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Ryegrass cv. Lema and guava cv. Paluma biomonitoring suitability for estimating nutritional contamination risks under seasonal climate in Southeastern Brazil

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ABSTRACT

The risks posed by nutrient deposition due to air pollution on ecosystems and their respective services to human beings can be appropriately estimated by bioindicator plants when they are well acclimated to the study region environmental conditions. This assumption encouraged us to comparatively evaluate the accumulation potential of ryegrass cv. Lema and guava cv. Paluma macro and micronutrients. We also indicated the most appropriate species for biomonitoring nutrient contamination risks in tropical areas of Southeastern Brazil, which are characterized by marked dry and wet seasons and complex mixtures of air pollutants from different sources (industries, vehicle traffic and agriculture). The study was conducted in 14 sites with different neighboring land uses, within the Metropolitan Region of Campinas, central-eastern region of São Paulo State. The exposure experiments with ryegrass and guava were consecutively repeated 40 (28 days each) and 12 (84 days each) times, respectively, from Oct/2010 to Sept/2013. Macro and micronutrients were analyzed and background concentrations and enrichment ratios (*ER*) were estimated to classify the contamination risk within the study region. Significantly higher *ER* suggested that ryegrass were the most appropriate accumulator species for N, S, Mg, Fe, Mn, Cu and Zn deposition and guava for K, Ca, P and B deposition. Based on these biomonitoring adjustments, we concluded that the nutrient deposition was spatially homogeneous in the study area, but clear seasonality in the contamination risk by nutritional inputs was evidenced. Significantly higher contamination risk by S, Fe, K and B occurred during the dry season and enhanced contamination risk by Mn, Cu and Zn were highlighted during the wet season. Distinctly high contamination risk was estimated for S, Fe and Mn in several exposure experiments.

1. Introduction

The industrial production, fuel combustion by light and heavy vehicles, energy production and fertilizer and herbicide applications in agriculture in major urbanized centers (such as metropolitan regions) are currently among the most important sources of anthropogenic nutrient deposition (Lehndorff and Schwark, 2010; Sawidis et al., 2011; Boian and Andrade, 2012). Several pollutants may deposit on natural and agricultural ecosystems and occur at toxic levels in their different compartments, even if they are essential macro and micronutrients (e.g. nitrogen, sulfur, iron, copper, zinc) (Lehndorff and Schwark, 2010). In such condition, reduced biomass production and disturbances in plant physiology and biochemistry may be expected due to both direct and indirect effects induced by nutritional imbalances and acidification. Disproportional ratios between nitrogen and phosphorus or mobile cations, for instance, are commonly reported in native plant species growing in forest ecosystems affected by anthropogenic N deposition (van den Berg and Ashmore, 2008; Huang et al., 2012).

The risks posed by anthropogenic nutrient deposition on natural or agricultural ecosystems due to air pollution can be estimated by bioindicator plants, which are able to react in a predictable and quantifiable way to environmental disturbances by changing their chemical composition or vital functions (Arndt and Schweizer, 1991; Fränzle, 2003; Markert et al., 2003; Abril et al., 2014). However, we may assume that the validity of risk prediction using bioindicator plant species will depend on their acclimation level to the regional natural conditions.

Ryegrass (*Lolium multiflorum* Lam. ssp. italicum Beck cv. Lema) has been well acclimated and appropriately used as a bioaccumulator of trace metals, sulfur, fluorine, and organic pollutants since the early 1970s in temperate regions. It has shown high tolerance against most air pollutants, without showing any visible

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injury due to environmental pollution levels (VDI, 2003; Klumpp et al., 2009). Some studies using ryegrass cv. Lema also highlighted its usefulness as a bioaccumulator in tropical regions (Klumpp and Klumpp, 1994; Klumpp et al., 1996; Domingos et al., 1998; Sandrin et al., 2008; Rinaldi et al., 2012; Nakazato et al., 2015).

Wild guava (*Psidium guajava*) also seemed to be efficient as an accumulator plant of nitrogen, sulfur, fluorine and some few heavy metals, mostly micronutrients, in a tropical environment (Moraes et al., 2002). In addition, some studies revealed that guava cv. Paluma is also interesting for biomonitoring toxic elements in tropical regions (Perry et al., 2010; Nakazato, 2014).

However, the satisfactory biomonitor properties of both species in tropical regions were only detected in areas with high water availability and affected by high levels of industrial air pollution, fact that raised the following question: which of them is more appropriate for biomonitoring anthropogenic nutrient deposition in tropical areas characterized by marked dry and wet seasons and generally by complex mixtures of air pollutants emitted by different sources, such as those found in the Metropolitan Region of Campinas (Southeast, Brazil)? In order to answer this question, the present study aimed at (1) comparing the accumulation potential of macro and micronutrients shown by ryegrass cv. Lema and guava cv. Paluma, indicating the most appropriate species for biomonitoring risks to natural and agricultural ecosystems associated with nutrient deposition in an area typically affected by alternate dry and wet seasons. (2) Verifying whether the risk posed by nutrient deposition varies among sites and seasons in the study region based on leaf accumulation in both cultivars.

2. Material and methods

2.1. Study area

The Metropolitan Region of Campinas (MRC), which is composed of 20 municipalities and located in the central-eastern region of São Paulo State, Brazil (Map, Supplementary material), was selected to be subjected to the field experiments because (a) It is the second most important economical center of São Paulo State, which is characterized by different land uses, among cities, highways, industries and extensive agricultural lands mainly devoted to sugarcane, orange and ornamental plantations (CETESB, 2013); (b) These activities emit considerable amounts of air pollutants (Tresmondi and Tomaz, 2004), which may potentially cause nutritional imbalances in crops and native plants growing in the Atlantic Semideciduous Forest remnants that still exist in MRC; (c) In addition to local emissions, the MRC is also affected by sizable emissions from the Metropolitan Region of São Paulo (MRSP), as a result of the predominantly southerly and southwesterly winds (Boian and Andrade, 2012); (d) Finally, more than three million people live close to these air, water and soil pollution sources in MRC, with consequent loss of ecosystem service quality.

The biomonitoring using *Lolium multiflorum* Lam. ssp. italicum Beck cv. Lema (ryegrass) and *Psidium guajava* cv. Paluma (guava) was performed in fourteen sites within the MRC, which were categorized according to their major neighboring land uses: I1, I2 and I3 were located near an industrial pole; I/A was placed near the industrial pole and agricultural crops; A1, A2, A3, A4, A5, A6 and A7 were predominantly surrounded by agricultural crops, mainly sugarcane plantation; A/U was located near agricultural crops and an urban area; and U1 and U2 were close to urban areas (Map, supplementary material). The last one was chosen due to its proximity to an automatic air quality and weather conditions monitoring station. The sites A1, A4, A5, A6, A7 and A/U were close to the last Atlantic Semideciduous Forest remnants, a subtype of the Atlantic forest domain in Southeastern Brazil.

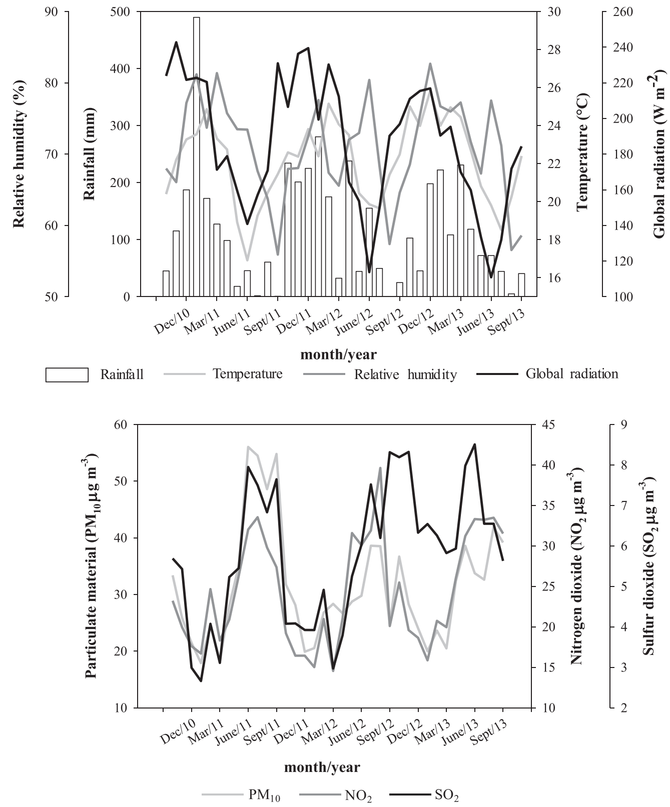

Fig. 1. Total monthly rainfall and monthly averages of global radiation, relative humidity, temperature, particulate material, nitrogen dioxide and sulfur dioxide in MRC from October/2010 to September/2013.

2.2. Plant cultivation and field exposure

The following procedures were repeatedly performed to produce similar lots of plants in all field experiments. Ryegrass cv. Lema seeds (0.8 g per pot) were germinated and cultivated in plastic pots (1 L) containing a mixture of standardized substrate (Tropstrato Hortaliça HT) and vermiculite (3:1 v/v). During cultivation, the plants (approximately 20 grown in each pot) were weekly excised to a height of 4 cm above the substrate and fertilized with macronutrient solution recommended by Epstein (1975) (40 cm^3 per pot), according to the protocol established by VDI (2003). Guava cv. Paluma saplings, with approximately 30 cm height and 12 leaves produced from the rooting of semi-herbaceous cuttings were taken from a specialized Brazilian producer. Fifteen days before the beginning of each field experiment, the saplings were transplanted into plastic pots (3.0 L) with the same standardized substrate used for the ryegrass (one sapling per pot). They were fertilized with 100 ml of a solution containing NPK (20:20:20) and insects and mites were controlled with tiametoxan 25% (Syngenta) applications.

The plants of both species were kept inside a greenhouse under charcoal-filtered air and ideal climatic growth conditions throughout the cultivation process. 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.

After growth, three pots of ryegrass cv. Lema plants were exposed in each of the fourteen sites for 28 days, following the methods suggested by VDI (2003). Ten potted saplings of guava cv. Paluma were exposed in the same sites on racks 1 m high from the soil surface for 84 days, following well-succeeded methods described by Moraes et al. (2002). These exposure experiments with ryegrass cv. Lema cultures and guava cv. Paluma plants were consecutively repeated 35 and 12 times, respectively, during the