrene. These findings have potential implications for the development of pollution abatement strategies and can minimize negative impact on health.

1. Introduction

The Amazon spans more than half of the Brazilian territory and this region has shown an advancing economic development, mainly agribusiness, ranching and infrastructure projects. The Brazilian Amazon region is extensive and has been intensively affected by deforestation and biomass burning, resulting in increased impacts on our climate and environment with adverse effects on public health (Oliveira et al., 2012; Sisenando et al., 2012).

Epidemiologic studies indicate strong association between exposure to particulate matters with aerodynamic diameter less than 10 μg and 2.5 μg and morbidity and mortality of cardiovascular and respiratory diseases (Pope, 2000).

PM is a complex mixture including inorganic and organic compounds. Size and chemical properties influence the site of deposition within the respiratory tract. In order to understand the properties of PM, such as its genotoxic effects, detailed knowledge of chemical composition is required. Moreover, studies have documented that one of the components that may be responsible for the observed health effects are organic PM, mainly carcinogenic/mutagenic compounds such as polycyclic aromatic hydrocarbons (PAHs)

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(Abou Chakra et al., 2007; Bonetta et al., 2009). In addition, analytical methods can be used to assess the composition and source of PM, for example, the identifying and quantifying of PAHs and *n*-alkanes (Vasconcellos et al., 2010).

Due to its low cost and high efficiency, cytogenetic methods, especially those based on identification of micronucleus (MCN), are the most extensively used technique to detect DNA damage induced by air pollutants (Fenech, 2002; Roubicek et al., 2007). A pioneering study of Alves et al. (2011b) associated chemical composition and the genotoxic effects of organic PM using *Tradescantia pallida* micronucleus (Trad-MCN) bioassay (*ex situ*) in the eastern Brazilian Amazon region. Besides, studies by Poma et al. (2006) with macrophages cell line, Wang et al. (2011) with human lung carcinoma cells and Oh et al. (2011) with human lung bronchial epithelial showed genotoxic effects through MCN biomarker after exposure to PM in urban areas.

There are limited study developments evaluating the health impacts resulting from biomass burning (Ignotti et al., 2010). Indeed, few studies have been developed in the Amazon, mainly on the genotoxicity and cytotoxicity of the PM associated with chemical composition. To date, there have been no published articles that had evaluated the genotoxicity of organic matter from biomass burning in Amazon region using in adenocarcinomic human alveolar basal epithelial cell line (A549). The use of this cell system *(in vitro)* is a good model to investigate genetic damage (Gminski et al., 2011; Könczöl et al., 2011; Tang et al., 2012).

The municipality of Alta Floresta located in the extreme north of the state of Mato Grosso (MT), southeast of the Amazon region, was selected for this study because of its extensive forest burning and its population has been increasing significantly in the last decade. We conducted a comparative analysis during intense and moderate biomass burning periods evaluating the following parameters: (a) PM_{10} and black carbon (BC) levels as well as fine and coarse fractions of PM; (b) characterization of the chemical composition of organic PM by identifying and quantifying *n*-alkanes and PAHs; (c) cytotoxicity of organic PM measured by mitochondrial dehydrogenase activity (MTT) in A549 cells and (d) genotoxicity of organic PM using in two bioassays: cytokinesis-block micronucleus (CBMN) in A549 and Trad-MCN (*ex situ*) assays.

2. Methods

2.1. Study area and sampling

According to the Brazilian National Institute for Space Research (INPE), among the states which constitute the deforestation arch in the Brazilian Amazon, MT had the highest concentration of heat outbreaks and of deforested area in recent years (Dubreuil et al., 2012). Alta Floresta covers an area of 9310.27 km^2. It is located 830 km from the capital of the state of MT-Cuiabá, 340 m above sea level, with latitude of 09°52′33″S (Fig. 1). This municipality was selected for this study due to the intense and extensive deforestation to wood exploration and to the preparation of the land for crops and pastures activities, consequently increasing the biomass burning.

In contrast to urban areas, where air pollution sources are independent of climate seasonality, biomass burning in the Amazon region occurs mainly during the dry season (July–October) when the highest inflammability of the forest is observed. We have devised a comparative study focusing on the physical and chemical characterization of the PM, associated with cytotoxic and genotoxic analyses. PM samples were collected during two well distinct periods: the dry season (August–October/2008) and the transition season (November/2008–January/2009). The former will be referred hereafter as intense biomass burning period whereas the latter as the moderate biomass burning period.

The PM was collected using two types of filters: Teflon and polycarbonate according to work described by Alves et al. (2011b). The PM_{10} was collected with Teflon filters (1 μm pore size, 47 mm diameter) for 48 h. A sampler coupled with an inlet was used to collect particles smaller than 10 μm. The sampling using polycarbonate filters was performed between 24 and 48 h using a stacked filter unit (SFU), separating fine and coarse particles ($PM_{2.5}$–$PM_{2.5-10}$).

2.2. PM organic extracts

The sample extraction method used in this study has previously been described in detail (Alves et al., 2011b; Sato et al., 1995). Briefly, the organic compounds from the Teflon filters were extracted by ultrasonication using dichloromethane (DCM). Excess solvent was eliminated in a rotatory evaporator and reduced to a final volume of 5 mL under a gentle nitrogen stream. Once the concentration of the extracted organic matter was determined for intense and moderate biomass burning periods, the samples were stored at −20 °C. After this, half of the material was re-suspended in *n*-hexane, where the extract was fractionated into individual compound classes using flash chromatography on silica gel. The other half was dissolved in dimethylsulfoxide (DMSO) and used in the cytotoxic and genotoxic tests.

2.3. Physical and chemical analyses

According to Maenhaut et al. (2002), the mass of Teflon and polycarbonate filters was obtained by gravimetric method. BC was measured by reflectance technique in accordance with Reid et al. (1998).

To analyze the alkanes and PAHs of samples and blank filters, the compounds were identified and quantified using a gas chromatograph with flame ionization detection (GC-FID, Varian 3800) (Gogou et al., 1998). A fused-silica capillary column, DB-5 (30 m × 0.25 mm i.d, 0.25 μm film thickness), was used for separation. The chromatographic conditions were as follows: temperatures used on the injector and detector were, respectively, 250 ºC and 290 ºC; temperature ramp: 40 °C (1 min); 40–150 °C (10 °C/min); 150–290 °C (5 °C/min); and 290 °C (30 min). Nitrogen was the carrier gas. A 1 μL sample was injected using the splitless mode (Vasconcellos et al., 2010). In this study, 18 PAHs compounds, listed as priority by the United States Environmental Protection Agency (US-EPA), were analyzed, as follows: naphthalene (NAP), acenaphthylene (ACY), acenaphthene (ACE), fluorene (FLU), anthracene (ANT), phenanthrene (PHE), fluoranthene (FLT), pyrene (PYR), chrysene (CHR), benz[a] anthracene (BaA), retene (RET), benzo[a]pyrene (BaP), benzo[e]pyrene (BeP), benzo [g,h,i]pyrene (BghiP), benzo[b]fluoranthene (BbF), benzo[k]fluoranthene (BkF), indene[1,2,3-c,d]pyrene (IND) and dibenz[a,h]anthracene (DBA).

2.4. Cell culture

A549 cell was cultured in Dulbecco′s modified Eagle′s medium (DMEM) – high glucose, supplement with 10% fetal calf serum, 100 U/mL of penicillin and 100 μg/mL of streptomycin. The cell was maintained in a humid incubator at 37 ºC with 5% CO_2. When the cells grew to approximately 85% confluence, they were released from the surface by trypsinization (10%) and centrifuged for 5 min at 1500 rpm. The cell precipitate was re-suspended with phosphate buffer saline (PBS). The A549 cells suspension was adjusted to an appropriate concentration for cytotoxic and CBMN assays.

2.5. Cytotoxic assay

Cell viability was determined by the mitochondria-dependent reduction of MTT (3-(4,5-dimethyl-thiazol-2y)2,5-diphenyl-tetrazolium bromide, Sigma) to formazan, following the protocol established by Wendy Hsiao et al. (2000), with some modifications. Cell cultures were seeded in 96-well culture plates at a density of 8×10^3 cells in DMEM and 24 h later the A549 cells were treated with 0.1, 0.5 and 1.0 mg/L of the organic PM from the intense and moderate biomass burning periods for 24 h. Each treatment was tested in 10 individual wells. After incubation for different concentrations, the cells were incubated with 100 μL of 1.0 mg/mL MTT for 4 h at 37 °C, followed by 15 min incubation at 37 °C with 100 μL DMSO. The reading of absorbance was performed in a wavelength of 570 nm by ELISA Microplate Reader. The results were expressed as a percentage of the absorbance of control cells.

2.6. Genotoxic tests

To investigate the genotoxic effect *in vitro*, we used the A549 cells. This assay was performed using the standard technique proposed by Fenech (2002), with some modifications. The test was initiated by seeding 1.5×10^5 cells into six-well culture plates and 24 h later cells were exposed to different concentrations (0.1 mg/L; 0.5 mg/L and 1.0 mg/L) of organic PM from the intense and moderate biomass burning periods. The cytochalasin B (Sigma) was added 24 h later at a final concentration of 5 μg/mL with the objective of accumulation of dividing cells at the binucleate stage, regardless of their degree of synchrony. Negative control cells were exposed to DMEM and DMSO 0.1%. The positive controls were treated with 10 ng/mL of a PAHs mixture similar to Roubicek et al. (2007). After 48 h of exposure, cells were harvested, fixed with ice-cold fixative solution (methanol–acetic acid 3:1), and subsequently stained with 10% Giemsa (stock solution diluted 1:10 in PBS) for 7 min, rinsed in distilled water and air-dried. For each treatment, the presence of MCN, nucleoplasmic bridges and nuclear buds were evaluated in 3000 binucleated cells (triplicate) in coded slides under a magnification of 400 × using an optical microscope (Fig. 2). In addition to genotoxicity studies, CBMN assess also cytotoxicity by the nuclear division index (NDI). The NDI was

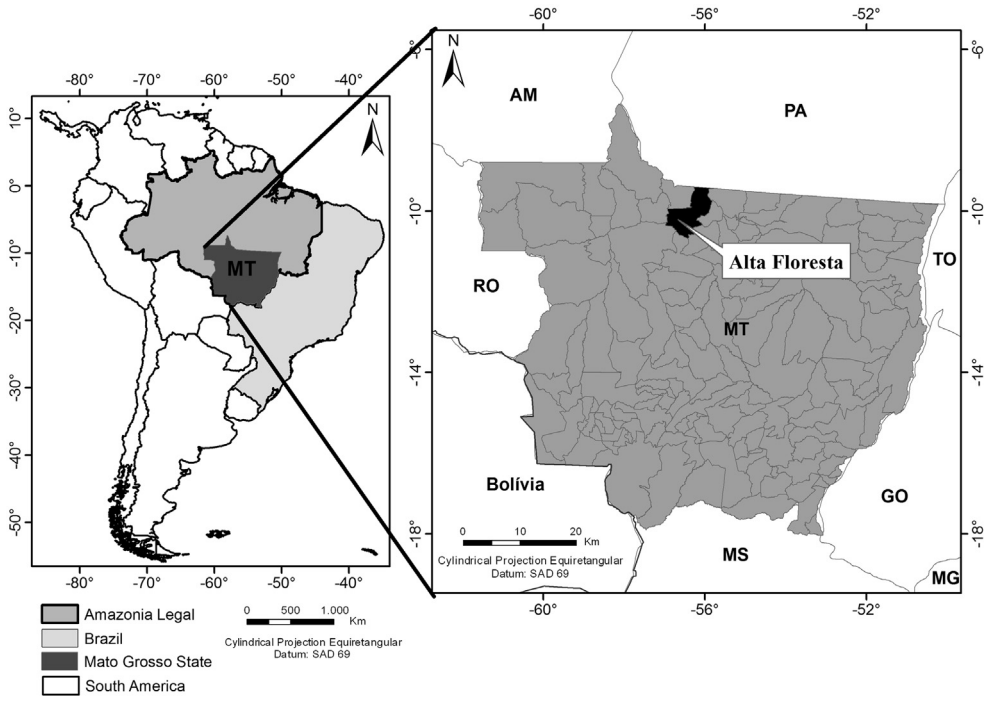

Fig. 1. Geographical location of the municipality of Alta Floresta, Mato Grosso State.

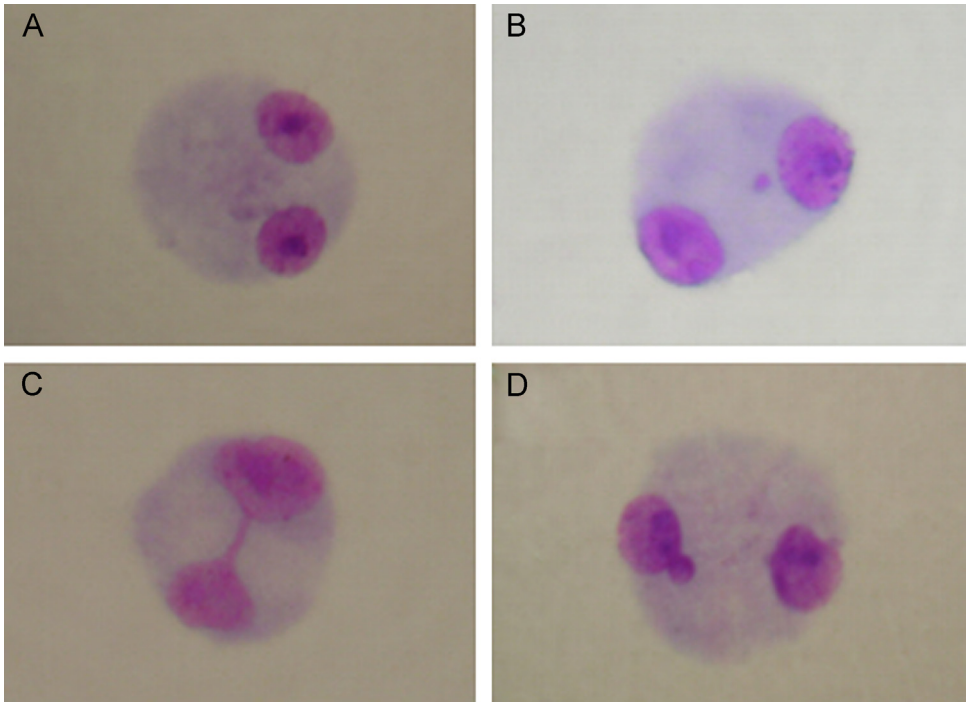

Fig. 2. Images illustrating the various end-points that were scored using the CBMN test in A549 cells. (A) Binucleated cell without MCN, (B) binucleated cell with MCN, (C) nucleoplasmic bridge and (D) nuclear bud.

determined using the formula NDI=(M1+2M2+3M3+4M4)/*N*, where M1–M4 represents the number of cells with 1 to 4 nuclei and *N* is the total number of cells score, as recommended by Fenech (1997).

The Trad-MCN assay (*ex situ*) was performed as described by Ma (1981). The plants were collected and kept for 24 h in the laboratory, using a nutritive Hoagland solution. Independent experiments were performed in triplicate. The organic PM were prepared at three different concentrations (0.1 mg/L, 0.5 mg/L, and 1.0 mg/L) for intense and moderate biomass burning periods. Negative (blank filters with DMSO 1%) and positive controls (0.2% formaldehyde) were also used in this bioassay. The period of exposure was of 8 h. Then, the plants were placed in Hoagland's solution for 24 h. The inflorescences were then fixed in a solution of acetic acid and alcohol (1:3 ratio) for 48 h and stored in 100% alcohol. The young anthers were removed, dissected and squashed on micro-slides in a solution of acetocarmine stain. The MCN present in a random set of 300 early tetrads per slide was scored under 400× magnification, analyzing a total of 3000 tetrads per each concentration. The results were expressed as the number of MCN/100 tetrads, taking into account that each MCN represented one mutation event.

2.7. Statistic analysis

The statistical computations were performed using Statistical Package for Social Sciences (SPSS) 15.0 and OriginPro 8, as follows: (i) an analysis of variance (ANOVA) of PM10 and BC data was performed, considering the simple linear regression;

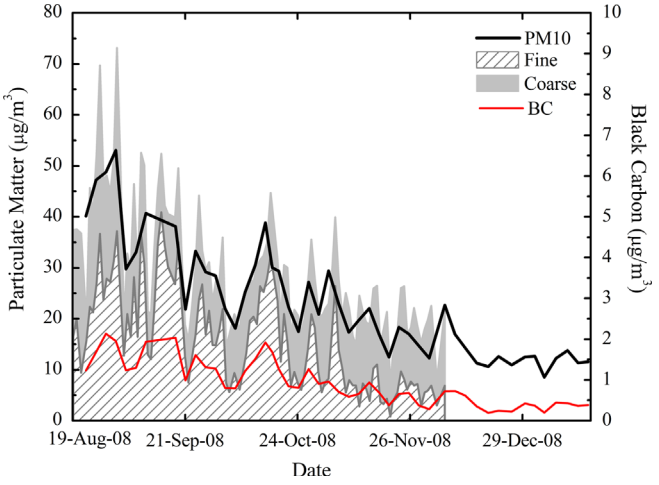

Fig. 3. Results of the mass concentration of coarse and fine fractions, PM_{10} and BC emissions from biomass burning in Alta Floresta.

(ii) one-way ANOVA was used in both cytotoxic and genotoxic tests; (iii) Dunnett's test was also carried out to determine the significance level between the treated and control group as well as the Tukey test for post hoc multiple comparisons. The mean differences and correlations were considered significant at $p < 0.05$.

3. Results

3.1. PM and BC concentrations

Fig. 3 represents a summary of the mass concentration of coarse and fine fractions, PM_{10} and BC of the samples collected during biomass burning in the Brazilian Amazon region. The PM_{10} concentrations, measured with two different filters (Teflon and polycarbonate), were similar. The highest concentrations of PM_{10} and BC were found in the intense biomass burning period rather than in the moderate and as expected, there is a strong correlation in this analysis ($p < 0.001$ and $R^2 = 0.88$). Evaluating fine and coarse fractions individually from the SFU dataset, a predominance of fine particles was observed in Alta Floresta for both periods of sampling.

PM_{10} average mass concentration in the samples collected were 34.3 μg/m^3 for the intense biomass burning and 16.7 μg/m^3 for the moderate biomass burning periods. It is important to point out that most of the PM_{10} concentrations did not exceed the limit established by the World Health Organization (50 μg/m^3) (WHO, 2005).

3.2. Chemical analysis of organic compounds

Total *n*-alkanes and individual PAHs concentrations of the samples collected in Alta Floresta are shown in Table 1. Aliphatic compounds comprised the *n*-alkane homologs series from C_{18} to C_{34}. The average concentration of the total *n*-alkanes during the intense and moderate biomass burning periods were 21.8 ng/m^3 and 103.2 ng/m^3, respectively. The highest concentration in both periods was observed at C_{29}. The Carbon Preference Index (CPI) is a diagnostic parameter, and high CPI indicates the major incorporation of biological constituents into the aerosol sample. The anthropogenic contaminants reduce CPI to values close to 1 (Omar et al., 2007). The CPI was calculated for each sample by using the following Eq. (1) (Ladji et al., 2009; Yassaa et al., 2001):

$$CPI = 0.5[(C_{24}+C_{26}+C_{28}+C_{30})/(C_{25}+C_{27}+C_{29}+C_{31}) + (C_{26}+C_{28}+C_{30}+C_{32})/(C_{25}+C_{27}+C_{29}+C_{31})] \quad (1)$$

In this study, the CPI values of the homologs were 0.9 and 1.4 in intense and moderate biomass periods, respectively. The isoprenoid hydrocarbons pristane and phytane were identified in the samples and ratios of these compounds were 1.30 and 1.09 for intense and moderate burning, respectively.

Table 1
Concentrations of organic compounds during intense and moderate biomass burning periods in Alta Floresta.

	Intense biomass burning	Moderate biomass burning
***n*-Alkanes (ng/m^3)**		
C_{18}	1.7	1.0
C_{19}	0.9	0.7
C_{20}	1.9	0.9
C_{21}	0.9	0.9
C_{22}	1.0	1.3
C_{23}	0.9	2.6
C_{24}	1.0	3.7
C_{25}	1.4	6.0
C_{26}	1.2	9.0
C_{27}	2.1	11.9
C_{28}	1.4	15.1
C_{29}	2.7	24.2
C_{30}	1.1	14.6
C_{31}	1.3	3.2
C_{32}	0.7	2.4
C_{33}	0.6	4.4
C_{34}	1.0	1.3
Total	21.8	103.2
Pristane (ng/m^3)	1.4	1.2
Phytane (ng/m^3)	1.1	1.1
CPI	0.9	1.4
C_{max}	C_{29}	C_{29}
PAHs (ng/m^3)		
NAP	0.10	0.10
ACY	0.15	< DL
ACE	0.52	0.40
FLU	0.36	0.18
ANT	0.10	< DL
PHE	0.10	0.05
FLT	0.09	0.05
PYR	0.06	0.04
RET	1.50	1.25
BaP	0.06	< DL
BeP	0.10	0.15
BghiP	0.09	0.10
BbF	0.12	0.10
BkF	0.05	0.22
IND	0.10	0.11
Total	3.50	2.75

< DL: below detection limit.

The relative contribution of biogenic emission was also evaluated for medium and low-volatile homologs ($> C_{23}$) using the Wax Normal Alkanes (WNA) index. Fig. 4 shows a source distribution of WNA during intense and moderate biomass burning periods. WNAs were estimated for individual compounds by means of Eq. (2) where any negative values were replaced by zero (Ladji et al., 2009; Simoneit et al., 1990):

$$WNA(C_n) = [C_n] - 0.5[C_{n+1} + C_{n-1}] \quad (2)$$

Besides alkanes, 15 PAHs compounds were identified and quantified in the filters. Retene was the most abundant aromatic hydrocarbon for both periods of this study. Several PAHs considered mutagenic according to the International Agency for Research on Cancer (IARC) have been identified, e.g. PHE, FLT, PYR, BeP and BghiP (IARC, 2010). Moreover, compounds considered mutagenic and carcinogenic have also been identified such as BbF, BkF, BaP and IND. It is important to stress that the BaP is highly mutagenic and carcinogenic, being listed as a Group 1 carcinogen to human by the IARC (IARC, 2010). The BaP was found only at the intense burning period samples. Those compounds pose an important risk for human exposure to biomass burning.

Source apportionment was carried out through PAHs diagnostic ratios analysis. The ratios of Flu/(Flu+Pyr), BFs/BghiP,

IcdP/(IcdP+BghiP), (BghiP/BeP) and BaP/BeP are listed and compared with those of other source in Table 2.

3.3. Cytotoxic and MCN tests

Comparative analysis of the genotoxic potential of the organic PM sampled in Alta Floresta was evaluated in two systems: *in vitro*, with the CBMN test and *ex situ*, with the Trad-MCN test. Considering that no work thus far assessed the MCN frequency of organic matter from Brazil's Amazon region using A549 cells, this research was conducted by comparing the genetic damage in both assays and the two different sampling periods.

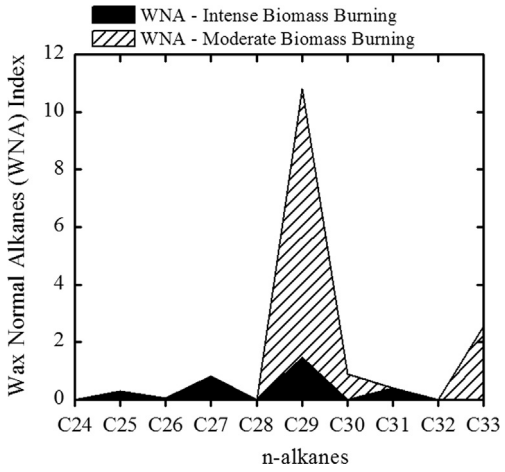

Fig. 4. Results of source distribution of WNA index during intense and moderate biomass burning periods.

The cytotoxic effect of organic PM was assayed using A549 cells. Table 3 shows that cell viability in the presence of the organic extract from both periods was slightly reduced in a concentration-dependent manner being significant at the highest concentration of the intense biomass burning period. Also, the concentration of 10 ng/mL of PAHs mixture (positive control) did not affect significantly the cell viability and, thus, this concentration could be used for CBMN analyses.

The results of the CBMN test are shown in Fig. 5. The NDI value as an index of cell proliferation inhibition was similar to the control group and ranged between 1.5 and 1.7. A significant increase in the MCN frequency, generated in a dose-dependent manner, was observed in A549 cells to non-cytotoxic concentrations of organic PM when compared with the negative control ($p < 0.001$) in the intense biomass burning period. In the other period, with moderate biomass burning, only at 0.5 mg/L and 1.0 mg/L of organic extract had the MCN formation significantly increased when compared with the negative control. This effect was also comparable to that induced by the positive control, PAHs mixture. Treatment with organic PM from Alta Floresta did not induce significant increase in the formation of nucleoplasmic bridges and nuclear buds compared with negative control (data not shown).

The Trad-MCN (*ex situ*) shows that in the intense biomass burning period there was a significant increase in MCN frequency at the tested concentrations. However, in the moderate biomass burning period, it was only significant at the highest concentration (1.0 mg/L) when compared to the negative control group ($p < 0.001$) (Fig. 6).

Table 2
Comparison of PAHs diagnostic ratios for different sources.

PAHs ratios	Intense biomass burning (this study)	Moderate biomass burning (this study)	Aerosol from wildfires	Aerosol from conifer	Aerosol from Tunel	Aerosol from urban area
Flu/(Flu+Pyr)	0.85	0.81	0.25–0.70[a] 0.51–0.68 [b] 0.53–0.86[c] 0.48–0.93[d] 0.65[e] 0.56[f]	0.18–0.88[g]	0.54[h]	0.40–0.85[i] 0.65–0.87[j] 0.13–0.95[l] 0.40–0.79[m] 0.64–0.97[n]
BFs/BghiP	1.89	3.28	3.2–12[a] 2.7–33[b] 3.5[c] 5.2–9.5[d] 3.6 [e,f]	–	0.79[h]	–
IcdP/(BghiP+IcdP)	0.53	0.52	0.15–0.49[a] 0.24–0.57[b]	–	0.31[h]	–
BghiP/BeP	0.96	0.67	0.87[c,d]	–	–	–
BaP/BeP	0.57	< DL	–	–	–	0.40–1.00[i] 0.40–0.80[j] 0.40–1.00[k] 0.40–0.70[l] 0.50–0.10[m]

< DL: below detection limit.

[a] Vicente et al. (2012) – $PM_{2.5}$.
[b] Vicente et al. (2012) – PM_{10}–$PM_{2.5}$.
[c] Vicente et al. (2011) – $PM_{2.5}$.
[d] Vicente et al. (2012) – PM_{10}–$PM_{2.5}$.
[e] Alves et al. (2011a) – $PM_{2.5}$.
[f] Alves et al. (2011a, 2011b) – PM_{10}–$PM_{2.5}$.
[g] Oros and Simoneit (2001).
[h] Oliveira et al. (2011).
[i] Vasconcellos et al. (2011) – Autumn/2003.
[j] Vasconcellos et al. (2011) – Wnter/2003.
[k] Vasconcellos et al. (2011) – Spring/2003.
[l] Vasconcellos et al. (2011) – Summer/2003.
[m] Vasconcellos et al. (2011) – Autumn/2004.

Table 3
A549 cells cytotoxicity of binucleated cells treated with organic particulate matter from two different periods in Brazilian Amazon region.

Concentrations (mg/L)	Intense biomass burning Organic PM in A549 cells (% viability ± SD)	Moderate biomass burning
DC[a]	107.41 ± 0.57	105.87 ± 0.41
0.1	102.07 ± 0.68	102.03 ± 0.51
0.5	87.59 ± 0.59	91.60 ± 0.53
1.0	75.90 ± 0.52**	94.03 ± 0.50
PC[b]	93.95 ± 0.68	93.17 ± 0.43

[a] DMSO 0.1% Control.
[b] Positive Control: 10 ng/mL of PAHs mixture.
** $p < 0.01$ Statistically significant compared to negative control (DMEM) according to Dunnett's test.

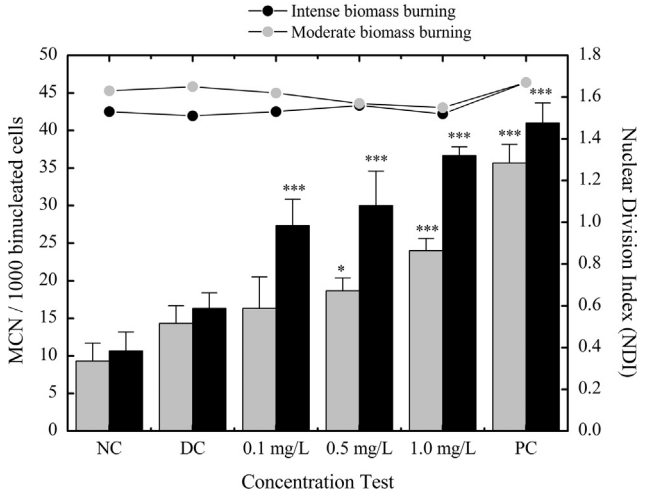

Fig. 5. Comparative results of frequency de MCN and NDI in A549 cells for different concentrations of organic particulate matter in Alta Floresta. Negative Control (NC) – DMEM, DMSO Control (DC) – DMSO 0.1% and Positive Control (PC) – PAHs mixture (10 ng/mL). *$p < 0.05$ and *** $p < 0.001$ Statistically significant compared to NC according to Dunnett's test.

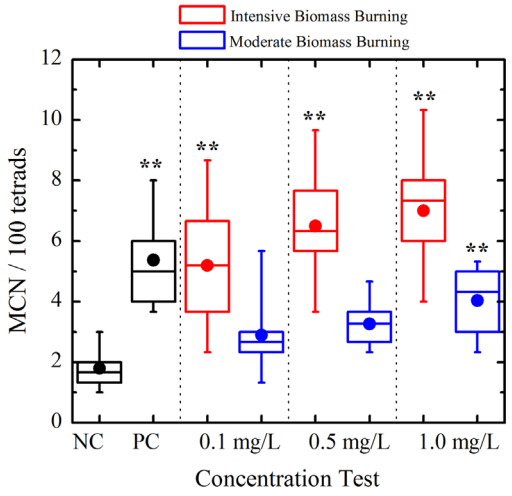

Fig. 6. Box plot diagram for the distribution of the values of MCN using Trad-MCN test. The box range indicates the 25th and 75th percentile, the whiskers show the 5th and 95th percentile and the line the distribution median. The mean values are shown as circles. Negative Control (NC) – DMSO 1% and Positive Control (PC) – 0.2% formaldehyde. **$p < 0.01$ Statistically significant compared to NC according to Dunnett's test.

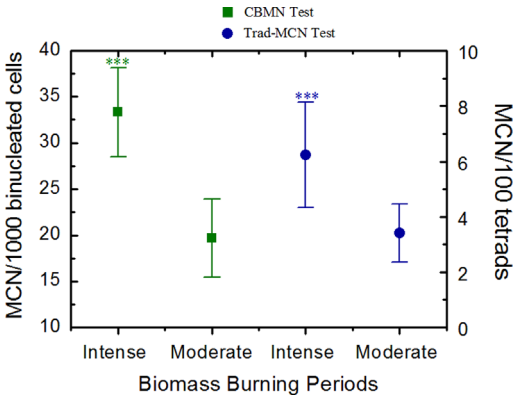

Fig. 7. Results of MCN frequency of CBMN and Trad-MCN tests during intense and moderate biomass burning periods in Alta Floresta. ***$p < 0.001$ Statistically significant between sampling periods according to Tukey test.

Moreover, the study with CBMN and Trad-MCN assays show that the mean MCN rate from the intense biomass burning period is significant compared to moderate biomass burning period ($p < 0.001$) (Fig. 7).

4. Discussion

The biomass burning that systematically occurs in the Amazon region as part of the deforestation process has an adverse effect on human health and environment (Alves et al., 2011b; Ignotti et al, 2010, Jacobson et al, 2012; Oliveira et al., 2012; Sisenando et al., 2011, 2012). However, in this region, there are few studies linking atmospheric pollution and genetic damage.

In the review done by Kelly and Fussel (2012), the authors related the largest gap in the knowledge of PM toxicity to the lack of knowledge about the components present in PM as well as about their physical and chemical characteristics. This work focused on the composition and genetic damage of PM and it is the first research that compared the potential genotoxic effect of organic PM in the intense and moderate biomass burning periods in the Amazon region using two bioassays with different bioindicators.

During the study in Alta Floresta, the PM_{10} concentrations showed great differences between intense and moderate biomass burning periods. In agreement with this result, satellite image of the region identified 182 fire spots during the intense biomass burning period and only 5 fire spots for the moderate biomass burning period (INPE, 2008). However, the PM_{10} concentrations were lower than the World Health Organization Air Quality Guidelines for 24 h (WHO, 2005). This result is very important because studies performed by Alves et al. (2011b) and Coronas et al. (2008) suggested a reassessment of the standards established by environmental and health agencies, in terms of chemical composition and genotoxic potential.

The data obtained in this research indicate a good correlation between PM_{10} and BC during the entire sampling period. Similar results were observed in Alta Floresta by Maenhaut et al. (2002). Furthermore, studies also showed that the BC probably contribute to various adverse health effects (Cançado et al., 2006; Hoek et al., 2002). Zanobetti and Schwartz (2006) conducted a study linking BC with increased risk of emergency myocardial infarction hospitalization.

Also, a large fine fraction in PM could be observed when the SFU were analyzed. According to Bräuner et al. (2007), PM may cause health effects including oxidative stress, resulting in damage to DNA and other macromolecules.

The chemical analysis has identified *n*-alkanes ranging from C_{18} to C_{34}, as well as the pristane and phytane compounds. The determination of C_{max} at C_{29} during intense and moderate biomass

burning periods shows biogenic emission. In addition, our data is in agreement with that obtained by Oros and Simoneit (2001) where they make a description of organic constituents in biomass burning aerosols in forested areas of California and Oregon. It is important to point out that *n*-alkanes from biomass burning are indistinguishable from plant wax alkanes in the ambient aerosol (Simoneit et al., 1996). Further evidence of biogenic contribution from the alkanes analysis arises from a pristane/phytane ratio higher than 1 during both sampling periods (1.30 and 1.09 for intense and moderate biomass burning periods, respectively).

The CPI values found during the intense burning period indicate an anthropogenic influence directly associated to biomass burning (CPI ~0.9). Similar results were found by Alves et al. (2011b) in Tangará da Serra (MT) in the intense burning biomass period. However, during the moderate burning periods, the CPI indicates a predominance of biogenic influence (CPI ~1.4). Besides, the WNA index show that biogenic emission is higher in the moderate biomass burning period, corroborating with results of CPI.

PAHs analysis results indicated the retene as the most abundant during both sampling periods. Retene is a well-known biomarker of wood combustion (Ramdahl, 1983). This result is in good agreement with other studies performed in wildfires areas (Alves et al., 2011a; Vicente et al., 2011, 2012). Carcinogenic and mutagenic compounds were identified during both sampling periods. However, during the moderate burning period BaP was below the detection limit, suggesting that other components are responsible for the genotoxic effect observed at the highest concentrations.

Previous studies have shown that PAH concentrations have large variations in the composition and different emission sources. Thus, some ratios between compounds are a powerful tool to understand the source origins including biomass and fossil fuel burnings, plants emissions, etc. (Vasconcellos et al., 2011; Wang et al., 2009). The ratios for Flu/(Flu+Pyr), BFs/BghiP and IcdP/(BghiP+IcdP) obtained for both periods of this study were in agreement with those reported in the literature for different biomass burning sources (Oros and Simoneit, 2001; Vicente et al., 2011, 2012). The diagnostic ratio for BghiP/BeP during the intense burning is 0.96 and during moderate burning is 0.67. Accordingly to Nielsen et al. (1996), these ratios have been used to differentiate between traffic (BghiP/BeP $\sim$2.02) and non-traffic sources (BghiP/BeP $\sim$0.80). The BaP/BeP ratio during intense biomass period is 0.57. Vasconcellos et al. (2010) reported that this ratio can be an important index for calculating the aging of the particles. Also, Vasconcellos et al. (2011) found BaP/BeP <1 in most samples in urban area (São Paulo, Brazil) and suggested that the PM be submitted to photochemical reactions during the transport. These reactions depend on solar radiation intensity, and it is expected to happen in warmer months in the tropics.

To assess the potential genotoxic of a sample is interesting to perform different tests. The bioassays chosen for this study were successful in detecting genotoxic compounds present in air. However, among the researches performed in the Amazon region, none have analyzed the genotoxic effect of PM using human alveolar cells (*in vitro*) and only one study, conducted by Alves et al. (2011b), used the Trad-MCN to evaluate the effects of organic extract from biomass burning in this region. It is important to emphasize that A549 cells line were used because the lung cells represent the main biological target for inhaled toxin and genotoxins. Besides, this cells system is a particular good tool to analyze chromosomal damage and has the ability to biotransform xenobiotics (Roubicek et al., 2007).

Our data of both tests, for the same concentrations, showed that the MCN frequency from the intense biomass burning period is more significant than the moderate biomass burning period. This result can be associated with the presence of BaP in the intense biomass burning period. Interestingly, the organic extracts from Tangará da Serra using Trad-MCN indicate similar results (Alves et al., 2011b). This study confirmed the genotoxic effect of organic PM in vegetable and human alveolar cells.

However, CBMN test indicated that in the moderate biomass burning period, at 0.5 mg/L and 1.0 mg/L of organic extract had the MCN formation significantly increased when compared with the negative control whereas Trad-MCN was only significant at the highest concentration. This result can be explained by the greater sensibility of cells in vitro and by metabolic activity of A549 cell, and/or by the presence of other compounds in this fraction which could be responsible for this activity.

5. Conclusion

The study using human alveolar cells associated with Trad-MCN, clearly confirmed that organic PM collected in the intense biomass burning period has genotoxic effects. A predominance of fine particles in the PM was observed. The BaP was found only at the intense biomass burning period samples. The retene was the most abundant PAH during both sampling periods. PAHs ratios obtained for both periods of this study were in agreement with those reported in the literature for biomass burning sources. The determination of C_{max} at C_{29} during both periods shows biogenic emission. The CPI values indicate that during the intense biomass burning period anthropogenic influences occur which are directly associated with biomass burning. During the moderate biomass burning periods were observed a predominance of biogenic influence. The analysis of WNA confirmed this result.

Several studies showed that concentration of PM below the WHO standard also poses a risk for human health. This study also should guide decision-makers on the assessment of environmental health-policy strategies linked with exposure to biomass burning in Amazon region, taking into account the exposure distribution in the population.

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