

## Genotoxicity and composition of particulate matter from biomass burning in the eastern Brazilian Amazon region

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### ABSTRACT

In the present study *Tradescantia pallida* micronucleus (Trad-MCN) bioassay was used to assess the genotoxicity of particulate matter with a mass median aerodynamic diameter less than 10  $\mu\text{m}$  ( $\text{PM}_{10}$ ) in Tangara da Serra (MT), a Brazilian Amazon region that suffers the impact of biomass burning. The levels of PM (coarse and fine size fractions) and black carbon (BC) collected were also measured. Furthermore, the alkanes and polycyclic aromatic hydrocarbons (PAHs) were identified and quantified in the samples taken during the burning period by gas chromatography with flame ionization detection (GC-FID). The PM and BC results for both fractions indicate a strong correlation ( $p < 0.001$ ). The analysis of alkanes indicates an anthropic influence. Retene was the most abundant PAH found, an indicator of biomass burning, and 12 other PAHs considered to be potentially mutagenic and/or carcinogenic were identified in this sample. The Trad-MCN bioassay showed a significant increase in micronucleus frequency during the period of most intense burning, possibly related to the mutagenic PAHs that were found in such extracts. This study demonstrated that Trad-MCN was sensitive and efficient in evaluating the genotoxicity of organic compounds from biomass burning. It further emphasizes the importance of performing chemical analysis, because changes in chemical composition generally have a negative effect on many living organisms. This bioassay (ex situ), using *T. pallida* with chemical analysis, is thus recommended for characterizing the genotoxicity of air pollution.

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### 1. Introduction

Particulate matter (PM) has been closely linked to biological response; however, the relationship between the physicochemical properties of PM and health effects remains unsolved. It may derive from both anthropogenic and biogenic emission sources, resulting in a complex mixture of extremely small particles and liquid droplets. Particle pollution consists of a number of components, including acids (such as nitrates and sulfates), organic chemicals, metals, soil dust particles, sea salt, geological disturbances, biological debris, forest fires and oxidation of biogenic reactive gases (Schlesinger, 2007).

Several studies show that a significant fraction of the total mass of PM is made up of organic compounds (Alves et al., 2001; Galarneau, 2008; Yassaa et al., 2001). Biomass burning introduces several pollutants into the atmosphere, including carcinogenic/mutagenic compounds such as polycyclic aromatic hydrocarbons (PAHs; Duan et al., 2009). Air emissions of these compounds have been associated with both acute and chronic adverse effects on human health and the ecosystem (Mariani et al., 2009; McGee et al., 2003; Quiao et al., 2006). Size is an important factor that influences how particles deposit in the respiratory tract and affect human health. Large particles are generally filtered in the nose and throat and do not necessarily cause problems. However, particulate matter with a mass median aerodynamic diameter less than 10  $\mu\text{m}$ , known as  $\text{PM}_{10}$ , has been shown to be potentially hazardous to health, due to the complex mixture of compounds and the fact that it can settle in the bronchi and lungs (Coronas et al., 2008; Holmes, 2007; Kaonga and Kagabi, 2010; Traversi et al., 2009).

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Fig. 1. Map of the deforestation arc in the Brazilian Amazon, showing the study site.

Tangara da Serra is located in the eastern Amazon and is on the path of pollutant dispersion resulting from deforestation of the Brazilian Amazon, where the deforestation radius in this region ranges from southeastern Maranhão to southeastern Acre (Fig. 1). This region had the highest concentration of heat outbreaks in recent years and almost all the fires are deliberately started for reasons such as selling wood, clearing pastures and preparing the land for crops. It still contains vast areas of sugarcane culture, causing negative effects on human beings and plants species. Moreover, epidemiological studies have demonstrated that this region suffers from high rates of mortality and morbidity caused by respiratory diseases, possibly associated to the effects of biomass burning (Ignotti et al., 2007, 2010; Rosa et al., 2008).

Many analytical methods have provided important information for identifying compounds released by emission sources that may be responsible for causing adverse health effects. However, few studies have aimed at identifying the compounds (mainly organic) generated from biomass burning. Alkanes and PAHs are two organic compounds that have been chemically characterized (Alves, 2008; Ladji et al., 2009). Santos et al. (2002) observed the levels of selected polar compounds and PAHs of atmospheric PM in Campos dos Goytacazes, a region near Rio de Janeiro, and concluded that sugarcane burning and vehicular exhaust are the two main sources of pollution affecting the urban environment. According to Umbuzeiro et al. (2008), chemical composition studies are essential to identify the compounds generated during sugarcane burning and correlate them with the levels of mutagenic activity detected.

Plant bioassays are best suited for assessing the effects of air pollution, which are generally highly sensitive in detecting the genotoxic effects of air pollutants (Claxton and Woodall, 2007; Villarini et al., 2009). Studies have used the *Tradescantia pallida* micronucleus (Trad-MCN) assay for screening mutagenic potential in the environment both in situ (Prajapati and Tripathi, 2008; Meireles et al., 2009; Villarini et al., 2009) and ex situ (Batalha et al., 1999; Carvalho-Oliveira et al., 2005; Lima et al., 2009; Mielli et al., 2009), but there is a paucity of papers reporting on pollutants from biomass burning because most studies were conducted in areas exposed to air pollution formed by emissions from industries and/or intense road traffic. To date, there have been no published articles using the Trad-MCN (ex situ) assay to assess the organic compounds of PM<sub>10</sub>, which justifies the present study in the Brazilian Amazon region.

The aims of this work were threefold: to use the Trad-MCN (ex situ assay) to assess the genotoxicity of three different concentrations of organic PM collected in Tangara da Serra,

between August and December 2008; to correlate PM<sub>10</sub> and black carbon (BC) levels as well as fine and coarse fractions of PM and to evaluate the correlation of Trad-MCN (ex situ) data with the concentration of atmospheric pollutants; and finally, to characterize the chemical composition of the particulate matter by identifying and quantifying alkanes and PAHs.

## 2. Materials and methods

### 2.1. Sampling site characteristics

Tangara da Serra is located in southwestern Mato Grosso (MT) state (14°37'10"S, 57°29'09"W), part of the eastern Amazon, where the period of most intense biomass burning occurs in the dry season (July to October).

### 2.2. Particulate matter collection and chemical analysis

The PM was collected during the second half of 2008 using two types of filters: teflon and polycarbonate. The PM<sub>10</sub> was collected with Teflon filters (1 μm pore size, 47 mm diameter) for 48 h, continuously. A total of 50 samples were collected on Teflon filters from August to December/2008. A sampler coupled with an inlet was used to collect all particles smaller than 10 μm. The sampling device used for polycarbonate filters, on the other hand, consisted of a stacked filter unit (SFU), which separates the aerosol into coarse (2.5–10 μm equivalent aerodynamic diameter) and fine (smaller than 2.5 μm) size fractions (Cançado et al., 2006). These samples were collected between 24 and 48 h, continuously. A total of 96 samples were collected on polycarbonate filters from July to December/2008. To separate the particles we used 8 and 0.4 μm pore size Nuclepore polycarbonate filters for the coarse and fine size fractions, respectively. The mass of both filters was determined by the difference in gravity analysis, in which sample weights were measured on an electronic micro-analytic 1 μg precision Mettler scale, before and after sampling. At this point, PM<sub>10</sub>, fine and coarse fraction concentrations and BC levels were determined. The Teflon filters were used not only to identify alkanes and PAHs, but also to conduct the genotoxicity test. BC was determined using a light reflectance technique, similar to that employed by Andreae et al. (1984) and Reid et al. (1998). It is important to point out that the blank filters (unsampled) were submitted to the same process as the other filters in laboratory. N-alkanes and PAHs compounds were extracted from the filters with dichloromethane using an ultrasonic bath. The different fractions were obtained using a silica gel column (2.5 g) and different solvents of increasing polarity following the procedure proposed by Gogou et al. (1998). Compounds were determined using a gas chromatography coupled to flame ionization detection (GC-FID, Varian 3800). 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 and 290 °C; 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. Limits of detection ranged from 0.12 to 0.41 ng m<sup>-3</sup> for n-alkanes and from 0.27 to 0.99 ng m<sup>-3</sup> for PAHs (Vasconcellos et al., 2010).

### 2.3. Sampling preparation and organic matter extraction

After the mass of PM<sub>10</sub> from the Teflon filters was determined, organic compounds were extracted by ultrasonication, using dichloromethane (DCM) at a proportion of 1 ml of solvent to 1 mg of PM<sub>10</sub>. Each filter was sonicated three times for 10 min. A pool was then made to obtain monthly samples. The extract volume was reduced to 5 mL, using a vacuum rotary evaporator and then dried using a gentle stream of nitrogen. Once the concentration of the extracted organic matter was determined, the samples were stored at -20 °C (Sato et al., 1995). One part of the extracted organic matter was resuspended in n-hexane, where the extract was fractionated into individual compound classes using flash chromatography on silica gel. The other part was dissolved in dimethylsulfoxide (DMSO), used at different concentrations in the genotoxicity test. It is important to stress that in both analyses the blank filters were used and put through the same extraction procedures as the exposed filters.

### 2.4. *T. pallida* micronucleus (Trad-MCN) bioassay (ex situ)

To evaluate the genotoxicity test, the extracted organic compounds were prepared at three different concentrations (0.1, 0.5 and 1.0 mg/L) for each monthly sample (August to December). Triplicate of two independent experiments with negative control (blank filters) and positive control (0.2% formaldehyde) were performed to compare the responses of plants.

Trad-MCN was performed using the protocols established by Ma (1981). To conduct the bioassay, the plants were collected and kept for 24 h in the laboratory using a nutritive Hoagland solution. They were then transferred to beakers containing the test solution (negative and positive control and the three concentrations of matter for each month) and kept there for 8 h. After the exposure period, 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) 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. Only micro-slides containing early tetrads were considered. Three hundred early tetrads were examined per slide under 400× magnification and micronuclei frequencies were calculated by dividing the total number of MCN by the total number of tetrads recorded and expressed in terms of MCN/100 tetrads. Micronucleus counting was done on coded slides and the key was revealed only after completion of the experiment.

### 2.5. Statistics analysis

Statistical analyses were performed using BioEstat, version 5.0, as follows: (1) an analysis of variance (ANOVA) of PM<sub>10</sub> and BC data was performed, considering simple linear regression; (2) two-way ANOVA was used in the Trad-MCN bioassay to analyze the different sampling periods and concentrations; (3) 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; (4) Pearson's correlation was conducted to verify the correlation between PM<sub>10</sub> and mean MCN frequencies measured in *T. pallida* during the period of most intense burning. The mean differences and correlations were considered significant at  $p < 0.05$ .

## 3. Results

### 3.1. PM<sub>10</sub> and BC collected in Teflon and polycarbonate filters

The distribution of PM<sub>10</sub> and BC concentration collected on the Teflon filters in Tangara da Serra are given in Fig. 2. The ambient PM<sub>10</sub> and BC mean measured during the experimental period was 25.2 µg/m<sup>3</sup> and 1313 ng m<sup>-3</sup>, respectively. The mean mass of PM<sub>10</sub> collected was 2.0 g. The highest levels of PM<sub>10</sub> (µg/m<sup>3</sup>) were obtained from August to October (period of most intense burning); however, concentrations did not exceed the limit established by the World Health Organization (50 µg/m<sup>3</sup> 24 h-mean). As expected, the results indicate a strong correlation between PM<sub>10</sub> and BC (Fig. 2A).

Polycarbonate filters were analyzed for coarse and fine fractions of PM emitted in Tangara da Serra. The average concentration of PM (27.9 µg/m<sup>3</sup>) and BC (1621 ng m<sup>-3</sup>) for both fractions in relation to the periods of highest burning rates showed greater concentration levels and good correlation (Fig. 2B). During all the sample months (July to November, 2008), we obtained an average coarse PM levels of 11.5 and 16.5 µg/m<sup>3</sup> for the fine PM levels

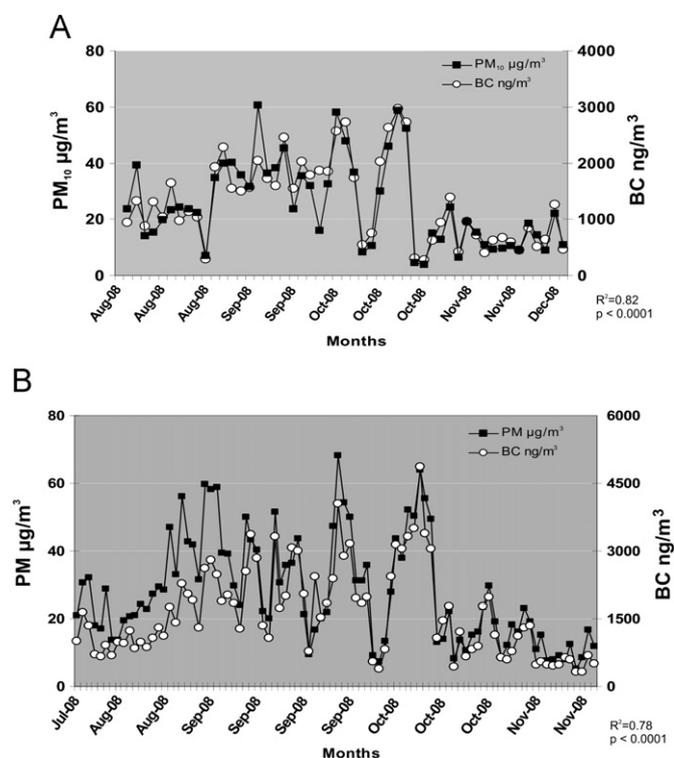


Fig. 2. Results of ANOVA and simple linear regression between PM<sub>10</sub> and BC collected in Teflon (A) and polycarbonate (B) filters in the second half of 2008 in Tangara da Serra, MT.

(Fig. 3A). Furthermore, we analyzed the mean concentrations of BC for coarse (213 ng m<sup>-3</sup>) and fine (1408 ng m<sup>-3</sup>) fractions (Fig. 3B). This data showed that the PM and BC fine fraction predominated during all the sample months.

### 3.2. Chemical analyses of n-alkanes and PAHs

Knowledge of the chemical composition of PM is still limited, mainly in relation to particles from biomass burning. In order to make a preliminary identification of the organic compounds during the period of most intense burning, we determined the n-alkanes and PAHs.

The total concentration of n-alkanes ranging from C<sub>16</sub> to C<sub>35</sub> is over 39 ng m<sup>-3</sup> (Table 1). It has been established that odd-carbon-number n-alkanes have predominance above even-carbon-number homologs when the emission is from epicuticular waxes of terrestrial plants. In contrast, vehicular emissions and other anthropogenic sources show no preference (Omar et al., 2007).

The carbon preference index (CPI) is used to determine the intensity of biogenic versus fossil fuel contributions. For n-alkanes this empirical parameter is expressed as a sum of the odd carbon number homologs over a range, divided by a sum of even carbon homologs over the same range. Emissions are considered biogenic when the CPI has values much greater than one and anthropogenic when CPI is equal to or near one. The maximum carbon number of the most abundant n-alkanes (C<sub>max</sub>) can also be used as an indicator of relative source input (Alves, 2008; Simoneit et al., 2007).

Homolog series of n-alkanes from C<sub>16</sub> to C<sub>35</sub> indicate anthropogenic and natural emissions sources. The low CPI values demonstrate the strong input of anthropogenic materials. The low end is representative of contaminant species and the high end demonstrates a major input of higher plant wax. For this work, the CPI was 0.9 in the C<sub>17</sub>–C<sub>23</sub> range and 1.0 in the C<sub>25</sub>–C<sub>35</sub> range.

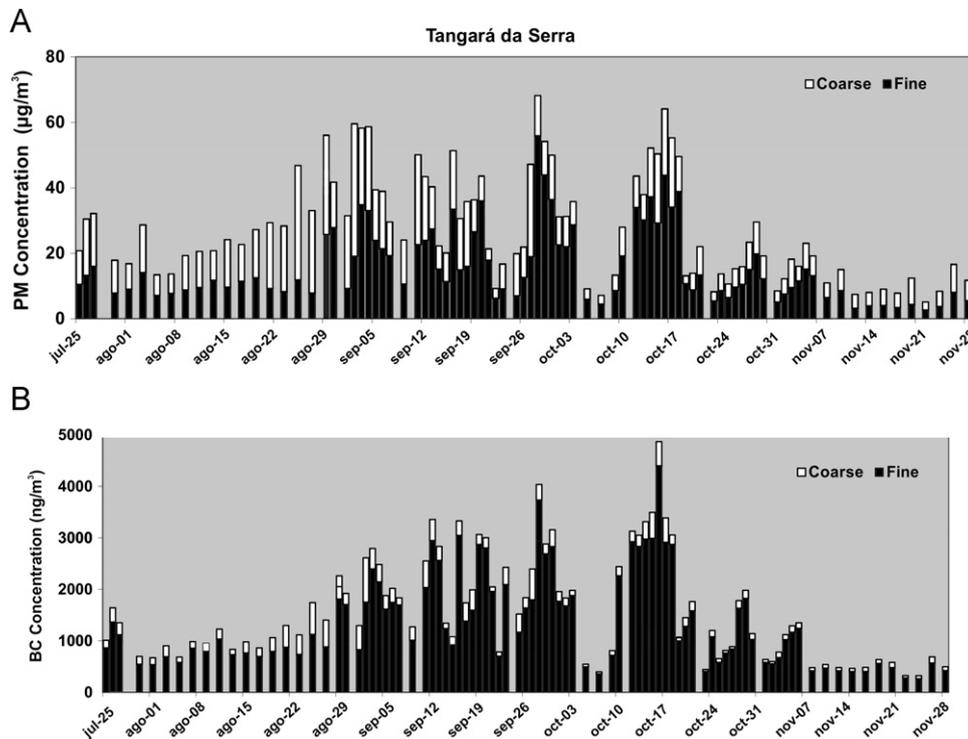


Fig. 3. Concentration of coarse and fine fractions of PM (A) and BC (B) collected in polycarbonate filters from July to November, 2008 in Tangara da Serra, MT.

Table 1

Results of alkanes identified and quantified in the organic compounds of Tangara da Serra during the period of most intense burning (August to October).

Results of alkanes from Tangara da Serra, MT					
Alkanes	Retention time (min)	C (ng m <sup>-3</sup> )	Alkanes	Retention time (min)	C (ng m <sup>-3</sup> )
C <sub>14</sub>	12.90	4.41	C <sub>24</sub>	31.56	1.09
C <sub>15</sub>	15.11	0.23	C <sub>25</sub>	33.11	0.57
C <sub>16</sub>	17.48	2.89	C <sub>26</sub>	34.60	1.86
C <sub>17</sub>	19.31	2.03	C <sub>27</sub>	36.04	2.73
Pristane	19.42	1.11	C <sub>28</sub>	37.44	3.48
C <sub>18</sub>	20.63	0.63	C <sub>29</sub>	38.79	3.76
Phytane	21.35	0.68	C <sub>30</sub>	40.09	2.33
C <sub>19</sub>	23.01	0.65	C <sub>31</sub>	41.86	2.44
C <sub>20</sub>	54.82	0.68	C <sub>32</sub>	43.06	1.42
C <sub>21</sub>	26.59	0.64	C <sub>33</sub>	44.15	1.11
C <sub>22</sub>	28.30	0.66	C <sub>34</sub>	47.04	1.05
C <sub>23</sub>	29.96	1.02	C <sub>35</sub>	50.77	1.35

Total: 39 ng m<sup>-3</sup>.

CPI: Carbon Preference Index.

CPI (C<sub>17</sub>–C<sub>23</sub>): 0.9 CPI (C<sub>25</sub>–C<sub>35</sub>): 1.0.

The pristane and phytane concentrations were 1.11 and 0.68, respectively (Table 1).

In this study 18 PAHs were detected, as follows: naphthalene (NAP), acenaphthalene (ACY), acenaphthene (ACE), fluorene (FLU), anthracene (ANT), retene (RET). Among the PAHs identified, phenanthrene (PHE), fluoranthene (FLT), pyrene (PYR), benzo[e]pyrene (BeP), and benzo[g,h,i]pyrene (BgP) are mutagenic, according to the International Agency for Research on Cancer (IARC, 2008). Moreover, benz[a]anthracene (BaA), chrysene (CHR), benzo[b]fluoranthene (BbF), benzo[k]fluoranthene (BkF), benzo[a]pyrene (BaP), indene[1,2,3-c,d]pyrene (IND) and dibenz[a,h]anthracene (DBA) are also considered to be carcinogenic and mutagenic (Fig. 4).

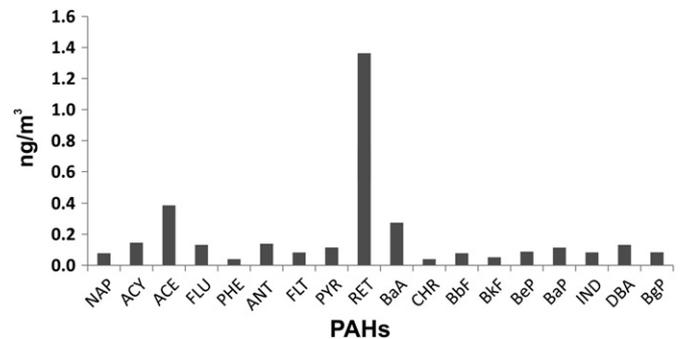


Fig. 4. Results of PAHs identified and quantified in the organic matter of Tangara da Serra during the period of most intense burning (August to October).

### 3.3. Genotoxic potential of organic matter using Trad-MCN

Considering that no work has thus far assessed the genotoxic potential of organic matter from biomass burning in Brazil's Amazon region using the Trad-MCN bioassay (ex situ), we conducted this research in the region of Tangara Serra, Mato Grosso state. Tests were performed with the following control groups: negative (blank filters) and positive (0.2% formaldehyde).

A significant increase in MCN frequency at the tested dosages was observed from August to September ( $p < 0.05$  and  $p < 0.01$ ). In October, it was only significant at the highest concentration ( $p < 0.01$ ). On the other hand, organic compounds from November and December exposed at the three different concentrations showed no significance with respect to the micronucleus rate, compared to the negative control group (Table 2). Thus, it was concluded that the mean micronucleus rate from the period of most intense burning is significant compared to the period of least burning (Fig. 5). We also concluded that there was no correlation between PM<sub>10</sub> concentration and mean micronucleus frequency during biomass burning, but it is important to

**Table 2**

Mean and standard deviations (SD) of micronucleus frequency in tetrads of *Tradescantia pallida* (MCN/100 tetrads) exposed for 8 h at three different concentrations during the months of most intense burning (August to October) and least intense burning (November and December).

Organic particulate matter				
Concentrations (mg/L) and Mean $\pm$ SD				
Months	0.1	0.5	1.0	B > ou B <
August	3.73 $\pm$ 1.16**	4.54 $\pm$ 1.19**	5.21 $\pm$ 1.30**	B >
September	3.46 $\pm$ 1.43*	3.69 $\pm$ 1.77**	3.73 $\pm$ 1.10**	B >
October	2.36 $\pm$ 1.13	3.16 $\pm$ 0.94	5.00 $\pm$ 1.24**	B >
November	1.45 $\pm$ 0.92	2.03 $\pm$ 0.86	2.13 $\pm$ 0.42	B <
December	1.53 $\pm$ 0.32	1.63 $\pm$ 0.55	2.20 $\pm$ 0.54	B <

Negative Control (blank filters): 1.80  $\pm$  0.54.

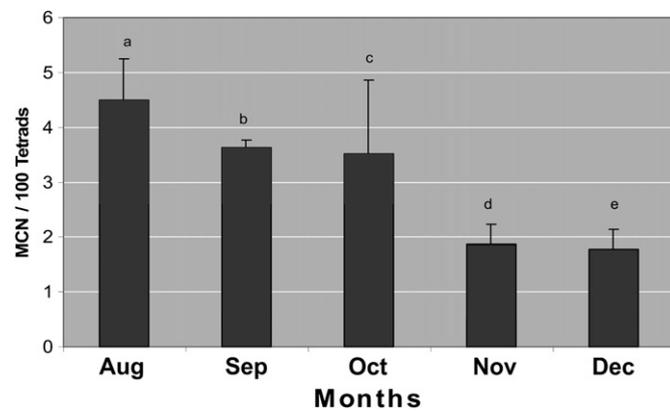
Positive Control (0.2% formaldehyde): 5.37  $\pm$  1.60.

B > : most intense burning

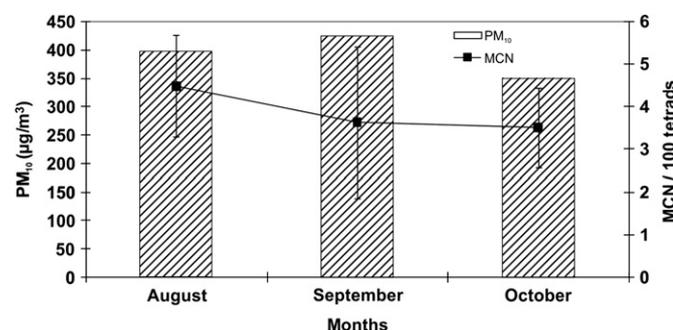
B < : least intense burning

\*  $p < 0.05$  statistically significant compared to negative control according to Dunnett's test.

\*\*  $p < 0.01$  statistically significant compared to negative control according to



**Fig. 5.** Results of Trad-MCN bioassay using organic extracts collected on Teflon filters. Tukey's test, used to analyze the monthly groups, showed that (a and d) and (a and e) have  $p < 0.01$  and that groups (b and d), (b and e), (c and d) and (c and e) are significant ( $p < 0.05$ ).



**Fig. 6.** Relationship between PM<sub>10</sub> and mean MCN frequencies measured in *Tradescantia pallida* in the period of most intense burning in Tangara da Serra, MT.

Dunnett's test.

emphasize that genotoxic damage was observed in plant cells even though air quality was considered fair, according to WHO air quality guidelines (World Health Organization, 2006; Fig. 6).

#### 4. Discussion

The effects of atmospheric pollution have become a matter of concern throughout the world. However, a major problem in

linking atmospheric pollution to health involves quantification, especially in the Amazon region, where infrastructure is very limited and the sparsely inhabited areas hinder systematic air quality monitoring. This is the first study to analyze the genotoxic effects of smoke exposure, with a particular focus on organic compounds in the Amazon region.

In the present study we found PM<sub>10</sub> concentrations within established limits set by the World Health Organization (50 µg/m<sup>3</sup> 24 h mean). However, the interactions of the various compounds found in our samples and their potential damage to the environment and human health continue to be questioned, as suggested by others' previous mutagenic studies (Coronas et al., 2008, 2009).

The results of this study indicate a strong correlation between PM<sub>10</sub> and BC. Moreover, analysis of polycarbonate filters in Tangará da Serra (MT) showed that the fine fraction predominated during all the sample months. Interestingly, similar results were also observed in Amazon regions during periods of intense biomass burning (Artaxo et al., 2000; Maenhaut et al., 2002). In addition, others studies made by Artaxo et al. (2002) and Viidanoja et al. (2002) have shown that most of the particles emitted by biomass burning in the Amazon region consist of the fine fraction of PM, composed of the mass of graphitic carbon, inorganic compounds and, primarily, of organic compounds.

The total concentration of n-alkanes found in this work, ranging from C<sub>16</sub> to C<sub>35</sub> is over 39 ng/m<sup>3</sup>. N-alkanes are released into the atmosphere by many anthropogenic sources such as automobile exhausts and biogenic sources, including plant waxes and microbial detritus in minor amounts. For this reason, Cheng et al. (2006), suggests that n-alkanes can provide information on aerosol origin and its characteristics.

The CPI values measured for Tangara da Serra (all range CPI ~ 1.1) indicate a major contribution from anthropogenic activities. Even so, the odd carbon number predominance, C<sub>max</sub> at C<sub>29</sub>, is characteristic of wax from plants as indicated by Simoneit et al. (1991). The isoprenoid hydrocarbons pristane and phytane were identified in this work. These compounds are generally present in diesel fuel, lubricating oil and in both auto and diesel engine exhausts (Simoneit et al., 2007). However, the presence of pristane and phytane in an area of the Brazilian Amazon may be influenced by long range transport of pollutants. Similarly, Vasconcellos et al. (2010) identified these alkanes in the Atlantic Forest.

Other studies have shown that numerous mass concentrations of anthropogenic aerosol particles are generated by Amazon burnings, especially during the period of biomass burning (Fuzzi et al., 2007; Vasconcellos et al., 1998). Pristane and phytane are two of the most common biogenic hydrocarbons produced (Mazquiarán and Pinedo, 2007).

Even though many genotoxic compounds have been identified in the atmosphere, only a few are regularly monitored. In Brazil, a database capable of establishing the amount and sources of PAHs emitted into the atmosphere has yet to be implemented. The results of the incomplete burning of organic matter have shown the mutagenic and/or carcinogenic potential of PAHs in a number of experiments. Several studies correlate the same compounds, identified in our samples, with lung cancer (Ravindra et al., 2008; Zhang and Tao, 2009). The compounds phenanthrene, fluoranthene, pyrene, benzo[e]pyrene, benzo[g,h,i]pyrene, benz[a]anthracene, chrysene, benzo[k]-fluoranthene, benzo[a]pyrene, indene[1,2,3-c,d]pyrene and dibenz[a,h]anthracene were also identified in our samples and are considered, in the literature, to be carcinogenic and/or mutagenic. This information is particularly important.

Phenanthrene, fluoranthene and pyrene were also identified in our samples. Magalhães et al. (2007) reported that these

compounds are emitted by the burning of grasses, an indication of biomass burning. The Benzo(a)pyrene, a mutagenic PAH, also found in Tangará da Serra, showed a lower concentration ( $0.1 \text{ ng m}^{-3}$ ) than that found by Cicciolelli et al. (1996) in Alta Floresta (over  $0.15 \text{ ng m}^{-3}$ ) during biomass burning.

Retene, established by Ramdahl (1983) as the molecular marker of coniferous wood combustion in ambient air (Lehndorff and Schwark, 2009b), was the most commonly found compound in the PAHs identified in this study ( $1.4 \text{ ng m}^{-3}$ ). This value is much higher than those found in previous studies conducted in the Amazon (from  $16$  to  $18 \text{ pg m}^{-3}$ , Vasconcellos et al., 1998) and recently at a sugarcane burning site ( $1.0 \text{ ng m}^{-3}$ , Vasconcellos et al., 2010). However, an additional source of retene in the study area has been attributed to open pit lignite mines (Lehndorff and Schwark, 2009).

We concluded that the mean micronucleus rate from the period of most intense burning is significant compared to the period of least burning. PAH analysis shows that some of the mutagenic compounds identified in these samples could be influencing this result. Rossnerova et al. (2009) used peripheral lymphocytes to evaluate the effects of exposure to carcinogenic aromatic polycyclic hydrocarbons and volatile organic compounds, observing that PAHs and xylene may influence the micronucleus rate.

Furthermore, there was no correlation between  $\text{PM}_{10}$  concentration and mean micronucleus frequency during biomass burning. This is an interesting result, because, in spite of the different PM emission sources, these data are similar to those of the comparative study conducted by Carvalho-Oliveira et al. (2005), where the authors observed that PM levels were similar on strike and nonstrike days. However, the quantified trace elements in the collected filters showed a difference in their composition and a greater micronucleus frequency in the non-strike period. Coronas et al. (2009) reported that adverse effects of air pollution increase concern about regulatory policies and encourage the development of new efficient air standards. Given this situation, we emphasize the need for a reassessment of the standards established by environmental and health agencies, in terms of PM composition, due to its complexity and genotoxic potential as an alternative to show the effects of exposure, because the current results indicate that the values established for air quality are not safe enough to ensure the integrity of the environment and human health.

## 5. Conclusion

In contrast to the large amount of information associating urban PM emitted from industries and/or intense road traffic to environmental and human health impacts, there are a limited number of studies evaluating the impact resulting from biomass burning. This is a pioneering study that evaluates the genotoxicity of organic compounds emitted by biomass burning using the Trad-MCN bioassay (ex situ) in the arc of deforestation of the Brazilian Amazon.

It was observed that the bioassay was sensitive and efficient in analyzing organic extracts from biomass burning. Mutagenic and carcinogenic compounds were also found in the analyzed samples and alkane analysis revealed an anthropic influence. Given these findings, the present study shows the need for measures that minimize biomass burning emissions. Furthermore, it may serve as a reference and incentive for further investigations on the composition and genotoxic potential of organic compounds emitted by such a source, to facilitate a more detailed diagnosis of environmental risk control.

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