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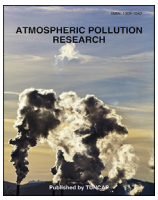

Responses of *Trichilia dregeana* leaves to sulphur dioxide pollution: A comparison of morphological, physiological and biochemical biomarkers

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ABSTRACT

Industrial zones in eThekwini, South Africa, such as the South Durban Basin (SDB) are often characterised by extremely poor air quality owing to industrial emissions. This study investigated the effects of SO_2 pollution on selected stress biomarkers in the leaves of *Trichilia dregeana* to assess the utility of this species as a bioindicator of this pollutant. Leaves were sampled from trees growing at three industrial (treatment) sites (Prospecton, Ganges and Southern Works) within the SDB and from greenhouse-located trees (*ex situ* control). Sampling accommodated for directional and seasonal wind effects and yielded a sample size of n = 24 for all four seasons. Ground-level SO_2 concentrations ($[SO_2]$) measured at each site were positively correlated with leaf sulphate contents and both $[SO_2]$ and leaf sulphate levels of the control were lower than the treatment sites. Values for the various biomarkers did not differ significantly for leaves from different cardinal points within sites, but seasonal variations were evident. Except for leaf chlorophyll fluorescence, all biomarkers could discriminate between treatment and control leaves. Though seasonal data for a number of these biomarkers were significantly correlated with leaf sulphate content, none of the biomarkers were sensitive enough to reflect differences in leaf sulphate levels across the treatment sites. Furthermore, the results suggest that leaf area and relative chlorophyll content should be measured in combination with each other. Leaf sulphate content is a reliable proxy for SO_2 pollution. All biomarkers, except for chlorophyll fluorescence, can be used to establish *T. dregeana* leaves as a bioindicator of SO_2 pollution.

1. Introduction

Since the beginning of the industrial era, the greenhouse effect has intensified due to the increase in atmospheric greenhouse gas emissions from anthropogenic activities and the growing demand for energy produced via the use of fossil fuels such as coal and oil (Jain and Hayhoe, 2008). South Africa depends on the energy sector which contributes to 15% of the country's GDP (Department of Energy and Minerals, South Africa, 2008); 70% of the total primary energy comes from coal, and 93% of electricity from coal-fired stations (Menyah and Wolde-Rufael, 2010). Coal sectors are therefore the major contributors to atmospheric pollution in South Africa, accounting for 96% of all atmospheric sulphur dioxide (SO_2), 94% of all atmospheric nitrous oxides (NO_x), and 87% of all atmospheric carbon dioxide (CO_2) (Menyah and Wolde-Rufael, 2010).

Globally, industries are the main contributors of air pollution (Lamego et al., 2000) and consume the most energy. Along the east coast of South Africa industrial zones such as the South Durban Basin (SDB) are characterised by extremely poor air quality as a result of industrial emissions (Matooane and Diab, 2001; Diab et al., 2002). Atmospheric SO_2 concentrations have been reported to be exceptionally high (15.28 ppb) in this area and have been used as an indicator of air quality within the SDB, as SO_2 contributes to two thirds of industrial emissions in the area (Diab and Motha, 2007). Those authors also emphasised the need for air quality monitoring in the SDB, given that SO_2 levels are likely to rise with the expansion of various industrial activities in the area.

In many parts of the world air quality data are usually based on

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one or a few monitoring stations at strategic sites across cities, making average pollution levels unrepresentative of specific regions (Moreno et al., 2003). This is often unavoidable since the installation of multiple air quality monitoring stations is not always financially feasible, particularly in countries within the developing world. An alternate approach is to supplement instrumental air quality monitoring with data collected using bioindicators, i.e. organisms that can be used to identify and determine the effect that biotic and abiotic stresses have on the environment (Conti and Cechetti, 2001). In this regard, Novak et al. (2003) argued that it is best to use indigenous species as bioindicators of air pollution, provided these species are able to withstand high pollution levels, have a wide geographical distribution, are abundant and easily accessible, and are negatively impacted on by the pollution (Conti and Cechetti, 2001).

The use of plants and trees in particular, as bioindicators of air pollution is a widespread strategy (Madejon et al., 2004) and the identification of reliable bioindicators of air quality may be useful in many countries across the developing world (Areington et al., 2015). Several authors report the value of using morphological and biochemical characteristics of leaves as biomarkers of environmental pollution (Bakker et al., 1999 and other studies reviewed in Madejon et al., 2004). This motivated the present study, which investigated the utility of leaves of the indigenous tree species, *Trichilia dregeana* as a bioindicator of SO_2 pollution within selected industrial areas in Durban (in eThekwini, KwaZulu-Natal), South Africa.

Vegetation represents passive sampling in biomonitoring and has the advantage of high spatial and temporal resolution due to widespread distribution of plants and low sampling costs (Sawidis et al., 1997). One of the prerequisites of using tree leaves as bioindicators is that they have to be susceptible to the pollutants investigated, but be tolerant enough to avoid mortality (Madejon et al., 2004). Literature suggests that one can measure a number of physiological, biochemical and morphological biomarkers of air pollution in tree leaves (Sawidis et al., 2011). For instance, as a consequence of air pollution and other environmental stressors, plants increase the production of reactive oxygen species (ROS) in their tissues (Vranova et al., 2002). The stress experienced by the plants and damage incurred is determined by the equilibrium between oxidative stress and antioxidant activity (Gill and Tuteja, 2010). During non-stressful periods, antioxidant defence systems are able to protect the organism from ROS (Gill and Tuteja, 2010); however, excessive production of ROS can lead to damage such as lipid peroxidation, protein oxidation and oxidative damage to nucleic acids. Lipid peroxidation damages plant cell membranes, the integrity of which can be measured in terms of leaf electrolyte leakage (Santamaria and Martin, 1997). Other parameters such as chlorophyll content and leaf area have also proven to be useful biomarkers of pollution stress (Areington et al., 2015). Leaf chlorophyll fluorescence, though rarely applied in pollution research, is a non-destructive measure of the efficiency of photosystem II (PSII) that can be used as an indicator of stress-induced damage in a number of plant species (Maxwell and Johnson, 2000).

In light of the above, the aim of the present study was to asses the utility of morphological (leaf area), physiological (relative leaf chlorophyll content and fluorescence) and biochemical (hydrogen peroxide production, total aqueous antioxidants activity and electrolyte leakage) parameters as biomarkers of SO_2 pollution in *T. dregeana* tree leaves.

2. Materials and methods

2.1. Study area: polluted (treatment) sites, ex situ control site, location of trees and geography of area

The geographic location of the three SDB industrial treatment sites, viz. Southern Works, Ganges High School; and Prospecton, is shown in Fig. 1. At each site, four *Trichilia dregeana* Sond. trees were selected for investigation, within 750 m of an air pollution monitoring station (the locations of which are also indicated in Fig. 1). The selection of *T. dregeana* leaves is based on the fact that this evergreen coastal forest tree grows naturally and is planted as a street tree in Durban and the province of KwaZulu-Natal as a whole (Pooley, 1993). Sampling sites were selected on the basis of the presence of air pollution monitoring stations (owned and operated by the eThekwini Municipality) that measured ground-level SO_2 levels on an hourly basis. Trees housed in a greenhouse on the grounds of the University of KwaZulu-Natal (Westville, Durban), a month prior to and for the duration of the study, served as the *ex situ* control. Recent studies (Areington et al., 2015; Appalasamy et al., 2016) have shown the utility of greenhouse-located control trees in studies of this nature, provided that the control and treatment trees are exposed to comparable light levels, which was ensured here as discussed below.

2.1.1. Measurement of ground-level SO_2 concentrations ($[SO_2]$)

Ground-level $[SO_2]$ (in parts per billion [ppb]) were measured hourly (by the eThekwini Municipality) at each of the monitoring stations (Fig. 1) and the data was made available for use in this study. The data used were collected in each of the four sampling seasons, between 2014 and 2015. Prior to any analyses, the data were cleaned (i.e. erroneous, zero, negative and blank values were deleted) and a yearly average $[SO_2]$ and seasonal averages were generated for each monitoring station. The air pollution monitoring stations that provided the data, measure the ground-level concentrations of SO_2, NO_2 and PM (amongst others) via continuous monitoring (using United States Environmental Protection Agency (USEPA) designated analysers, North Carolina). Sulphur dioxide levels were also measured within the greenhouse and at three random points (on the university campus) less than 1 km from where the greenhouse control site is located. These measurements were carried out using a HORIBA PG-350E (HORIBA Europe GmbH, Julius-Kronenberg-Strasse 9, Leichlingen, Germany) atmospheric monitoring system. The SO_2 detector functions with a cross-flow modulation, non-dispersive infrared (NDIR) absorption method (according to the European standard, DIN EN 15267-3, DIN EN 14181).

Data for NO_2 and PM were only available for two sites, viz. Ganges and Southern Works, and furthermore only collected at the monitoring stations in spring and summer. Nevertheless, this limited dataset was also analysed here in the interest of gaining some insight into the levels of pollutants other than SO_2 at the treatment sites. The NO_2 and PM results were not related to the biomarkers directly but rather used to interpret some of the trends observed at specific treatment sites.

2.1.2. Leaf sulphate content

Leaf material from three treatment sites and the control were tested for sulphate content according to Lau and Luk (2001). Leaf material was dried at 80 °C for 48 h, weighed to l.0 g dry weight (DW) for each of three replicates, and placed into a furnace at 500 °C for 2 h. The ash produced was moistened with 1–2 drops of distilled water, and 2 ml of 1:1 nitric acid was added. Subsequent to evaporation on a hot plate, the residue was heated at 500 °C for 1 h, cooled and dissolved in 2 ml of warm 1:1 hydrochloric acid. The solution was then filtered and diluted in a flask to reach a final volume of 25 ml (Lau and Ho, 1993). The sulphate concentration in the solution was thereafter determined for each sample using a tubidimetric method (American Public Health Association, 1992).

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