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Physiological traits and antioxidant metabolism of leaves of tropical woody species challenged with cement dust

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ABSTRACT

Tropical woody species occurring in limestone outcrops are frequently exposed to particulate material from cement factories. The effects of 60-day cement dust exposure on physiological traits and enzymatic antioxidant system of young plant leaves of *Guazuma ulmifolia* Lam., *Myracrodruon urundeuva* Allemão and *Trichilia hirta* L. were investigated. Cement dust (2.5 or 5 mg cm^{-2}) was applied to the leaf surface or soil or both (leaf plus soil) and plants were maintained at greenhouse. Cement dust barely affected the mineral nutrient levels, except for iron whose content was decreased in leaves/leaflets of all species studied. The incident light was partly blocked in cement dust-treated leaves, regardless of the plant species, causing a decrease in the photosynthetic pigments in *M. urundeuva.* The chlorophyll *b* content, however, increased in *G. ulmifolia* and *T. hirta* leaves upon cement dust treatment. The potential quantum yield of photosystem II in challenged leaves of *G. ulmifolia* was 3.8% lower than that of control plants, while such trait remained unaffected in the leaves of the other species. No changes in leaf stomatal conductance and antioxidant enzymes activities were observed, except for *M. urundeuva*, which experienced a 31% increment in the superoxide dismutase activity upon 5 mg cm^{-2} cement dust (leaf plus soil treatment), when compared with control plants. Overall, the mild changes caused by cement dust in the in physiological and biochemical traits of the species studied indicate that such species might be eligible for further studies of revegetation in fields impacted by cement factories.

1. Introduction

Particulate-material-caused pollution, particularly the one emitted from cement industries, represents one of the greatest threats to the environment and human health (Grantz et al., 2003; Rai, 2016; Salem et al., 2015). Brazil is among the top five cement producing countries in the world (SNIC, 2016), in which such activity is known to provide great risk to native vegetation since cement dust can adversely affect plant growth and development (Abu-Romman and Alzubi, 2015; Maletsika et al., 2015; Rai, 2016).

Although the number of studies that addresses the impact of particulate cement material on plants has been growing (Dziri and Hosni, 2012; Farmer, 1993; Işikli et al., 2006; Maletsika et al., 2015; Mandre, 2014; Paal et al., 2013), researches focusing on the impact of this abiotic stress on Brazilian woody species native to limestone outcrops are incipient (Siqueira-Silva et al., 2016a, 2016b). The lack of knowledge on the flora of cement-dust-affected Dry forests (Arruda et al., 2013; Melo Júnior et al., 2015) is also another factor that makes researches of this kind difficult to be performed. These factors, combined with the exploitation of limestone, the main raw material for cement production, may result in vegetation losses at cement-affected sites and surrounding ecosystems.

Exposure to cement dust is a global problem and has been shown to cause multiple damages to plants (Lukjanova et al., 2013; Mandre and Lukjanova, 2011; Mutlu et al., 2013). In addition to calcium (Ca) (Bermudez et al., 2012; Branquinho et al., 2008; Lee and Pacyna, 1999), metals such as Cu, Mn, Fe, Cr, Zn, Hg, Cd and Pb, among others, can be found in cement dust in varied amounts (Ogunbileje et al., 2013) according to the raw material and type of fuel used for producing the cement (Brown et al., 2014). Therefore, caution must be taken when using cement dust for remediation of contaminated soils (Hale et al., 2012), as fertilizer for plants (Klõšeiko et al., 2014) and for the treatment of wastewater for irrigating crops purposes (Salem et al., 2015), as cement dust usually contains toxic metals (Märkl et al., 2017).

Exposure to particulate materials can affect plant metabolism, particularly photosynthesis and transpiration as it facilitates the uptake of

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phytotoxic pollutants (Semhi et al., 2010) and accumulation of essential and non-essential elements in leaf tissues (Lau and Luk, 2001). Additionally, particulate materials have been shown to negatively affect chlorophyll levels (Lukjanova and Mandre, 2010; Maletsika et al., 2015; Nanos and Ilias, 2007; Rai, 2016) and boost the deficiency of essential elements (Mutlu et al., 2013). Indeed, the determination of chlorophyll content in plant leaves has been used as a tool to evaluate the effects of airborne pollutants because the decrease of such pigments leads to lower rates of plant growth (Rai, 2016). Other effects resulting from the deposition of particulate materials on leaves surface include the blockage of photosynthetic active light, alteration of gas exchange rate, leaf abrasion and warming (Farmer, 1993; Grantz et al., 2003; Hirano et al., 1995; Pereira et al., 2009).

It is documented that the first events triggered by cement dust on plant cells are lipid peroxidation and stimulation of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT) and peroxidase (POX) (Dziri and Hosni, 2012) as a result of an oxidative burst (Apel and Hirt, 2004; Bhaduri and Fulekar, 2012). It is well known that the activity of antioxidant enzymes can be differentially affected following the duration and intensity of an abiotic stress, plant developmental stage and plant resistance to the abiotic stimulus (Horta et al., 2014).

The study herein presented focused on the investigation of the potential of three tropical plant species for further use in programs of conservation, revegetation and recovery of areas degraded by cement factories. The species *Guazuma ulmifolia* Lam. (Malvaceae), *Myracrodruon urundeuva* Allemão (Anacardiaceae) and *Trichilia hirta* L. (Meliaceae) were selected because they are heliophytic woody species native to Brazilian limestone outcrops that are widely found in different physiognomies. The tree habit exhibited by such species, the relatively fast growth and the easy management for cultivation also supported the selection of the referred plant species. To achieve the goal, leaf surface of young individuals of *G. ulmifolia* (5-month old), *M. urundeuva* (6-month old) and *T. hirta* (9-month old) and/or the soil in which plants were cultivated were challenged with cement dust under controlled conditions to verify if the treatments affected some physiological traits, the activity of some antioxidant enzymes and plant nutrition.

2. Material and methods

2.1. Experimental conditions for plant growth

Seeds of *Guazuma ulmifolia* Lam. (Malvaceae), *Myracrodruon urundeuva* Allemão (Anacardiaceae) and *Trichilia hirta* L. (Meliaceae) were harvested from mother plants located in the Pampulha campus of the Federal University of Minas Gerais (UFMG, Brazil) (19°52′25.2″S and 43°58′21.2″W) and the Ecological Park District Attorney Francisco Lins do Rego (19°51′34.1″S and 43°59′46.1″W). The dormancy of *G. ulmifolia* seed coat was overcome essentially as described by De Araújo Neto and De Aguiar (2000) while the arillus of *T. hirta* seeds was manually removed following by seeds drying at room temperature. No treatment prior to germination was required for *M. urundeuva* seeds.

Plants of *G. ulmifolia*, *M. urundeuva* and *T. hirta* were grown in a greenhouse (19° 52′ 09.3″ S and 43° 58′ 00″ W) for five, six and nine months, respectively, in pots (5 L) with a contamination-free eutrophic soil that was harvested from typical Dry forests in Minas Gerais, Brazil. The soil in which plants were cultivated was irrigated every two days at 90% field capacity.

2.2. Cement dust deposition

Amounts of 2.5 or 5 mg cm^{-2} of cement dust (Argos' Portland Cement, LPC Argos, São Paulo, Brazil) were chosen to simulate the air pollution that occurs in the vicinity of cement factories under full operation in Minas Gerais based on a previous report (Siqueira-Silva et al., 2016b). The size of cement particles averaged 75 µm with less than 10 wt% of 2 µm and 90 µm particules.

One group of plants had cement dust applied manually to the soil only (Soil treatment), another group had dust applied on the leaf surface only (Leaf treatment) and a third one had dust applied to both soil and leaf surfaces (Leaf/Soil treatment). For the latter two treatments, dust was applied using a chamber essentially as described by Siqueira-Silva et al. (2016b) to simulate the natural deposition of solid particulate material. The nominal concentrations (2.5 or 5 mg cm^{-2}) of the cement dust were estimated by gravimetry using Petri dishes that placed inside the chamber, along with the plants. Plant shoots and the soil were manually sprayed with deionized water after each application of cement dust (9 a.m. to 12 p.m.) and every five days thereafter to simulate the natural conditions of the impacted area and to ensure the adhesion of the particules to the surface. The control group did not receive any cement dust. The soil used to cultivate plants under Leaf treatment was covered with a polyethylene film to avoid cross-contamination. Two applications of cement dust were performed throughout the experiment (60 days): one at the beginning of the experiments and another one 22 days later (Siqueira-Silva et al., 2016b). Afterwards, plants were maintained at a greenhouse under a nylon net of 14 Mesh and 0.3 mm thread, a maximum photosynthetic active radiation of 1290 µmol m^{-2} s^{-1} and maximum and minimum temperatures of 27.1 °C and 17.3 °C (in average). The local maximum and minimum relative air humidity were 86% and 46%, in average.

The experiment employed a completely randomized design with four replicates per treatment.

2.3. Quantification of dust deposited on leaf surface

After 60 days of exposure to cement dust, the particulate material deposited on leaf surfaces was quantified according to Kuki et al. (2008). Three leaves of *G. ulmifolia* from the third node and five leaflets of *M. urundeuva* and *T. hirta* from the fourth node were randomly harvested from treated and untreated plants for the measurement of leaf area using AxioVision 4.9.1 software (Carl Zeiss MicroImaging GmbH, Jena, Deutschland). Leaves/leaflets were individually washed prior to the measurement of leaf area using soft-bristle brush with distilled water. The wash water was collected and maintained in a non-ventilated oven at 40 °C until dryness. The resulting particulate matter was weighed and the results expressed in terms of mg of solid per cm^2 of leaf area.

2.4. Determination of the chemical composition of soil and cement dust and quantification of leaf nutrients

The chemical composition of the cement dust used in the present study was determined after digestion of samples with a solution of nitric-perchloric acid. For the treatments with application of the cement dust, soil fertility was assessed by the end of the experiments, according elsewhere (Defelipo and Ribeiro, 1981; EMBRAPA, 1997). After 60 days of exposure to the treatments, the surface of leaves/leaflets were then washed with tap water to remove the cement or other particles (in the case of plants that did not receive the aerial dust treatment) and then submitted to mineral nutrient analysis following Tedesco et al. (1995). Quantification of Zn, Fe, Mn, Cu, Ca and Mg in leaves was performed using a SpectrAA-20 Atomic Absorption Spectrometer (Varian, Victoria, Australia) and the content of K was determined using a B462 Flame Photometer (Micronal, São Paulo, Brasil). The levels of B, P and S were determined using an E225D Spectrophotometer (Celm, São Paulo Brasil) and the quantification of total N was carried out according to Malavolta et al. (1997). The soil and cement dust pH values were determined in water (1:2.5; solid/water, respectively) using an AJX 511 Digital Microprocessor-based pH Meter (Micronal, São Paulo, Brazil).