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Cashew nut roasting: Chemical characterization of particulate matter and genotocixity analysis

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ABSTRACT

Background: Particulate matter (PM) is potentially harmful to health and related to genotoxic events, an increase in the number of hospitalizations and mortality from respiratory and cardiovascular diseases. The present study conducted the first characterization of elemental composition and polycyclic aromatic hydrocarbon (PAH) analysis of PM, as well as the biomonitoring of genotoxic activity associated to artisanal cashew nut roasting, an important economic and social activity worldwide.
Methods: The levels of $PM_{2.5}$ and black carbon were also measured by gravimetric analysis and light reflectance. The elemental composition was determined using X-ray fluorescence spectrometry and PAH analysis was carried out by gas chromatography–mass spectrometry. Genotoxic activity was measured by the *Tradescantia pallida* micronucleus bioassay (Trad-MCN). Other biomarkers of DNA damage, such as nucleoplasmic bridges and nuclear fragments, were also quantified.
Results: The mean amount of $PM_{2.5}$ accumulated in the filters (January 2124.2 $\mu g/m^3$; May 1022.2 $\mu g/m^3$; September 1291.9 $\mu g/m^3$), black carbon (January 363.6 $\mu g/m^3$; May 70 $\mu g/m^3$; September 69.4 $\mu g/m^3$) and concentrations of Al, Si, P, S, Cl, K, Ca, Ti, Cr, Mn, Fe, Ni, Cu, Zn, Se, Br and Pb were significantly higher than the non-exposed area. Biomass burning tracers K, Cl, and S were the major inorganic compounds found. Benzo[k]fluoranthene, indene[1,2,3-c,d]pyrene, benzo[ghi]perylene, phenanthrene and benzo[b]fluoranthene were the most abundant PAHs. Mean benzo[a]pyrene-equivalent carcinogenic power values showed a significant cancer risk. The Trad-MCN bioassay revealed an increase in micronucleus frequency, 2–7 times higher than the negative control and significantly higher in all the months analyzed, possibly related to the mutagenic PAHs found.
Conclusions: This study demonstrated that artisanal cashew nut roasting is a serious occupational problem, with harmful effects on workers' health. Those involved in this activity are exposed to higher $PM_{2.5}$ concentrations and to 12 PAHs considered potentially mutagenic and/or carcinogenic. The Trad-MCN with *T. pallida* was sensitive and efficient in evaluating the genotoxicity of the components and other nuclear alterations may be used as effective biomarkers of DNA damage.

1. Introduction

About 31 countries worldwide produced 4.20 million metric tons of cashew nuts in 2011. The major cashew nut producing countries and their production figures are Vietnam (1.27 million tons), Nigeria (0.81 million tons), India (0.67 million tons), Ivory Coast (0.45 million tons), Brazil (0.23 million tons) and the Philippines (0.13 million tons). In 2010, major exporters of shelled cashew nuts included Vietnam, India and Brazil and the main importing countries were the USA (119.11 metric tons), the Netherlands (41.27 metric tons), Germany (25.44 metric tons) and China (23.44 metric tons) (FAO, 2013).

The semi-arid region of Brazil is characterized by an annual rainy season and an extremely dry period. Cashew nut roasting is a socially accepted and financially viable alternative, since, in addition to being a year-round activity, it produces an easily commercialized product. However, the lack of assistance provided to workers,

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the informality of the activity and the lack of knowledge on the part of society in general about the conditions under which cashew nut roasting takes place, hamper control of the potential harmful effects associated to this type of enterprise.

One of the main issues in the cashew fruit productive chain are the conditions under which cashew roasting takes places to obtain the kernel. The nuts are oven-roasted, where the shell itself acts as the fuel. The fuel generated from cashew nut roasting releases cashew nut shell liquid, a highly flammable caustic phenolic oil that releases compounds into the atmosphere (Agila and Barringer, 2011; Chandrasekara and Shahidi, 2011). The smoke generated during cashew nut roasting is inhaled daily by family groups that take part in the activity for periods that may exceed 10 h/day. The roasting process generally occurs between 2:00 AM and 12:00 h PM.

Biomass burning releases several air pollutants, especially particulate matter (PM), a complex mixture including inorganic and organic compounds. Fine particulates ($PM_{2.5}$) are potentially harmful to health and may affect the lower airways, reaching the pulmonary alveoli. These particles are related to genotoxic events, an increase in the number of hospitalizations and ambulatory visits, and mortality from cardiovascular and respiratory diseases (Alves et al., 2011; André et al., 2012; De Oliveira et al., 2012; Da Silva et al., 2012). The organic fraction of PM has recently caused significant environmental concern due to the high mutagenic and carcinogenic potential displayed by its components (Vasconcellos et al., 2010). Data on the composition of PM smoke are important in order to understand the organic component contributions of biomass burning emissions to atmospheric chemistry, in addition to complementing existing research on the characterization of direct (natural) organic emissions from biomass sources (Simoneit, 2002).

Plant bioassays are best suited to assessing the effects of air pollution and are generally highly sensitive in detecting the genotoxic effects (Villarini et al., 2009; Alves et al., 2011, 2014; Sisenando et al., 2011). The Tradescantia micronucleus bioassay (Trad-MCN) has been extensively used in genotoxicity studies, due to its high sensitivity to such compounds (Mišík et al., 2011). However, in tropical countries Tradescantia clones, mainly BNL-4430 and KU-20, do not grow well in the high temperature, humidity and rainfall conditions typically found in these regions, often exhibiting inhibited growth and flowering (Suyama et al., 2002). An appropriate alternative to the environmental conditions found in tropical countries is the use of *Tradescantia pallida*. Several studies have demonstrated its sensitivity to the genotoxic compounds from air pollutants *in situ* (Batalha et al., 1999; Guimarães et al., 2000; Carreras et al., 2006; Prajapati and Tripathi, 2008; Mariani et al., 2009; Meireles et al., 2009; Savóia et al., 2009; Sisenando et al., 2011; Pereira et al., 2013) and *ex situ* (Carvalho-Oliveira et al., 2005; Alves et al., 2011, 2014; Carreras et al., 2013).

Owing to this exposure, the aim of the study was to assess the concentration, elemental composition and polycyclic aromatic hydrocarbon (PAH) analysis of PM, as well as the genotoxic potential associated to artisanal cashew nut roasting during the dry, rainy and intermediate seasons in a semiarid region of Brazil.

2. Materials and methods

2.1. Study area

Two sites were chosen as test areas: (1) The community of Amarelão, located on the rural perimeter of the municipality of João Câmara, Brazil (05°30'51.81"S; 35°54'17.13"O), site of the cashew nut roasting. (2) Santa Luzia farm (05°33'6.72"S; 35°46'10.75"O), situated 13 km from the roasting location and exhibiting the same environmental conditions, but without the influence of cashew nut processing. The two sites are not directly affected by vehicular emissions or industrial pollutants. Fig. 1 shows the location of the sampling site, which is downwind from the two areas.

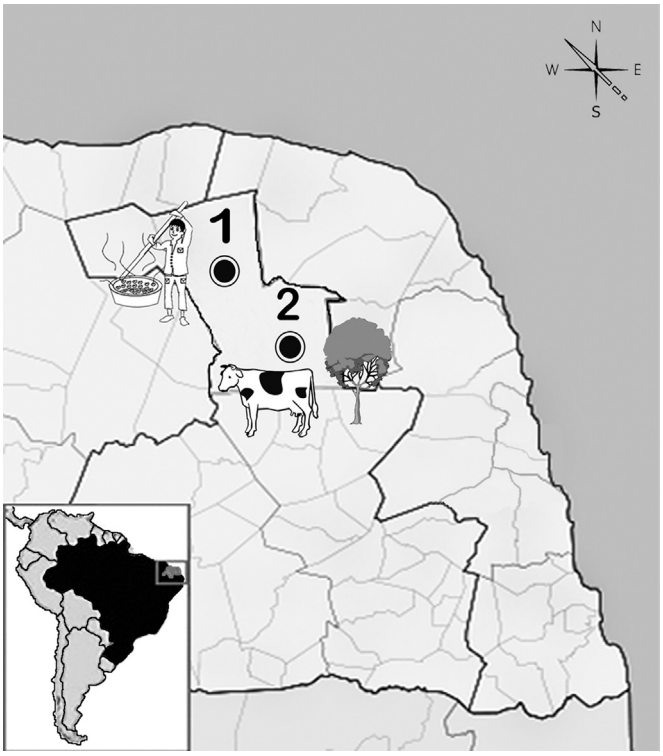

Fig. 1. Location of the artisanal cashew nuts roasted. Exposure sites of *Tradescantia pallida* and the $PM_{2.5}$ collection sites. Site 1 (Amarelão Community) and Site 2 (Santa Luzia farm). The arrow indicates the prevailing wind direction.

2.2. Fine particulate matter sampling

Fine particulate matter ($PM_{2.5}$) was sampled in January (dry season), May (rainy season) and September (intermediate season) 2009, using a portable sampler (manufactured by the Harvard School of Public Health) equipped with a $PM_{2.5}$ probe and at a flow rate of 1.8 lpm, with a polycarbonate membrane (diameter of 37 mm, pore 0.8 μm, Millipore-USA). Each campaign sampled 10 filters per site, obtaining a total of 60 filters.

Each filter was submitted to gravimetric analysis using a Mettler MT5 microbalance (Mettler-Toledo, Greifensee, Switzerland), with a minimum resolution of 1 μg, to estimate average daily mass concentration. Black Carbon (BC) concentration was estimated by optical reflectance using a Smoke Stain Reflectometer (Model 43D, Diffusion Systems LTD, London, UK) (Reid et al., 1998).

2.3. Total suspended particle sampling

Total suspended particles (TSP) were sampled in March 2013 only at the Amarelão site, using a Handi-vol sampler (Energética, Brazil) operating at a flow rate of 230 lpm, with a quartz fiber filter (EQTZ diameter of 110 mm, Energética, Brazil). Each filter was previously conditioned in an oven for 8 h at 800 °C, to avoid contamination during PAH laboratory analysis.

2.4. Elemental composition of fine particulate matter

The elemental composition of $PM_{2.5}$ was determined by energy dispersive X-ray fluorescence spectrometry analysis (ED-XRF) using an EDX-700HS model (Shimadzu Corporation Analytical Instruments Division, Kyoto, Japan). A low power Rh-target tube, with voltage of 5–50 kV and current from 1 to 1000 μA, was used in the analyses. The characteristic X-ray radiation was detected by a Si (Li) detector. Analysis was performed in a vacuum atmosphere on a 10 mm diameter surface, for the elements Al, Si, P, S, Cl, K, Ca, Ti, Cr, Mn, Fe, Ni, Cu, Zn, Se, Br and Pb.

2.5. Organic compounds of total suspended particles

Organic compounds were extracted from the filters by ultrasonic bath (3 cycles of 20 min), with 80 mL dichloromethane (DCM) as solvent for each cycle. Sample extracts were concentrated to 5 mL by a rotary evaporator and then under gentle N_2 flux. The different fractions were obtained using a silica gel (3.4 g) and alumina (1.8 g) column. The column was pre-washed with 20 mL of *n*-hexane. The first fraction containing the *n*-alkanes was eluted from the silica gel column with 40 ml