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Leaves of *Lolium multiflorum* 'Lema' and tropical tree species as biomonitors of polycyclic aromatic hydrocarbons ☆

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ABSTRACT

This study extends the current knowledge regarding the use of plants for the passive accumulation of anthropogenic PAHs that are present in the atmospheric total suspended particles (TSP) in the tropics and sub-tropics. It is of major relevance because the anthropic emissions of TSP containing PAHs are significant in these regions, but their monitoring is still scarce. We compared the biomonitor efficiency of *Lolium multiflorum* 'Lema' and tropical tree species (*Tibouchina pulchra* and *Psidium guajava* 'Paluma') that were growing in an intensely TSP-polluted site in Cubatão (SE Brazil), and established the species with the highest potential for alternative monitoring of PAHs. PAHs present in the TSP indicated that the region is impacted by various emission sources. *L. multiflorum* showed a greater efficiency for the accumulation of PAH compounds on their leaves than the tropical trees. The linear regression between the logBCF and logKoa revealed that *L. multiflorum* is an efficient biomonitor of the profile of light and heavy PAHs present in the particulate phase of the atmosphere during dry weather and mild temperatures. The grass should be used only for indicating the PAHs with higher molecular weight in warmer and wetter periods.

1. Introduction

The occurrence of polycyclic aromatic hydrocarbons (PAHs) in the environment has recently received considerable attention due to the harmful effects they may cause on humans. These compounds are among the most widely distributed carcinogens in the environment (Ohura et al., 2004). PAHs can be formed during the incomplete combustion of organic materials, such as oil, gas and wood, and they are emitted primarily by anthropogenic sources in all around the world, inclusively in Brazil (Netto et al., 2007; Ravindra et al., 2006; WHO, 2002). However, they are also emitted from natural sources, such as volcanic activity and forest fires (Boitsov et al., 2009).

The partition of PAHs between the particulate and gaseous phases, which is an important aspect for determining their transport, transformation and deposition in the atmosphere, depends on meteorological factors, on the characteristics of the particulate material and on the concentration and molecular weight of these compounds (Tasdesmir and Esen, 2007).

Vegetation plays an important role in the global cycle of PAHs (Collins et al., 2006); however, the processes of accumulation, transport and transformation within the plant have not yet been fully established (Lin et al., 2007).

Lichens and various groups of plants (e.g., aquatic plants, grasses, vegetables and trees) have been recommended as biomonitors of PAH because they may accumulate these species in their organs (Augusto et al., 2010; De Nicola et al., 2011; Lehndorff and Schwark, 2010, 2004; Orecchio et al., 2008; Orliński, 2002; Piccardo et al., 2005; Rodriguez et al., 2010). The magnitude of this accumulation and the efficiency of plants as biomonitors depend on their morphological and physiological characteristics (De Nicola et al., 2005; Franzaring and Van der Eerden, 2000; Wild et al., 2006) in addition to the physicochemical properties of PAHs and the local meteorological conditions (St-Amand et al., 2008).

Although there are few initiatives for using plant species as bioaccumulators of PAHs emitted from human activities in the tropics and sub-tropics, an ideal model for biomonitoring has not yet been established. Furthermore, the knowledge of the sources and distribution of PAHs in these regions is limited, although it is important to understand the global and temporal dynamics of these compounds (Jones and De Voogt, 1999; Wilcke et al., 2000; Kraus et al., 2005).

☆ *Capsule: Lolium multiflorum 'Lema' was the most efficient biomonitor of the profile of light and heavy PAHs present in the particulate phase of the atmosphere in the region of Cubatão.*

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Lolium multiflorum spp. *italacum* 'Lema' is one standardized and frequently used cultivar for the biomonitoring of inorganic substances adsorbed on the atmospheric particulate matter emitted in large urban centers (Klumpp et al., 2009; Rodriguez et al., 2010; VDI (Verein Deutscher Ingenieure), 2003). It has also been recommended by the VDI (Verein Deutscher Ingenieure) (2003) as a biomonitor of PAHs. *T. pulchra* and *P. guajava* are tropical tree species, which have shown a high capacity to accumulate sulfur, fluorides and metals in several studies performed in the vicinity of the Industrial Complex of Cubatão in southeastern Brazil (Domingos et al., 2003; Furlan et al., 2007; Klumpp et al., 1996; Moraes et al., 2002). These plant species may also be appropriate PAH biomonitors.

Thus, the objectives of the present study were: (1) to compare the efficiency of PAH accumulation in the particulate matter on the leaves of *L. multiflorum* 'Lema', *T. pulchra* and *P. guajava* 'Paluma' growing in an intensely polluted site in the region of Cubatão; (2) to establish which species among the investigated has the highest feasibility for alternative monitoring of PAHs.

2. Materials and methods

2.1. Plant cultivation

Plants of *L. multiflorum* spp Italicum ''Lema'' were cultivated from seeds that were germinated in pots with a standard substrate (a mixture of a commercial substrate primarily composed of the bark of *Pinus* and fine vermiculite in a 3:1 ratio) following the VDI (Verein Deutscher Ingenieure) (2003) protocol. During the 28 day after germination, the plants were excised and fertilized weekly with a nutrient solution. Seedlings of *T. pulchra* and *P. guajava*, acquired from commercial producers with a height of 20 cm and 4–6 leaves on the main stem, were transplanted into plastic pots with the same standard substrate used for *L. multiflorum* at least 15 day before the beginning of each field experiment. Throughout the cultivation process, plants of all three species were kept inside a greenhouse under filtered air and ideal climatic growth conditions. They were continuously watered by nylon strings inserted into the bottom of the pots at one end and immersed in water reservoirs at the other. These procedures were performed repeatedly to obtain similar lots of plants for the field experiments.

2.2. Study site and field experiments

The field experiments were conducted in a location (Center for Training and Research Environment belonged to the University of São Paulo) near the industrial complex in the city of Cubatão (southeastern Brazil). The city, with an area of 142 km^2, is situated 44 km from São Paulo and 12 km from Santos (a coastal city) between latitudes 23°45′ and 23°55′ and longitudes 46°15′ and 46°30′. The territory is surrounded by the mountain range regionally known as Serra do Mar and by the estuary of Santos (Domingos et al., 2009). This complex is composed of chemical, petrochemical, fertilizer, non-metallic minerals, paper and cement industries that sum approximately 230 air pollutant emission sources. Under normal weather conditions, winds blow toward the ocean late at night and in the morning, dispersing the pollutants. The wind direction reverses during the warmer period of the day, carrying moisture from the ocean and industrial pollutants directly to the slopes of Serra do Mar, which is covered by the Atlantic Forest. These conditions are prevalent, but they may change, particularly with entering cold fronts (Alonso and Godinho, 1992; CETESB, 2010).

The Center for Training and Research Environment (CEPEMA) where the plants were placed is strongly influenced by particulate matter emitted by both the petrochemical industry and by light and heavy vehicles because the site is next to a highway with heavy traffic.

The plants of *T. pulchra* and *P. guajava* were exposed in the location during two consecutive periods of 12 weeks each (April 27 to July 20 and July 20 to October 15 of 2009), and the cultures of *L. multiflorum* were exposed during nine consecutive periods of 4 weeks each (April 27 2009 to January 04 of 2010). Eight replicates were used per field experiment.

The pots containing the cultures of *L. multiflorum* were maintained under field conditions on apparatuses framed in accordance with the guidelines of the German Association of Engineers (VDI (Verein Deutscher Ingenieure), 2003), and those containing the saplings of the tree species were maintained on apparatuses modeled by Arndt and Schweizer (1991). Only the saplings of *T. pulchra* were shaded (50% reduction in solar radiation). During the field experiments, these plants were also irrigated using nylon strings.

2.3. Sampling of particulate material

The total suspended particles (TSP) were sampled at the same site (CEPEMA) and during the same period of plant exposure to compare the concentrations trends of PAHs present in plants to those found in the particulate matter. The TSP collection was performed once a week, for 24 h, on glass fiber filters previously treated and inserted in a high air volume sampler. These collections occurred only during periods without rain.

2.4. Extraction of PAHs from plant samples and particulate matter

At the end of each field experiment, the leaves of *L. multiflorum*, *T. pulchra* and *P. guajava* were cut and assembled to obtain four composite samples for each species and each exposure period. Each composite sample was divided into two parts: one for the extraction of PAHs and the other for determining the dry weight.

The plant samples and filters containing TSP were weighed, wrapped in aluminum foil for protection against light and then stored in a freezer until PAH extraction.

The procedure for PAH extraction from both types of material was modified from the technique recommended by Orecchio et al. (2008). PAH extraction was performed in a Soxhlet apparatus with 250 mL of HPLC grade dichloromethane and 50 mL of HPLC grade hexane for 24 h.

The plant extracts were concentrated to about 10 mL with a rotary evaporator and mixed with 0.2 g of sodium sulfate to remove water following the protocol described by Lehndorff and Schwark (2004), with modifications. Each sample was centrifuged twice at -2 °C at 3500 rpm for 15 min to separate the fraction of dichloromethane containing the compounds under investigation (the epicuticular wax and sodium sulfate). The resulting extracts were then evaporated, and the dry residue was dissolved in 2 mL of HPLC grade acetonitrile and frozen for later analysis.

The resulting TSP extracts contained in the filters were then dried with a rotary evaporator, and the dry residue was dissolved in 2 mL of HPLC grade acetonitrile and frozen for later analysis.

For analysis, the extracts of both materials were filtered through an Acrodisc filter made of recovered cellulose (0.45 μm pore size) and diluted in HPLC grade acetonitrile.

All this procedures were based on preliminary tests which pointed a PAH recovery ranging from 82% to 105%.

2.5. Analysis of PAHs

All final plant extracts and TSP were analyzed by high performance liquid chromatography (HPLC) with a fluorescence detector set at wavelengths of 275 nm (extinction) and 395 nm (emission). The separations were performed with a 25 cm × 4.6 mm (length—i.d.) 5 μm particle Supelcosil LC-PAH coupled with a guard column. The mobile phase used in the analysis was composed of water and acetonitrile with a gradient elution at a flow rate of 1.5 mL/min. The volume of sample injection was 20 mL. The following PAHs were analyzed: naphthalene (NAP), acenaphthene (ACE), fluorene (FLU), phenanthrene (PHE), anthracene (ANT), fluoranthene (FLT), pyrene (PYR), benzo[a]anthracene (BaA), chrysene (CRY), benzo[b]-fluoranthene (BbF), benzo[k]fluoranthene (BkF) and benzo[a]pyrene (BaP).

2.6. Monitoring of meteorological conditions during the experimental period

The rainfall data during April 2009 through January 2010 was provided by the Metropolitan Company of Water and Energy of São Paulo (EMAE). The temperature and relative humidity were monitored in an area near the study by the Estate Technological Company of Environmental Sanitation (CETESB), and the data were obtained from its website (http://sistemasinter.cetesb.sp.gov.br/Ar/ar_dados_horarios.asp).

2.7. Statistical analyses

Pearson correlation analyses were applied to ascertain the relationships between the sum of the PAH concentrations (ΣPAH) and the TSP concentrations.

The concentration of each compound analyzed in the three plant species for each 12-week exposure period was individually gathered in box plots. The plots for *L. multiflorum* represented results gathered from all three four-week periods that coincided with each exposure period for *P. guajava* and *T. pulchra*. Nonparametric analyses of variance followed by Dunn's test were applied to find significant differences ($p < 0.05$) between the concentrations of each compound analyzed in the three plant species from when contemporaneously exposed.

2.8. Calculation of the bioconcentration factors

The bioconcentration factor (BCF) was estimated by the ratio between the concentrations of each PAH measured in the plants and in the TSP multiplied by the density of air to obtain unscaled values, following the formula described by Ötvös et al. (2004). Linear regression curves were then drawn between the