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Bryophytes in Areas Subject to Exposure to Sulfur Dioxide

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A B S T R A C T

Plants may provide signs indicating environmental problems such as the presence of pollutants. Thus, this study aimed to identify species of bryophytes in Cerrado fragments in the municipality of Cezarina - GO, exposed to sulfur dioxide (SO_2) emitted by the cement manufacturing process. The bryophytes were collected at different points surrounding the cement plant. The idea was to identify the species sensitive to sulfur and, at the same time, evaluate the degree of sulfur accumulated in species by each geographical position. We identified 14 species belonging to 10 families. Of these families the most representative were Frabroniaceae with 7 individuals, Hypnaceae with 6 and Helicophyllaceae with 5. *Chryso-hypnum diminutivum* and *Helicophyllum torquatum* stood out because of the amount of sulfur present in their tissues, demonstrating to be sulfur accumulating and tolerant species.

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INTRODUCTION

The municipality of Cezarina is located in the southwestern region of the state of Goiás, has a total area of 416 km^2, in the Rio dos Bois valley microregion, where its geographical position can be found at 16°58’30” latitude and 49°46’30” longitude. The total population is 7,545 inhabitants, of which 5,595 live in the urban zone (IBGE, 2010).

The cement factory in the city of Cezarina was built 42 years ago. The region has important limestone deposits, which influenced the installation of the industry in the city, besides being located in a strategic position in the center of the country and being cut by one of the most important highways, facilitating production flow (SOUSA, 2003).

Brazil occupies the 4th place in the world ranking in cement production, producing 69 million tons per year, and being the 5th largest consumer of the cement in the world. The center west region occupies the 4th position in the country, having produced, in 2010, a total of 5.6 million tons of cement (SNIC, 2012).

Despite the economic importance of this sector in the Brazilian industry, the cement production process has high potential to generate pollution. Throughout its manufacturing process there is a wide range of pollutants generated, causing health damage and a range of environmental impacts (SALOMON, 2002).

The burning of fossil fuels and industrial discharges have contributed to air pollution in the urban-industrial environment. With the increase of vehicles and industries, the concentration of pollutants has increased significantly, highlighting sulfur dioxide (SO_2), nitrogen oxides (NO), carbon monoxide (CO) and heavy metals. The implementation of control measures is necessary due to the growth of these pollutants in the air, especially in urban centers (MARTINS MAZZITELLI; MOTA FILHO and FILGUEIRA, 2006).

Sulfur in the form of SO_2, is considered an essential element, but with increasing anthropogenic emissions coming from industries that burn various compounds, damage has been caused to different plant species (MANNINEN *et al*., 1996; 2000 MANNINEN and HUTTUNEN). The plants that absorb atmosphereic SO_2 suffer from sulfur

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accumulation effects in leaves, where changes are observed in stomatal movement, inhibition of various photosynthesis steps and activity of various enzymes (Deepak and Agrawal, 1999). Dispersion of these chemical components into the atmosphere causes physiological, metabolic and morphological alterations in the plants and may provide some changes in the development of exposed plant communities (SCHMIEDEN and WILD, 1995).

Regarding organisms with potential for environmental quality bioindication, studies show that bryophytes are among the principal biological indicators of air pollution (LISBOA and ILKIU-BORGES 2001).

The bryophytes have been widely used as indicators of environmental quality because some species are very sensitive to air pollution (VISNADI and MONTEIRO, 1990). Many of these species have the ability to accumulate pollutants due to the abundance of cation exchange sites on the walls of their cells (GERDOL *et al*., 2000). These organisms remove nutrients from the air by absorbing particles deposited on them, from the soil and via anthropogenic emissions (ADAMO *et al*., 2003). Their presence is directly related to air quality and changes resulting from urbanization. Today there are several methods to calculate contaminant concentrations and effects, however, bioindication has been the most common method used in recent decades (FILGUEIRAS and PEREIRA, 1993).

In the different cement processing stages, bryophytes are subjected to the effect of released pollutants, not only species present in areas very close to the industry, but also in remote areas, since these pollutants can travel long distances and even reach remote areas (GIODA and GIODA, 2006).

Thus, bryophytes can be used as bio-indicators because they provide signs which are quick and easy to evaluate, indicating environmental problems and anticipating the identification of pollutants (LOUZADA, 2001).

Therefore, the aim of this work was to evaluate the potential of bryophyte bio-indicator species in Cerrado fragments in the municipality of Cezarina - GO, exposed to sulfur dioxide (SO_2) emitted by the cement manufacturing process.

MATERIAL AND METHODS

Collection and identification:

The Bryophytes were collected in nine cerrado fragments margins of BR 060 between the geographical coordinates 16 ° 56,117 'S, 049 ° 40,218' W and 17 ° 06 387 'S and 049 ° 54,814' W, where the points were marked with GPS Garmin etrex model and compiled into field sheet every 5 km (Figure 1).

Fig. 1 Map of bryophyte collection points. The red line represents the BR060; the points are separated every five kilometers having the Cimpor cement factory as base point, four points were distributed toward Goiânia-GO and four toward Indiara-GO.

The Bryophytes were taken from trunks of living trees, rotting logs and rocks in a total of four samples for each species. Since, when collected in trees it was done on the basis on trees up to 50 cm in diameter using a stainless steel spatula or knife. Once collected, the samples were placed in kraft paper bags of 1 kg and placed in the sun to pre-dry. The identification was carried out in the bryophyte laboratory at the University of Brasilia with the help of keys and specialized bibliography: Buck (1998), and Gradstein Ilkiu-Borges (2009), Gradstein; Churchill and Salazar (2001), Gradstein and Costa (2003), by experts in order to confirmed the identification of the bryophytes.

Sulfur accumulation degree:

The collected bryophyte samples were dried at 60°C in an oven with air circulation, ground in a knife mill and digested according to Krug *et al*. (1983): 500 mg of sample were weighed and placed in a glass tube adding 10 ml of HNO_3, it was then heated at 120°C until almost dry in a block digester and then received 5 ml of $HClO_4$ and more heating at 120°C for another hour. After cooling, the solution was transferred to polypropylene tubes and the volume adjusted to 25 ml with deionized water.

Sulfur was analyzed in the laboratory for agricultural and environmental analysis Inc. in Botucatu SP through the turbidimetric determination of sulfate based on the turbidity formed by precipitation of sulfur by barium chloride in the form of barium sulfate, turbidity this measure in a spectrophotometer at absorbance form (A or OD) (MALAVOLTA *et al*., 1997).

Statistical procedures:

Statistical analyzes were conducted using Assistat Software Silva (1996). Three repetitions with 250 mg each were conducted for each species collected per collection point through a completely randomized design (CRD). The significance of the average sulfur accumulations was determined by the Tukey test at 5% probability.

RESULTS AND DISCUSSION

Analyzing the characteristics of the vegetation in the city of Cezarina it can be observed that most plants have leaf necrosis. According to Rao and Singh (1988) and Posthumus (1982), the emergence of leaf necrosis is can be related to high SO_2 concentrations. On the other hand, low concentration for a long period result in chronic damage, for example, gradual disappearance of chlorophyll. These leaf damage differences among species may occur due to variations in sensitivity and pollutants absorption (AGRAWAL; SINGH and RAO, 1991).

The identification of bryophytes allowed the identification of 14 species belonging to 10 families, as shown in Table 1. Of these families, the most representative in terms of specimens collected were Fabroniaceae with 7 individuals, followed by Hypnaceae 6, and Helicophyllaceae with 5 individuals.

Table 1: List of bryophyte species occurring in the region of Cezarina - GO in different collection points.

Species	Family	Substrates	Points
Callicostella pallida	Pilotrichaceae	Stone	2
Cheilolejeunea sp	Lejeuneaceae	Tree	1
Chryso-hypnum diminutivum	Hypnaceae	Tree, Stone	7 3 5
Entodon beyrichii	Entodontaceae	Tree	2 9
Entodontopsis leucostega	Stereophyllaceae	Tree	3 1
Entodontopsis nitens	Stereophyllaceae	Tree	4 3
Erythrodontium longisetum	Fabroniaceae	Tree	4 3 2 1
Erythrodontium squarrosum	Entodontaceae	Tree	9
Fabronia ciliares	Fabroniaceae	Tree	6 3 2
Helicophyllum torquatum	Helicophyllaceae	Tree	2 5
Macromitrium contextum	Orthotrichaceae	Tree	1
Octoblepharum albidum	Calymperaceae	Tree	3 8
Sematophyllum subpinnatum	Sematophyllaceae	Tree	9
Sematophyllum subsimplex	Sematophyllaceae	Tree	8

Bryophytes include very sensitive species and with little frequency in disturbed areas. In the collections made, *Callicostella pallida* and *Sematophyllum subsimplex* were found at Points 2 and 8, a characteristic that may be related to the pollution of the site. Lisbon and Ilkiu Borges (2007) noted that these species are quite common in the Amazon and their development may be linked to the characteristics and quality of the environment.

Chryso Hypnum diminutivum has the largest distribution per collection point, with presence at 3 points of the analyzed area. This species is common at Point 5, the area closest to the cement factory, and found both in trees and on stones. According to Neto (2011), this specimen is quite common in Brazil and can be found in various areas of the cerrado, especially on dead trunks and trees. For presenting wide distribution in different environments, this specimen was considered tolerant to air pollution.

Fabronia ciliaris was collected at three points, 6, 3 and 2, being found in trees. It has very wide distribution in America (Buck, 1998), with greater presence in disturbed areas, mainly in urban centers, because it is a pollution-resistant species of large cities (PERALTA, 2005). According to Vital and Bononi (2006), this species has been found in cemetery tombs and can adapt to certain adverse conditions, such as air pollution. Other species that have wide distribution and present pollution tolerance features are *Octoblepharum albidum* and *Sematophyllum subpinnatum* (BORDIN, 2009). However, in the points studied, these were collected at Points 3, 8 and 9, between 10, 15 and 20 km away from the cement plant, indicating that they may be finding it difficult to development and reproduce in the studied locations. According to Angelo and Santos (2004), these species do not show a preference for substrates and weather conditions.

The species *Erythrodontium longisetum* and *Sematophyllum subpinnatum* have wide distribution in the study area. However there is no data to show their bioindicator potential, since the first registeration of such specimens in the state was cited by (CARVALHO and SANTOS, 2005).

Entodontopsis leucostega has little distribution in the collection areas, being found at points 3 and 1, which may reflect a greater sensitivity to the conditions of these urban environments, such as substrate heating, low humidity and mineral nutrient availability, high pollutant concentrations and other consequences of anthropogenic action (CARVALHO, 2004).

*Entodontopsis niten*s was found at Points 3 and 4, 15 and 20km away from the cement plant, showing difficulty to develop in areas closer to the factory. According to Carvalho (2005), this species is found quite often in protected areas, as they provide more appropriate conditions and enable further development of this species a factor that may explain the presence of this species in only two collection points.

Helicophyllum torquatum, a species occurring in large quantities in the state of Espírito Santo and in the southern and eastern regions of Minas Gerais state, have low frequency in the state of Goiás (YANO, 1979 and 1984). In this work, *Helicophyllum torquatum* was found in front of the Cezarina cement factory and at Point 2, occurring in dense woods with preservation characteristics.

The species *Cheilolejeunea sp* and *Macromitrium contextum*, also mentioned in the work of Yano (2005), have no registrations in anthropogenic environments. In the collections, it is observed that these species were only found at the fourth point, which may be sensitive to exposure to air pollutants.

Comparing the data obtained in this work with the data of Soares (2011), *Entodon beyrichii* has the same habitat characteristics, being found in gallery forest interiors, always near watercourses, differentiating only tree substrate, so that it has no relation with air quality, it is only mentioned as a species present in humid environments of dense woods.

The sulfur quantification was only possible to be evaluated in the areas between Indiara and Cezarina - GO, due to the amount, in mass, of each species in the area and elsewhere not being sufficient to quantify the sulfur of the samples. Table 2 presents the data obtained from the cumulative sulfur concentration analysis of 7 different species divided into five distinct points, and in Figure 2 shows by the sulfur average per species regardless of location.

Table 2: Average sulfur concentration in the exposed Bryophytes samples between Indiara and Cezarina – GO.

Species	Sulfur (g kg^{-1})	
	Point	Averages
Cheilolejeuna sp	1	2.36 b
Chryso hypnum diminutivum	5	2.68 a
Crhyso hypnum diminutivum	3	1.50 de
Entodontopsis leucostega	3	1.97 c
Erythrodontium longisetum	1	1.21 e
Erythrodontium longisetum	4	2.56 ab
Fabronia ciliares	3	2.51 ab
Helicophyllum torquatum	5	1.75 cd
Helicophyllum torquatum	2	1.43 e
Macromitrium contextum	1	1.91 c
CV%	5,24	

The averages followed by the same letter are not statistically different from each other. The Tukey test was applied at 5% probability. CV - Coefficient of variation.

The results obtained confirm the sulfur accumulation capacity of the bryophyte species analyzed, highlighting the specimens of *Crhyso hypnum diminutivum* and *Helicophyllum torquatum* (Figure 3), which were found at Point 5 at sulfur concentrations greater than 1.75g kg^{-1} and at Points 2 and 3 at 10 and 15 km away from the cement plant, with sulfur concentrations less than 1.50 g kg^{-1}, showing that the closer the proximity of the specimens to the cement plant, the higher the sulfur level found. Therefore, one can consider these species as accumulative sulfur bioindicators. According to Costa (2007), the species can be considered bioindicators of air pollution by accumulating anthropogenic influences without showing damage that can be recognized over a short period, being called accumulative bioindicators.

The species *Cheilolejeuna sp* and *Macromitrium contextum* have a very high level of sulfur because they are located within Point 1 (20 km from the cement plant), with characteristics of an accumulator species. However, at points closest to the plant it was not possible to find these specimens, possibly because they are sensitive to exposure to high pollution levels as they were not found closer to the cement factory areas. According to Rennenberg (1984) and Burton (1986), plants living in industrial regions are able to accumulate sulfur, typically presenting a direct negative correlation between sulfur concentration in the leaves and the distance from the emission source.

According to Medeiros; Santos and Tertullian (2008), sulfur content in cerrado plant species may vary between 1.4 g kg^{-1} at 2.4 g kg^{-1}, and comparing to the data of the present study, almost identical results were obtained in terms sulfur content in plants ranging from 1.4 g kg^{-1} to 2.7 g kg^{-1}, different form the study by Silva (2011), which had an average of 4.94 g kg^{-1}. This difference in sulfur levels can be related to the use of other species or higherr exposure to the pollutant.

The CONAMA Resolution No. 03/90 Brazil (1990), regulates the following parameters: total suspended particulates in smoke, inhalable sulfur dioxide, carbon monoxide, ozone and nitrogen dioxide particles. With respect to sulfur dioxide, 80 µg/m³ is considered as primary standard and 40 µg/m³ as a secondary standard, as the annual arithmetic average. Since the values set by CONAMA Resolution No. 03/90 considers the concentration of substances in a volume of sampled air, unlike the passive biomonitoring performed in the present work, it is not possible to compare the results. However, the results show an increasing accumulation of sulfur dioxide in the samples as a function of distance to the cement plant, showing the efficiency of bryophytes as bioindicators.

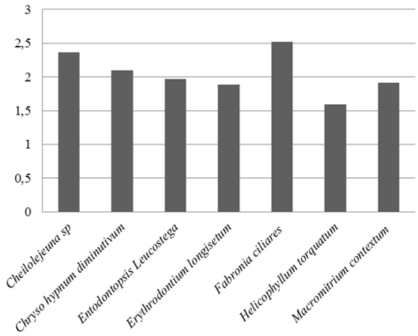

Fig. 2: Sulfur accumulation in bryophyte species in (g kg^{-1}).

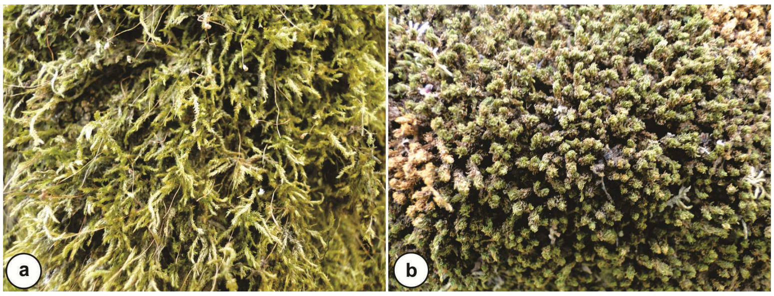

Fig. 3: (A) *Crhyso hypnum diminutivum* (B) *Helicophyllum torquatum.*

Conclusions:

The bryophyte species identified have proved to be excellent cumulative sulfur dioxide monitors, or sensitive to it.

Species that were found in locations near the factory and at distant points showed different sulfur accumulation levels.

The evaluation of air quality in the city of Cezarina is suggested, using bryophytes as bioindicators in controlled environments, where the level of sulfur can be calculated more precisely, enabling the creation of sulfur tolerance limits for each bryophyte species studied.

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